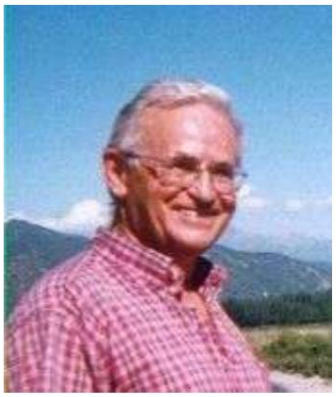
ARTISANAL SPIRULINA GROWING MANUAL

(Revised February 2, 2018)

(Details on the LATEST REVISIONS)
(Summary in English: ENGLISH)
(Summary in Spanish: CASTELLANO)

SUMMARY

PRESENTATION



JP Jourdan shares here his experience of nearly twenty years of practicing spirulina cultivation and also shows how to apply chemical engineering methods to perfect this production even on a small scale and without sophisticated technical means. It gives a wealth of details allowing the construction of the culture installation and its operation in very varied circumstances, but also to evaluate and optimize the cost price of this micro-algae so currently in demand for its nutritional virtues. or others.

A graduate of MIT, JP Jourdan made his career in the chemical industry before devoting his retirement in the south of France to the development of spirulina for children in the Third World. After having been a "spirulina student" with Ripley D. Fox and Francisco Ayala, he is a member of Technap and has actively collaborated with Antenna Technology and several other NGOs in the field of spirulina.

PRELIMINARY

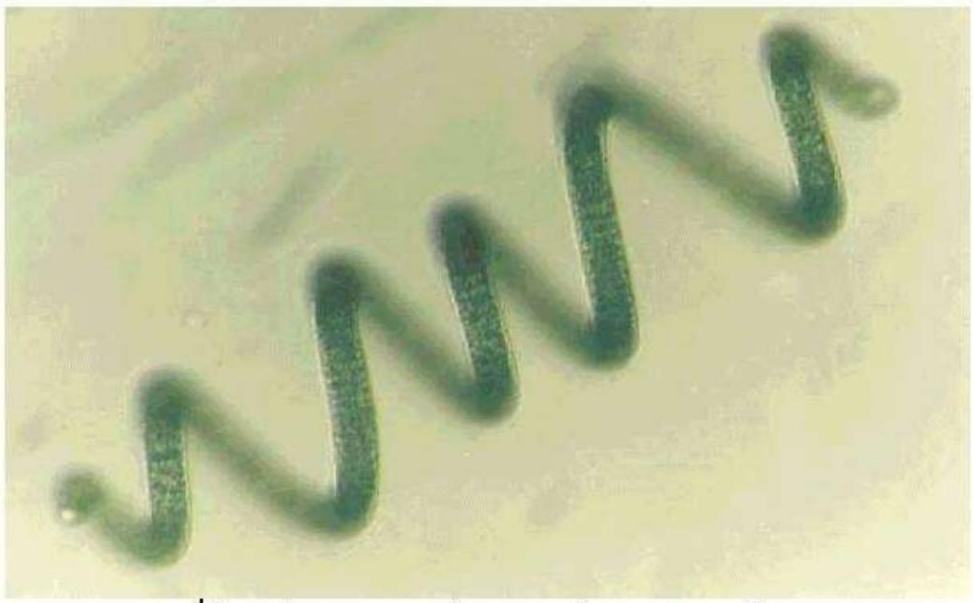
The objective of this manual is to train trainers to disseminate and make the cultivation and consumption of spirulina accessible to a greater number of people, and to help future "gardeners" (gardeners of the future?) to control a certain number of parameters. to produce, on a family, cooperative or community scale, a food whose nutritional qualities are recognized today, but which remains practically inaccessible to them, at least in the fresh state. It is not necessary to provide the necessary elements for an operation meeting only criteria of commercial profitability, especially in countries with expensive labor, because the proposed process is very labor intensive because of its almost zero mechanization rate.

We have been growing spirulina on a small scale since 1991, with the aim of bringing it within reach of those who really need it. We hope that this document, essentially based on our personal experience and that of a few colleagues working in a similar way, can initiate you, guide your first steps in this new type of culture.

We advise you to start your culture on a small scale, to get your feet wet, better understand natural phenomena that are actually very simple, and handle working tools that will be all the more familiar to you as you have made them yourself.

Let us add that without being able to guarantee the quality of the spirulina produced in such a place, in such a climate, in such conditions, we can affirm that we have never had knowledge of a case of toxicity of a spirulina produced by craftsmen in latitudes (between 0 and 45°) where we worked.

WHAT IS SPIRULINA?



Arthrospira platensis

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"SPIRULINA"

It is a small aquatic being (0.3 mm long), as old as the world whose scientific name is "cyanobacterium *Arthrospira* platensis" (not to be confused with the marine cyanobacterium scientifically called "Spirulina subsalsa"), which lives by photosynthesis like plants and thrives naturally in saline, alkaline lakes in warm regions of the globe. Traditional food of the Aztecs of Mexico and the Kanembous of Chad, richer in protein than meat, spirulina is now cultivated in large factories in the USA, India, China, Thailand, etc., because it is always discovered more interesting qualities for food and health, both for humans and for animals. By

example a child suffering from kwarshiorkor (malnutrition) can be restored by giving him a spoonful a day of spirulina for a month. Spirulina strengthens the immune defenses and alleviates the suffering of people with AIDS.
It allows tuberculosis patients to better tolerate their treatment. Spirulina is also used as an active ingredient in cosmetics.

In nature, spirulina only needs to "grow" a clay basin retaining brackish and alkaline water, in a hot climate, and some animal waste. The flamingos of the "minor" species (the most numerous) provide the supply of excreta and the agitation necessary to ensure the growth of the natural spirulina which is their exclusive food, especially in lakes in East Africa. East (Rift Valley).

Spirulina comes in the form of filaments made up of juxtaposed cells.
The reproduction of spirulina, asexual, is done by division of the filaments.

For details on the characteristics, virtues, industrial manufacture and market of spirulina, we refer you to the most recent works available on these subjects, including the classic "Earth Food Spirulina" by Robert Henrikson, published by Ronore in the USA (1997) and those of Jacques Falquet: “Spirulina, Nutritional Aspects”, Antenna Technology, Geneva (2006) http://www.antenna.ch/documents/AspNutr2006.pdf, D. Fox: “Spirulina, Production & Potential” , Editions Edisud (1999), without forgetting “Spirulina Platensis (Arthrospira), Physiology, Cell biology and Biotechnology”, by Avigad Vonshak, Editions Taylor & Francis (1997). “Earth Food Spirulina” is now available at http://www.spirulinasource.com/ with permanent update. “Spirulina in Human Nutrition and Health”, by ME Gershwin and Ahma Belay, CRC Press (2008) is highly recommended.

The Hawaiian plant is described in http://www.cyanotech.com/.

See also, of course, the publications of Antenna Technologie on www.antenna.ch.

CLIMATE INFLUENCE

The two fundamental parameters that contribute to constituting the climate are temperature and rainfall.
However, do not neglect the prevailing winds, for example the mistral in the Rhône valley, which can have significant consequences on the evaporation of a culture basin, on the temperature of the water or the "pollution" of this basin by all the debris and dust that it may carry.

Similarly, certain elements such as hedges, the presence of rocky bars, forests, etc. can have significant consequences on the microclimate, consequences that should be assessed before setting up a pond... like a vegetable garden.

2.1) Temperature
The first benchmarks concerning temperatures are about the same as for humans, 37°C: ideal temperature for growing. Above, it's too hot (43°C can be deadly). Below, the rate of multiplication decreases with temperature. At 20°C growth is practically stopped. The temperature of the culture medium must therefore be between these two temperatures. The longer the “season”, the longer the harvest period. Continental or high altitude climates are disadvantaged.

The handicap of a climate that is too cold can be compensated artificially, as for all plants. The construction of ponds under greenhouse can be all the more interesting as this shelter constitutes not only a protection against the cold, the evaporation, the insects and the dust but also against the diluvian rains, like the storms, which can make overflow the basins and therefore cause a loss, or at least a dilution

of the culture medium.

2.2) Rainfall
Running culture ponds requires a minimum of water resources. Rainwater is interesting because it is clean and neutral (no minerals in solution). In climates with low rainfall, or with a long dry season, it may be necessary to provide a cistern to store rainwater and thus compensate for evaporation from the basins. Again, there needs to be a “middle ground”. Excess rainfall will have to be anticipated by constructing deeper basins or protecting them. The lack of water is obviously prohibitive. The lack of rainwater can be compensated by the use of water from various sources, and more or less "loaded" (river or river, groundwater, waste water...). It will then be necessary to take into account the quality of the water in the development, then the maintenance of the culture medium.

The presence of a translucent cover above the basins to avoid dilution of the culture medium is a good solution in regions with heavy rainfall (see § 3.2 cover).

2.3) Ideal climate
There are ideal climates where it is never cold and where the rains are evenly distributed and compensate for evaporation, such as certain points on the eastern slope of the Andes. Another type of ideal climate is the desert at the foot of mountains that ensure a large supply of water, such as the Atacama Desert in Chile. The water consumed by a basin is mainly used to maintain the culture below 40°C, by evaporation. In a desert climate without water cultivation is impossible (except to import water), while in a cool climate greenhouse cultivation is easy with low water consumption.

2.4) Seasonality (See Appendix A25 wintering)
In temperate regions, winter is usually too cold to grow spirulina, except with expensive artificial heating and lighting. Even in hot regions, an annual shutdown may be made necessary by heavy rains or drought or by sandstorms in certain seasons.

The cultivation of spirulina will therefore often be seasonal.
During the bad season, a "strain" of spirulina must imperatively be kept in its culture medium. The containers (jars, carboys, basins) must allow light to pass through and be stored in a bright place but in the shade, or be under electric lighting. Even though spirulina cultures survive temperatures below 10°C, or even brief frosts, it is prudent not to store them below 18°C for long periods of time, as the risk of contamination increases.

The fact that spirulina thrives in a very alkaline environment has two major advantages: - better absorption of carbon dioxide from the air - protection against contamination.

This protection was involuntarily demonstrated to us in the spring of 1997. We had two 10 m² spirulina basins side by side, one in the open air, the other protected from the rain. The unprotected basin having overflowed was drained and filled with rainwater, which was colonized by unicellular green algae (chlamydomonas) and many animals (red worms, mosquito larvae, swimming insects). The other basin has kept its spirulina without contamination. However, it should not be believed that only spirulina can grow in its culture medium: other algae, microorganisms and animals can live there, hence the need to monitor cultures from the point of view of contaminants, especially at changes in seasons.

BASINS

Where should the pools be located? It is necessary to respect some rules which are not always obvious: not under trees, nor in a place subject to flooding, nor near a road or an industry (pollution). Safe from the curious, often ignorant and not always well intentioned. Flat ground will make the work easier, as will proximity to water, etc. It is worth thinking about before deciding.

3.1) Construction of culture basins

For family or artisanal production, we can be satisfied with small basins, without paddle wheel agitation, without median baffle. There are then many ways to build a suitable pond, varying according to local conditions.

The pool should not have sharp angles, but rounded shapes (at least at the ends in the case of rectangular pools). The bottom should be as level as possible,

with a very slight slope towards a more hollow place of easy access (to facilitate emptying).
The edges of the basin must be above the level of the ground, to reduce the entry of dust and animals, and at least 20 to 40 cm above the bottom: it is better to provide a fairly deep depth, to collect the rains , facilitate transfers between basins and possibly the biological self-purification of the culture medium. The pools, especially the deeper ones, must be taken care of to prevent access by small children. We must also make sure that we cannot confuse the basins with a dump, a mishap that has unfortunately happened in several countries.

One of the biggest difficulties in making a pond successful is leveling the bottom: in fact, this is where the main surface limitation lies for a craftsman with only ordinary tools (pickaxe, rake, ruler and spirit level) . For large pools, companies use lasers, which make the job much easier.

A variant, which will not be described here because it is not very suitable for artisanal conditions, consists in cultivating in a sheet of water flowing down an inclined plane.

3.1.2) In plastic sheeting

A film thickness of 0.25 mm minimum, and preferably 0.5 mm, is recommended. The film (polyethylene, EVA, PP, EP, PVC, EPDM rubber), food grade (or at least non-toxic), plasticizer-free and UV-resistant, can be simply attached to a wooden or steel tube frame or PVC, or supported by a low wall made of boards, bricks, concrete blocks (preferably cemented and on a concrete foundation), possibly stabilized raw earth (rammed earth, “banco”). In fact, the hard wall solution is the best if there is a risk of attack by rodents or termites. Avoid as much as possible the folds in the angles giving zones which would not be well agitated or ventilated. It is recommended to cement the ground supporting the basin or to cover it with a layer of well-tamped river sand or crushed laterite. If you have to use thin plastic film, protect it from direct contact with the ground and masonry, for example with a "geotextile" type felt or two or three layers of used film. There is a PVC film, food grade, 1.2mm thick and 2m wide, which can be assembled by welding with a special hot air gun (requires electricity). The EPDM rubber film, which can stick, is a good but luxury solution. Thick, weldable or glueable films reduce wrinkles and make it easier to install a center baffle that can simply be welded or glued to the bottom of the pond, but these films tend to stay out of reach for small growers. The baffle can be a pocket filled with sand, or a large tube placed under the tarp.

When installing films and tarpaulins, take into account the high coefficient of thermal expansion of plastic films (if installed in hot weather, there will be significant shrinkage in cold weather, and vice versa).

If using a film of unknown quality, do a culture test to check that it is not toxic and that it is resistant to the culture medium (see quality).

If there are termites it is recommended to put a bed of sand on a layer of ash under the plastic and to use a hard wall, or at least to treat the wood, unless you have naturally unassailable wood; you can also put the film on a slab of dried clay or better still cement, or protect it with metal. Note that African quackgrass is able to pierce plastic. Sometimes the plastic does not leak even if it is

pierced with a small hole, which closes spontaneously.
A small hole can be repaired with a tacky (dry) black putty sold commercially for this purpose, or even with a "patch" of waterproof tape.

Rodents can be formidable dangers for unprotected plastic film ponds. For years I didn't have this problem at Mialet, then in the winter of 2000-2001 (very mild) 4 pools were drilled with multiple holes on the unprotected edges. There are electric ultrasonic devices that effectively repel rodents.

To be able to drain and clean a basin consisting of a plastic tarpaulin supported by a hard wall, an easy way is to make a hole in the ground near the edge of the tarpaulin to form a drain point (sump pit).

Photo of one of the first pools (in PVC coated polyamide fabric) of the Ecopark, Madurai, Tamil Nadu (India), 18 m², 1998:

3.1.3) “Hard” (concrete, breeze blocks, bricks)

The bottom of a cement basin must be built in the form of a reinforced concrete slab of at least 10 cm thickness, of very good quality, on well-compacted ground. The edges of the basin can be made of bricks, blocks or reinforced concrete. Avoid sharp angles. Take care of the waterproofing coating (a waterproofing adjuvant or an epoxy paint are practically essential, or else paint the cement coating with lime – in this case leave the lime in place before impounding). It is good to wait a few days, basin full of water, before inoculating with spirulina (otherwise the excessive alkalinity of lime or fresh cement can very quickly turn the spirulina yellow). There are techniques for building very long pools (50 to 100 m) without expansion joints. The combination of concrete and plastic film is also a solution, either for the film to line the concrete to seal it, or for part of the

basin either in plastic film and the other in concrete (with concrete-film connection as practiced with success by Bionor in Chile). Concrete cracks can be repaired with silicone sealant.

Pictures :

At the Camilian Fathers in Davougon (Benin), 8 m², 1994:

In a village near Madurai, Tamil Nadu (India), 1 m², 1996:

3.1.4) In clay (if there really is no other possibility):

Dig 20 cm and make a well-packed slope of 20 cm as well. If the ground is not naturally clayey, cover the surface with a layer of good quality moist clay, 3 to 5 cm thick, well packed to avoid cracks. Line the edges with fired tiles or bricks, or with plastic to prevent cracking when the level drops. Spirulina grows very well in a clay basin, but its bacteriological purity must be monitored more closely (increased risk of the presence of anaerobic microorganisms at the bottom because the bottom cannot be stirred). The sealing is not complete, but it can be improved with even a very thin plastic film placed under the clay.

3.2) Coverage of the culture basin

In the absence of any protection on the basin, a good availability of water (to compensate for evaporation), the absence of torrential rains (rains of more than 200 mm/day) and low temperatures are necessary.

It is in fact often useful, even necessary, to install a greenhouse or at least a roof over the pond, to protect it against excessive rain, sun or cold, and against falling leaves, droppings of birds, sandstorms and various debris, while allowing it to "breathe". The roof can be made of white tent canvas or white PVC coated polyamide fabric allowing some light to pass through but capable of stopping the rain sufficiently. It can also be made of translucent plastic: anti-UV treated polyethylene film used for the construction of horticultural greenhouses, or sheets of polycarbonate or gel-coated fiberglass-polyester (to prevent the fibers from coming out). If the roof is opaque, it must be high enough so that the pool receives enough light from the edges. The roof is preferably completed with a translucent closure or mosquito nets on the sides. If the rain is tolerable, the roof can be replaced by simple shade (shade net, canisse, woven palm leaves). The roof can be floating (but not in contact with the crop) if the pond is too large to build a fixed structure to support it.

Installing a greenhouse consists of covering the basin with a translucent film with a slope and sufficient tension or supports to prevent the formation of pockets of rainwater and resist

to storms. The film can be supported by rigid uprights or iron wires or netting (underneath and sometimes also over). Ventilation and/or access openings must be provided and equipped with mosquito nets. It is generally necessary to also provide a shading device (black woven plastic shade net for example). Untreated wood and galvanized steel are suitable materials for greenhouse structures. Avoid cadmium-plated screws (with yellow reflections). Also avoid any paint that does not resist well to the culture medium (epoxy paint is suitable). Prohibit anti-rust paints based on minium (lead). Lay and stretch the film in hot weather to prevent it from stretching in hot weather. It should be noted that certain woods are attacked by the medium of culture, and that according to the country the use of wood of certain essences only is authorized in the industry or the food craft industry. An economical embodiment of a greenhouse pond consists of making a low wall of rigid elements (cemented or uncemented concrete blocks or bricks, boards screwed to steel stakes), laying the waterproofing film covering the low wall and l burying on the edges then stretching over a greenhouse film itself buried on the edges. A slight slope (4%) of the greenhouse film is sufficient for water to run off the film even in very heavy rains without accumulating there, on the express condition that the film is stretched (in hot weather) like the skin of a cow. drum or umbrella cloth; the slope may be provided by wooden beams or rafters forming a framework over the basin, if wood is permitted. NB: with a low slope, it is likely that the greenhouse will not withstand a heavy snowfall or hail. To access such a pool and ventilate it, it is necessary to install at least one point (but preferably two) an access "door", a simple vertical frame on which rests the edge of the film which remains unburied at this place ; the door can be closed with a mosquito net (not only against insects but also dead leaves). Plan the construction so that the horizontal component of the tension of the film does not tilt the low wall. A variant of this method of construction consists in doubling the outer film with an unstretched inner film with a low point, which allows better insulation the greenhouse and collect the condensation water. The most economical embodiment of a greenhouse pond uses the same film (greenhouse film) for the bottom, sides and cover. With a standard-width greenhouse film (6.5 m), pools up to 30 m² can easily be made. The ridge, facing East-West, can be a 6 x 8 cm wooden rafter, 5 m long fixed at about 1.5 m high. The film is stapled to the ridge on one side, then on the other before being fixed by battens to the ridge. At both ends, place a ledge made of planks or concrete blocks on which the edge of the film is raised and firmly fixed, and two access "doors" to be provided with mosquito nets are arranged. The cost of materials is $5/m² if the protective underlay is made of recycled plastic film (used), excluding shading, side protection and agitation. Experience has shown us that wild boars do not attack these plastic film structures, but side protection against the risk of drilling is still recommended: place it at least 50 cm from the edges if it is made of raw material that can damage the film when moving in high winds. To ensure stability in high winds, fill the pool by at least 20 cm. It is recommended not to leave the sides of the pool exposed to light as this could encourage the development of foreign algae on the illuminated walls. This type of greenhouse allows the automatic recovery of condensed water on the greenhouse film (important especially at night in desert climates). At night you can put on a soft insulating blanket.

Example of realization in 20m² (in Mialet, in 2000):

Watch out for snow if the slope is low and the beam is too low and/or too long between two supports! A variant of this system makes it possible to produce in winter. We put a fixed insulation under the bottom and on the sides up to the water level, and the slopes are insulated by a flexible aluminized multilayer insulation, as well as the doors. The flexible insulation that covers the South face can be rolled up during the day to let in the light and especially the sun which will be reflected by the North face remaining in place. Additional heating is provided by the stirring pumps. Waterproof fluorescent lamps can be hung under the rafter to provide additional light from 5 a.m. to 9 a.m. and from 5 p.m. to 9 p.m.

The use of greenhouse film raises the question of its quality from a food point of view. There doesn't seem to be a problem. Some films (those that are slightly yellow) are stabilized against UV by a cadmium-based compound but according to our analyzes the cadmium does not migrate from the plastic to the culture medium and does not pollute the spirulina.

A watertight greenhouse basin has the advantage of being able to be supplied with carbon dioxide from the combustion of gas or fermentation (compost) but aeration remains necessary if only to maintain a non-toxic oxygen level for spirulina.

A shady and airy greenhouse is ideal in all climates because it allows maximum control of temperature, light, rain and evaporation as well as insects and other animals, dust, dead leaves; it is the most effective protection for reducing water consumption as much as possible in an arid climate. In greenhouses with openings fitted with mosquito nets, there is generally no implantation of Ephydra fly larvae in the crops. And if an infestation does occur, it is easy to let the temperature rise to 42-43°C long enough to kill the larvae without killing too many spirulina.

At Bionor, near La Serena, Valle de Elqui (Chile), 1997: only the heads of the basins are made of cement, the rest is made of semi-rigid ethylene-propylene film (end embedded in concrete), the greenhouses have a wooden frame .

3.3) Number and area of pools

It is better to build two or more small basins than one large one: this way one can be emptied (to clean or repair it for example) without losing its contents, and if one of the cultures becomes contaminated, is not in good health or dies, another pond will allow to continue and reseed. It can also be practical to draw from one basin to filter on another. A service basin is also useful for preparing the culture media and carrying out transfers, or for evaporating purges with a view to recycling the salts, or else for purifying the culture medium, but it is not absolutely necessary.

One m² of pool covers the spirulina needs of one to 5 people depending on the dose. The investment cost per m² decreases when the unit surface and the surface/perimeter ratio of the pools increase. On the other hand, narrow pools (width less than 3 m) are easier to stir and cover. A unit surface of 5 to 20 m² seems practical at the family level or for a dispensary (depending on the daily dose of spirulina, and according to the productivity of the basins). For artisanal production, the total area of the pools will hardly exceed 300 m², in general, but a “semi-artisanal” level is possible, possibly exceeding 1000 m² (Appendix 28).

3.4) Pelvic agitation

Agitation is necessary to homogenize, promote the elimination of oxygen and ensure a good distribution of light among all the spirulina. Except in the event of very strong sun, one can with the rigor be satisfied with discontinuous agitations, more or less frequent (a few minutes every hour, at least 4 times per day), manual with a broom or an oar, or by pumps not damaging spirulina (propeller, screw, vane, diaphragm or vortex pumps). A magnetic drive aquarium pump of 1 to 3 m3l/h, running 15 minutes per hour or half hour (clock programmer) or even continuously, is sufficient.

to agitate 5 to 10 m² of pool if it is well positioned (orientation of its jet in elevation and in azimuth) and if the edges of the pool are regular and its angles rounded. A median baffle can facilitate circulation, but it must generally be supplemented by corner baffles redirecting the flows from the edges towards the centre, which complicates installation: in a small pool of well-chosen dimensions, the central baffle is completely useless. The installation of a median baffle in pools made of plastic sheeting (covering the baffle) poses the problem of creases which must be avoided as much as possible. This problem is minimized if the baffle is low in height (20 cm) and its ends are rounded. But some prefer baffles fixed on marble or granite slabs placed on the tarpaulin; in this case care must be taken to minimize the by-pass under the baffle. Another embodiment of the baffle is to weld a strip of tarpaulin to the bottom, and support it with strings to the greenhouse structures. There are also baffles made of bags filled with sand and suspended by strings. You can also put a big tube under the tarp. The efficiency of the pumps is improved by having their jet pass through a "Venturi" tube, but this complicates the firm installation of Consac (Charente Maritime) are agitated with this venturi system. All the basins of the

From time to time, clean the strainers of the pumps and the recesses of the body of the pumps (I prefer to remove the cover which only serves to embellish the aquarium pumps). With “wavy” stems (Paracas) ordinary cellar pumps can be used without risk of breaking the filaments; such a pump can agitate a square or round pool of 50 m²; but these pumps are not magnetically driven and therefore include a seal, which can cause sealing and corrosion problems after a while). Warning: 220 Volt pumps require precautions to avoid electrocution, especially in humid greenhouses (aquarium pump manufacturers ask that you unplug before touching the water); it is recommended that the power supply system be connected to a grounded “insulation screen” transformer (system used for shaver sockets in bathrooms); safety can be supplemented by a 30 mV differential circuit breaker. 12 or 24 Volt pumps are preferable…

Paddle wheel agitation remains preferred for medium to large sized ponds.

Better a discontinuous energetic agitation than continuous but weak. Even a vigorous agitation will be more effective if it is intermittent because at each restart there is mixing, whereas continuously the mass of water tends to move in one block (unless baffles are installed across the running). It is good practice to agitate the pond at least once a day, especially if it is deep enough, and to brush the bottom and sides once a day.

The large, very long industrial basins are always equipped with a median baffle and agitated by a paddle wheel. Their maximum unit area is 5000 m². The technique of construction of paddle wheels deserves a special chapter but will not be treated here, but only briefly discussed in Appendix 24.

Cultures in layers of water on an inclined plane (see § 3.1) are agitated by the turbulence due to the flow. Their construction is delicate and the cost of pumping is heavy.

Another mode of agitation, the compressed air bell applies well to small rather deep basins, preferably round. It consists of bringing a flow of compressed air (from an aquarium compressor) under a heavy bell placed at the bottom of the basin (a Pyrex dish works well): the bell is raised on one side, at regular intervals, producing a

large air bubble; by falling the bell causes a certain circulation of the liquid. On a round basin of 7 m² equipped with a 300 l/h compressor in continuous operation, the agitation proved to be good. An important advantage of this mode of agitation is the absence of electric wires. In practice, this mode of agitation is limited to basins or small basins but can be of real service.

It is necessary to emphasize that the culture medium is very corrosive to metals. Practically only galvanized steel and type 304 stainless steel resist quite well.

3.5) Containers, basins, sheaths

It happens that translucent containers such as bottles, carboys, basins, plastic film sheaths, fruit juice containers (there are 1000 liters) are used as small basins. It should be noted that the rate of photosynthesis will appear to be faster in such containers because the culture medium there receives light from several sides and also heats up more quickly. This can be advantageous, but the temperature and pH need to be monitored more closely than in ordinary ponds. Stirring in such containers is preferably done by compressed air (aquarium compressor).

NB These are in fact variants of “photobioreactors” with a large surface/volume ratio making it possible to achieve high biomass concentrations.

3.6) Repair of plastic films

It is possible to repair small holes in the films: clean and dry an area around the hole then stick a soft and sticky product (food grade) sold for this purpose, resembling chewing gum. PVC can also be repaired with glued or welded patches, or with a waterproof adhesive strip. Some adhesive tapes also apply to polyethylene films. Warning: use food grade products.

4) CULTURE MEDIUM

[NB MEDFEED software exists to facilitate calculations of media and food; see end of this chapter]

4.1) Preparation of the culture medium

Spirulina live in both salty and alkaline water. The water used for the culture medium should preferably be drinkable (but not strongly smelling of chlorine) or at least filtered (on filter candle or sand filter) and sometimes UV sterilized, the most important being the elimination of foreign algae. Rain, spring or borehole water is generally of adequate quality. If the water is hard, mineral sludge will be produced (more or less abundant depending on the calcium, magnesium and iron content), which settles quickly and is not particularly troublesome for the crop, provided however that the initial sowing in spirulina is quite concentrated. If the water is too hard, it is better to treat it to avoid annoying sludge.
The permitted alkalinity (or basicity, the two terms are interchangeable) and salinity limits are quite broad but we generally place ourselves towards the minimum, for reasons of economy (unless the source of alkali is very cheap), with a total salinity of 13 g/litre and an alkalinity of 0.1 gram-molecule/ litre (b = 0.1); but these concentrations can be doubled without inconvenience. It may even be beneficial to work at double alkalinity to smooth out pH fluctuations.

in the afternoon, especially on the surface or in the corners of the pool when the agitation is deficient. A case where b = 0.2 is preferred is that of an open basin started in the dry season: dilution by rain can bring b back to 0.1 or even below during the rainy season.

The alkalinity is usually provided by sodium bicarbonate, but the latter can be partly replaced by caustic soda or sodium carbonate which have the advantage of raising the initial pH of the culture medium (for example 5 g/l of sodium bicarbonate + 1.6 g/l of soda gives a pH of 10); sodium carbonate or caustic soda can even be the only source of alkalinity provided they are bicarbonated with carbon dioxide or by exposure to the air before use [be careful not to confuse caustic soda and "soda crystals" of commerce which are sodium carbonate decahydrate]. Natron or trona can also be used (see natron). The additional salinity is provided by the various fertilizers and salt (sodium chloride). Iodized and fluorinated kitchen salt may be suitable but often it contains up to 2% insoluble magnesia: it is better to use a salt that does not contain any, to avoid an excess of mineral sludge. Similarly, if the salt provides too much soluble magnesium (sulphate for example), there will be formation of insoluble mineral salts, especially at a fairly high pH; excessive mineral sludge can be very troublesome for a culture that is seeded with a low concentration of spirulina: this is in fact easily carried away by the flakes of sludge at the bottom of the basin without it being possible to recover it.

This is also a reason that militates against adding calcium at the start of a new crop. Furthermore, the use of a low-refined salt can be recommended because of its content of beneficial trace elements.

In addition to salt and soda, the culture medium contains fertilizers to ensure the growth of spirulina, as in usual agriculture: nitrogen (N), phosphorus (P), potassium (K) are the three main elements, but sulfur (S), magnesium (Mg), calcium (Ca) and iron (Fe) must also be added if they are not provided in sufficient quantity by water, salt and fertilizers. An analysis of water and salt is useful to calculate the dose of Mg, Ca and Fe to add because an excess of these elements can be harmful (loss of soluble phosphorus, formation of sludge). Water, salt and fertilizers often provide enough trace elements (boron, zinc, cobalt, molybdenum, copper, etc.), but as these are expensive to analyze, we prefer, when possible, systematically add trace elements, at least the main ones except molybdenum which is always sufficient.

The preferred nitrogen sources of spirulina are ammonia and urea, but these products are toxic beyond a limit concentration (urea hydrolyses little by little into ammonia). This is why it is often preferred, at least during the preparation of the culture medium, to use nitrate, of which a high dose can be added without danger, thus constituting a long-term nitrogen reserve. Spirulina will first consume ammonia or urea if available. A slight passing smell of ammonia reveals that we are approaching the authorized limit; a persistent and strong odor indicates that it has surely been exceeded and that a poor state of the culture must be expected (transient or irreversible depending on the dose of ammonia).

Spirulina fed with urea for a long time loses its ability to consume nitrate. If such a strain is used, it will therefore be necessary to start the culture with urea but without exceeding the permitted doses, i.e. it will be necessary to put it in small frequent doses (guided by the increase in the amount of spirulina in the pool: put a maximum of 0.3 grams of urea per gram of spirulina present).

Note: Urea is the common name for carbamide; some people confusing urea and urine, and may feel a certain reluctance to eat spirulina made with "urea", it may be preferable for them to replace the term "urea" with its scientific synonym: "carbamide" , just as correct but less evocative. However, urea is a very clean and odorless product, widely used in agriculture, and very generally available in the Third World.

Nitrate is not really without risk because it can be transformed spontaneously into ammonia under certain conditions (in the presence of sugar for example and undoubtedly of exopolysaccharides secreted by the spirulina itself). Vice versa ammonia (from urea for example) oxidizes more or less

quickly into nitrate by the natural phenomenon known as nitrification.

Phosphorus is provided indifferently by any soluble orthophosphate, for example monoammonium phosphate ($NH_4H_2PO_4$), dipotassium phosphate (K_2HPO_4) or trisodium phosphate (Na_3PO_4, 12 H_2O), or even phosphoric acid itself or sodium tripolyphosphate (which will slowly hydrolyze to orthophosphate). Similarly, potassium can be provided either by potassium nitrate, potassium chloride, sulphate or dipotassium phosphate. The usual source of magnesium is magnesium sulfate called Epsom salt ($MgSO_4$, 7 H_2O). The calcium that may be necessary is provided by a little slaked lime or plaster (calcium sulphate), or, better, a soluble calcium salt (nitrate, chloride); it is necessary to put in enough to saturate the medium with calcium at a pH close to 10, but not more, that is to say until a slight white cloudiness forms. In case of sowing a new culture with little spirulina, it is better to refrain from adding calcium at the beginning to avoid losing the seed entrained in the mineral sludge.

[Note : The addition of small amounts of acid products (phosphoric acid for example) in a medium containing sodium bicarbonate and sodium carbonate does not reduce its alkalinity but lowers its pH, i.e. transforms a part of the carbonate into sodium bicarbonate without loss of CO_2. This applies both to additions when preparing culture medium and when adding food to a culture. But if you prepare a mixture with a high acid content, there will be a loss of alkalinity and CO_2, which is unfortunate. So put the acid directly into the basin.]

Note the possibility of providing several elements at the same time by the same product, for example N and K by potassium nitrate, P and K by dipotassium phosphate, or S and Mg by magnesium sulphate.

We see the importance of having basic chemistry to be able to juggle between the different products according to their availability and their price. Basically, you just need to know the molecular weights and make a rule of three. It is also possible to dispense with the concept of molecular weight and only work with the % of elements given in Appendix A16.

The iron is provided by a solution of iron sulphate acidulated, preferably with citric acid, or by iron associated with a chelating agent as is commonly sold for horticultural uses.

Do not use ordinary agricultural fertilizers intended to be poorly soluble (and containing many impurities), but only soluble fertilizers (see § 6.1, NB e and f granules) or the corresponding pure chemical products. If in doubt, analyze the spirulina produced to check that it does not contain too much mercury, lead, cadmium, or arsenic).

The permissible concentration limits for the different elements in the culture medium are given in Appendix 18. Here is an example of analysis of a typical culture medium for a pond in production:

Carbonate = 2800 mg/l
Bicarbonate = 720 mg/l
Nitrate = 614mg/l
Phosphate = 25mg/l
Sulfate = 350 mg/l
Chloride = 3030 mg/l
Sodium = 4380 mg/l
Potassium = 642 mg/l
Magnesium = 10 mg/l
Calcium = 5mg/l

Ammonium + ammonia = 5 mg/l
Iron = 1 mg/l

Total salinity = 12797 mg/l
Density at 20°C = 1010 g/l
Alkalinity = 0.105 N (molecule-gram/l) pH
at 20°C = 10.4

The medium must also contain all the necessary trace elements, generally provided by the water and by the impurities of the salts, but it is prudent, when possible, to add a supplement, at least with regard to zinc (see Annex 26). A little clay can be a useful addition.

Here is a formula for new culture medium (pH close to 8, see § 4.7: pH) suitable for waters with zero or low hardness:

Sodium bicarbonate = 8 g/l
Sodium chloride = 5 g/l
Potassium nitrate = 2 g/l (optional)
Dipotassium sulphate = 1 g/l (optional; 0.1 minimum)
Monoammonium phosphate = 0.2 g/l
Magnesium sulphate MgSO4, 7H2O = 0.2 g/l
Calcium chloride = 0.1 g/l (or Lime = 0.07 g/l)
Urea = 0.01 g/l (or 0.034 g/l for culture extension, for example basin “with geometry variable”); optional if there is nitrate and if the strain is used to consuming nitrate Solution at 10 g of iron/litre = 0.1 ml/l Solution of trace elements (according to Appendix 26.2) = 0.05 ml/l

The iron can be provided in the chelated form by 0.008 g of Fetrilon 13 or Ferfol 13, or by 0.005 g of iron sulphate FeSO4.7H2O per liter of medium. If the phosphorus is provided by phosphoric acid or an ammonium-free phosphate, the urea increases to 0.035 g/l (or 0.070 g/l in the event of a pool extension).

Potassium nitrate is actually not necessary, but it makes the job easier by providing a reserve of nitrogen and potassium. Conversely, if nitrate is added, urea can be omitted (if the strain is used to consuming urea, it may take 3 to 4 days to get used to nitrate). If nitrate is omitted, potassium is provided by dipotassium sulphate. If the water is rich enough in sulphates, the dipotassium sulphate can be reduced to 0.1 g/l and if in addition potassium nitrate is added it can even be omitted.

The total dose of sodium chloride + potassium nitrate + potassium sulphate depends on the alkalinity b; it must be approximately equal to: 12 – (40 xb), in g/l, with a minimum of 4 g/l. However, this rule is not absolute since the Zarrouk medium contains only one gram of NaCl per litre.

The alkalinity of 0.1 can be provided by 5 g/l of sodium carbonate or by 4 g/l of soda, which must be allowed to carbonate before use (about 15 days in the air in a layer of 15 cm); sodium bicarbonate can also be mixed with sodium carbonate or caustic soda (see Annex 12 and A13 Annex 13). Note that a 50/50 mixture of carbonate and sodium bicarbonate gives a pH close to 10 which, at a dose of 7 g/l corresponding to an alkalinity of 0.1, is very suitable for starting a new culture. . Sodium sesquicarbonate Na2CO3.NaHCO3.2H2O, a natural product called “trona” in the USA, can be used at 8 g/l and gives a pH of 10.15 which is also suitable (see § 4.7: pH). African natron is an impure trona whose use as is is not always recommended. The best natrons are generally the least colored. Before using natron, it must be tested: check that a 20 g/litre solution filters well (on coffee filter paper) and is not

not too colorful or cloudy; measure alkalinity and sulphates. We often find up to 30% insolubles (sand) and only 30% carbonate/bicarbonate. The sand is easily removed by settling.

When the pH of a medium being prepared from sodium bicarbonate and hard water must be raised by adding soda, sodium carbonate or natron, it is important not to add the phosphate until after soda, sodium carbonate or natron, to avoid the formation of a flake precipitate that is very difficult to settle or even tends to float, as tests carried out in October 2005 in Montpellier (water at 116 ppm this side).

Chilean potassium nitrate ("salitre potásico", granules colored pink with iron oxide), a natural product, can advantageously replace potassium nitrate by providing a rich dose of trace elements, as well as sulfur and magnesium but no toxic heavy metals; at least that was the case in 1998 (see analysis in Appendix A16.1). Chile also exports purified potassium nitrate and sodium nitrate.

When the medium simultaneously contains ammonium (NH4), magnesium (Mg) and phosphate (PO4) ions, the concentrations of these ions are sometimes (depending on the concentrations and the pH) interdependent because the solubility of struvite, a mixed phosphate of ammonium and magnesium, is extremely low. The insolubilized mixed phosphate nevertheless remains available for spirulina since it redissolves as soon as the conditions allow it, but if there is an imbalance the concentrations of one or two of the three ions involved can be very low, which slows growth and can even kill the crop (both for lack of magnesium and phosphate). Mixed phosphate crystals are normally deposited with the sludge, but they can sometimes be found on the surface under certain conditions and even sometimes in harvested spirulina. This is not serious. These crystals redissolve immediately by acidification (as is the case in the stomach!). It should be noted that in the absence of ammonium the same phenomena tend to occur as well, magnesium phosphate also being very insoluble at pH > 9. It is recommended to maintain a concentration of Mg ions approximately equal to that of the PO4 ion.

When the water used is calcareous and especially very calcareous (100 and up to 500 mg of Ca/l, or even more), the phosphate tends to precipitate in the form of calcium phosphates (very insoluble), and this all the more more than the pH and temperature of the culture will be high. But insoluble phosphates can remain in supersaturation (in solution) without precipitating for a very long time, especially in the presence of organic matter, and even if calcium carbonate precipitates at the same time. It is therefore very difficult to predict when the phosphate in solution will be insufficient for good growth of spirulina. This is why it is recommended to check the phosphate content of the culture medium quite often if the water is very calcareous. Kits for measuring phosphate can be found in aquarium shops. During cultivation, especially in the event of weak growth or problems, it is a good idea to measure the phosphate content of the filtered medium and, if it is < 5 mg/l, to add phosphate; if you don't have a phosphate test, you can try to add phosphate to revive growth. In the case where the water is calcareous, the culture medium formula given above (formula) should preferably be adapted: reduction or elimination of the addition of calcium (this addition is equivalent to 36 mg of Ca/litre in the formula), and increasing the addition of phosphate (for example for each mg of excess Ca add 0.5 mg of P, i.e. 1.6 mg of phosphoric acid). The insolubilized Ca phosphates can be said to constitute a reserve of Ca and P, since they can redissolve when needed; however this possibility is limited by the organic sludge and the imperfections of the agitation near the bottom or in the angles of the basin. The medium calculation program MEDFEED (see below) takes this phosphate supplement into account. There is an alternative: add 80 ppm of EDTA as in the Zarrouk medium, but one may be reluctant to add such a quantity of this chelating product, 10 times the dose contained in Ferfol, especially since its action is not guarantee.

The water can also be treated to reduce its calcium content before use, which is a bit complicated but can be profitable (see Annex 31).

Precautions for the storage of new culture medium: see § 4.8 storage.
Precautions for the storage of treated water: see storage.

Precautions in handling the culture medium: the skin is at pH 5.5 and some individuals do not tolerate contact with the alkaline culture medium badly. The remedy is to wear gloves that also protect against electrical hazards.

4.2) "Zarrouk" medium (Zarrouk thesis (Paris, 1966), page 4)

Zarrouk's standard medium, very often cited and serving as a reference, but not very economical, is made from distilled water and contains, in g/litre:

NaHCO3 = 16.8; K2HPO4 = 0.5; NaNO3 = 2.5; K2SO4 = 1.0; NaCl=1.0; MgSO4 , 7 H2O = 0.2; CaCl2 = 0.04; FeSO4.7 H2O = 0.01; EDTA= 0.08; "Solution A5" = 1.0; "Solution B6" = 1.0.

Composition of "solution A5", in g/l: H3BO3=2.86; MnCl2.4H2O = 1.81; ZnSO4.7 H2O = 0.222; CuSO4.5H2O = 0.079; MoO3 = 0.015.

Composition of "solution B6", in g/l: NH4VO3 = 0.02296; K2Cr2(SO4)4.24 H2O = 0.096; NiSO4.7H2O = 0.04785; Na2WO4.2H2O = 0.01794; Ti2(SO4)3 = 0.04; Co(NO3)2.6 H2O = 0.04398.

It can be noticed that the solubility product of the tricalcium phosphate is very largely exceeded in this formula, but the EDTA prevents it from precipitating.

4.3) What if we don't have any chemicals?

In this case, or if you want to produce a "100% organic" spirulina, use natural products.
For example, natural American sodium bicarbonate, trona or natron or wood ash lye can be used, and everything else can be replaced by 4 ml of urine (Bibliography: Jourdan) per litre, plus salt and, if necessary, iron. See the chapter "Food" (urine) for the precautions involved in the use of urine. If urine is prohibited for one reason or another, use is made of Chilean nitrate and phosphoric acid extracted from calcined bone powder (natural phosphate and superphosphate contain too much cadmium); unfortunately Chilean nitrate has been declared "non-organic" in Europe despite its natural origin; then there is still a possibility: the leaves of inexpensive edible plants (example nettle) which are soaked in carbonated detergent and which provide all the elements including carbon, but their harmlessness is not proven and they tend to smear the medium. You can also use "leaf manure", but their smell is rather unpleasant. Or distilled ammonia from biogas digestates.

Note that filtered seawater (at a pinch raw salt) is a good source of magnesium and also provides calcium, potassium and sulphur.

Note also the possibility of putting in the culture medium products deemed insoluble but which in fact allow the progressive solubilization of elements consumed by spirulina; mention may be made of calcined bone powder (supply of phosphorus and calcium), crushed limestone and dolomite (supply of calcium and magnesium), residual sludge from ash water (supply of magnesium, calcium, sulfur and trace elements) and clay (supply of trace elements). These products will settle at the bottom of the basin where they risk being covered fairly quickly by sludge and thus losing their effectiveness. Broom agitation may help; but it is necessary to provide for the renewal of these additions each time the pool is cleaned.

Preparation of ash water

The wood ash used must be clean (white and soot-free) and rich in soluble salts. The best woods are (in Europe) those of poplar, elm, lime, birch, pine, eucalyptus; the branches are richer than the trunks. Certain parts of the palm trees, particularly rich in potash, are traditionally used in Africa for the extraction of potash, in particular for the manufacture of soap (there are also high temperature ovens specially built to obtain white ash for this purpose). In France, there are so-called “Turbo” wood stoves producing white ash (see for example http://rocles03.free.fr). To make ash lye, the following device is used, for example: a basin with a perforated bottom, a layer of pebbles on the bottom, a canvas, and 30 to 50 cm of ash in the canvas; the water is poured over the ash (approximately 5 liters of water per kilo of ash, and this several times in succession) and it is made to percolate through the layer of ash; at the beginning the juice flows concentrated and very caustic; protect yourself from it because it quickly attacks the skin and must never reach the eyes (in the event of damage, rinse immediately with plenty of water). We can recycle the first juices. Discard the old ash when it is exhausted and start again with new. Wait a fortnight for the carbonation of the lye to take place in the air, in a basin about 15 cm thick of liquid. During this period, ensure that the air is renewed and shake, stirring occasionally. The carbonation time being inversely proportional to the thickness, if you want to go faster, just spread the solution in a thinner layer; another possibility to save time is to neutralize with a little sodium bicarbonate (see Annex 13) or concentrated carbon dioxide.

Preparation of an ash water medium

Measure the salinity (see Annex 3) or better the alkalinity (see Annex 5) of the carbonated ash water. Dilute and salt: The normal dilution is 8 g/l of ash salts (or alkalinity = 0.1), plus 5 g/l of cooking salt, but in the event of a shortage, the dose of salts can be considerably reduced. of ashes while keeping the total salinity at 13 g/l by adding more salt. Don't forget to add iron. For better understanding, here is an example giving a culture medium for 4 m², ready to be sown: Leach 20 kg of ashes with 3 times one hundred liters of water Carbonate the lye in the air for fifteen days under low thickness Dilute to density (20°C) = 1.005 with 300 liters of water Salt with 3 kg of salt Add the missing elements: 80 g of iron syrup, and 2 liters of urine.

If urine is impossible, replace it with the desired amounts of nitrogen, phosphate, magnesium and calcium, but which will not always be "organic" and may include NPK fertilizer and urea.

Preparation of magnesium sulphate from wood ash

After extracting the soluble salts from the ash (as just described), the residual filter cake can be used to make a magnesium sulfate solution. Here is a recipe which has given good results (tried in Montpellier in February 2006): - Dilute 1 kg of wet residual paste (residue from the manufacture of ash water) in 4.5 liters of water.
- Gradually add 32% sulfuric acid [Warning: handle the acid with care, always having water on hand to wash immediately in case of contact with the skin]: a lot of carbon dioxide, be careful not to overflow the container. Stop the addition of acid when there is no more gas evolution (in our example it was necessary to add 1.16 kg of acid). The pH is then close to 5, but it rises to 7.5 in a few days when gas evolution ends. Decant and filter the solution obtained, approximately 6 liters, which contains: 1.75 g of Mg / liter, i.e. in MgSO4.7 H2O equivalent: 18 g / liter 0.38 g of Ca / liter, in the form of calcium sulphate 0.015 g of phosphorus / liter 2.6 g of sulfur / liter

The residue consists largely of dirty (brownish) plaster (hydrated calcium sulphate).
The use of this solution as a source of Mg brings quantities of Ca, P and S which can generally be neglected.
The 6 liters of solution obtained are enough to make 1000 liters of medium or, used in food formula, to produce 10 kg of spirulina (therefore you need about 40 g of ash + 40 g of sulfuric acid (counted as 100%) per kg of spirulina).

Preparation of phosphoric acid from bones (Method of Jacques Falquet, December 2003) - with sulfuric acid **Material:** Bones (from any animal, even old bones are suitable)

Something to make a
good fire A mortar A
kitchen scale A basin
or a plastic bucket (metal is not suitable, unless it is enamelled) with a capacity of at least 10 litres.

Battery acid but new (= 25% sulfuric acid). Warning: NEVER take the acid that is in a battery: only use new acid, sold in bottles.
Containers for storing the resulting liquid (glass or plastic, metal is not suitable)
Method:
Strongly calcine bones in an ember fire After
cooling, carefully remove the bones (take as little ash as possible)
Reduce these bones to powder (if the bones have been well calcined, they are white-gray and very easy to grind)
In a plastic basin (and out of reach of children!):

For 1 Kg of calcined bone powder, add 4 liters of battery acid, stir and leave for at least two days (stirring occasionally).
Warning: handle the acid with care, always having water at hand to wash immediately in case of contact with the skin.
Then add 4 liters of water, stir and leave to stand for a few hours.
Gently scoop out as much of the clear liquid as possible and store it in a plastic container or in glass bottles. [Editor's note: we prefer to filter the white mud obtained, then wash it on the filter with the same quantity of water; by pressing the filter cake, the yield can then be close to 100% and the volume obtained is doubled]

Attention ! This liquid (let's call it "bone extract") is corrosive: keep this product out of the reach of children or people outside the project. Label and write a warning sign on each bottle!

The " **bone extract"** contains approximately 50 grams of phosphoric acid
per liter To prepare fresh spirulina culture medium, use (instead of phosphate) two liters of bone extract for 1000 liters of medium. culture.
To feed the spirulina after harvest, we will use as a source of phosphorus: **1**
liter of bone extract per kg of dry spirulina harvested.
This, of course, in addition to other products (nitrate, etc.)

- with lemon
juice **Material:**
Bones (of any animal, even old bones are fine) and what to make a good fire.
A mortar, a kitchen scale.
A pot

Lemon juice
Method:
Strongly calcine the bones in an ember fire
After cooling, carefully remove the bones (take as little ash as possible)
Reduce these bones to powder (if the bones have been well calcined, they are white-gray and very easy to grind)
In a pot, mix 100 g of bone meal per liter of lemon juice Boil gently for
15 minutes Leave to stand for at least a day, stirring occasionally.

Filter through a fine
cloth. The recovered liquid contains approximately 20 g/l of soluble phosphate. If necessary, it can be concentrated by prolonged boiling.

Use :

To prepare new spirulina culture medium, use (in replacement of phosphate) **five liters** of this juice for **1000 liters** of culture medium.
To feed the spirulina after harvest, we will use as a source of phosphorus:
2.5 liters of juice per kg of dry spirulina harvested.
This, of course, in addition to other products (nitrate, etc.)

(NB 1: be wary of calcined bone powders sold on the markets, in Africa for example, whose quality can be questionable; it is better to make it yourself!)

NB 2: These methods of preparing phosphoric acid are applicable to natural calcium phosphates resulting from the decomposition of guano, such as the product called PHOSMAD in Madagascar.

4.4) Renewal of the culture medium/purge The
culture medium must remain lightly colored and slightly cloudy, as low in organic matter as possible, to ensure the best performance. Normally bacteria and zooplankton take care of the mineralization and recycling of biological waste. But it happens that the production of waste exceeds its elimination (especially in shallow basins with high productivity); it is also possible that the medium is depleted in trace elements or that the salinity tends to become too high (in the event of carbonaceous feeding in the form of sodium bicarbonate or nitrogen feeding in the form of nitrates for example) , or even if the make-up water is highly mineralized: the culture medium must then be replaced or purged. This purge is preferably done from the bottom (by pumping or siphoning) while eliminating sludge at the same time, or else during harvesting by not recycling the filtrate. If the rains raise the level of the basin to the point where it risks overflowing, a purge must also be performed to lower the level. Return the quantity of salts contained in the purge to the pool (except, of course, those whose concentration you want to lower). If we purged because the level was too high because of the rain, we obviously only add the salts, without water. Ideally one should never purge.

If a pool proves to be too rich in an element (excess urea for example) and if its level is low enough, new medium deprived of the excess element can be added to it, so as to dilute it.

Walking without renewal or purification of the culture medium for several years is possible if the trace elements are regularly added, and if the productivity is not excessive in relation to the depth of culture (the depth expressed in cm must be at least quadruple the average productivity expressed in g/day/m²) and preferably if agitation is maintained at night to improve oxygenation. In practice, however, a certain rate of renewal of the medium helps to keep the concentration of possible contaminants (chemical or biological) negligible and to ensure the supply of trace elements (by the traces contained in the make-up water or the salts). It is wise to count on at least one renewal every 2 kg of spirulina produced per m² of pond, i.e. every 6 to 18 months depending on productivity, all at once or, better, gradually. To avoid having any trouble, if you can afford it, it is better to renew the medium every 3 months (or

purge of 1%/day), but be aware that this is not a necessity and it is not good for the environment.

NB a) Running without or almost without renewal requires closer monitoring of possible contaminants.

b) The non-recycling of the pressing juice is equivalent to a purge rate of the order of 0.02%/day. If half of the nitrogen is brought by the nitrate, this one brings about the alkalinity lost by this purging.

4.5) Purification and recycling of the culture medium It is generally recommended, for ecological reasons, not to dispose of the purged medium in the environment but either to use it in animal feed or as fertilizer for halophilic plants (palm trees for example). example), or to let it evaporate to dryness in a separate basin acting as a "salt marsh", preferably sheltered from the rain in a greenhouse. The recovered salts, similar to natural natron, can certainly be purified by calcination at high temperature (pay attention to the correct adjustment of the temperature and the supply of air, to avoid blackening by charring) or by recrystallization, then recycled, but this remains to be tried. With dry evaporation, a renewal every 3 months would require an evaporation surface of one third of the surface of the basins.

It is also possible to recycle the culture medium after partial purification (process used by F. Haldemann in Equateur: see his publication at the Embiez Colloquium, May 2004, page 86): in this case, the relation of § 4.4 above is dispensed with (depth = 4 x productivity); this purification consists of a combination of filtration, decantation and anaerobic then aerobic biological treatment by natural flora, protected from light, in deep basins of 1 to 2 m. with an overall residence time of 2 to 4 weeks. Another way to proceed, less good: send the purges in a "natural" basin with little or no agitation, of surface equal to a third of that of the active basins and 2 m deep, recover for animal feed the (very beautiful) spirulina that develop there on the surface and recycle the medium after possible sterilization (in the event of contamination by foreign microorganisms) by UV rays or by heating. A simple storage of the culture medium for 6 months at 20°C, without agitation and away from light, purifies it fairly well: in a temperate zone, for example, the culture medium purifies itself markedly during winter when production is zero, despite the low temperature.

Another purification process is being developed in 2013/14, already used at Peter Schilling in the Canary Islands: by skimming (skimming). It extracts a highly colored concentrate containing proteins and EPS and provides a very clean recycled medium. It is planned to sterilize it before recycling, in order to eliminate foreign cyanobacteria and the smallest spirulina to avoid contamination and strain drift. The use of this process is highly recommended.

Rather than building a purification installation, it seems simpler, at the artisanal level, to increase the surface area and/or the depth of the basins to carry out biological purification "in situ", while ensuring the maintenance of the pH by the Atmospheric CO2, at the cost of lower productivity, but with a very low or even zero purge rate from the environment. Annual sterilization is recommended.

Another possible solution: use of the purges as fertilizer by agricultural spreading or on compost heaps. The high sodium concentration of the culture medium is troublesome for many plants, but not for all (for example not for the coconut palm). It is also possible to replace in the formula of the culture medium the maximum number of sodium ions by potassium ions. Ash water (high enough in potash not to require more than two or three grams of salt per litre) is suitable. Otherwise, a medium containing 10 g of potassium bicarbonate + 2 g of potassium nitrate + 1 g of dipotassium sulphate + 3 g of salt per liter can be used (the rest as in § 4.1). To obtain a medium with a pH close to 10, the 10 g of potassium bicarbonate can be replaced by 6 g of potassium bicarbonate + 2 g of caustic potash (caution: same safety precautions as with soda!) or by 3 g of potassium bicarbonate + 4 g of potassium carbonate. A potassium-rich medium is at least twice as expensive as a sodium-rich medium, but it has the added benefit of yielding spirulina which may be useful for some "sodium-free" diets; this advantage could more than offset the additional cost of the environment.

4.6) Use of sea water

Using seawater to establish and maintain a culture of spirulina, without prior treatment of the seawater other than filtration, is possible but on condition of working at a regulated pH with a

great precision close to that of seawater, which is technically difficult for artisanal producers. Seawater contains an excessive amount of calcium and magnesium which, at high pH, cause abundant precipitation of carbonates and phosphates. On the other hand, the high salinity of this water (35 g/l) prohibits its use as make-up water to compensate for evaporation, unless this is kept very low by judicious use of greenhouse basins.

Ripley Fox developed the concept of a (giant) spirulina farm using seawater treated with soda ash, itself produced on site from electrolytic soda. Chlorine and hydrogen by-products of electrolysis are transformed into hydrochloric acid used to generate pure CO2 from soda ash. The problem of compensating for evaporation is solved by rejecting the culture medium (previously neutralized) into the sea when its salinity has become too high. This concept may be applied one day, but it requires large means, beyond the reach of a small producer. In addition, it requires a real chemical factory which some people would not like.

On the other hand, sea water can be used with profit, in small quantities, to provide magnesium and sulphur. And desalinated seawater is already widely used to produce spirulina (Canary Islands for example).

4.7) optimum pH

The optimum pH of a new culture medium to be made depends on its use.
If it must be inseminated to start a new culture, its pH must be at least 9, as close as possible to that of the strain used: if it is too low, the culture risks starting badly, with the formation of lumps. or precipitation of spirulina at the bottom. Natron or the carbonate + sodium bicarbonate mixture, or carbonated ash water are therefore well suited to this case.

On the other hand, if the new medium is to serve as a supplement to an existing culture, its pH can advantageously be close to 8, which contributes to maintaining the pH of the culture sufficiently low by adding sodium bicarbonate. This is typically the case for basins being extended (“with variable geometry ”). In this case the medium must be based on sodium bicarbonate alone, if the latter is available. If the medium is at low pH, it will be easier to use non-deammoniated NPK without risking killing the spirulina, because what is dangerous is NH3 (at low pH it is NH4 which dominates).

4.8) Storage of new culture medium and treated water

It is not recommended to store new culture medium, even away from light, because it constitutes by nature a "culture broth" where undesirable micro-organisms could develop. This remark applies especially to low pH media.

It is also strongly advised not to store fresh water, for example water treated to eliminate excess hardness, in the presence of light because in a few days foreign algae and cyanobacteria would develop there. However, among the latter there are highly toxic ones (case of certain freshwater lakes).

4.9) “MIDIEU” calculation software

To facilitate the calculation of the media and the mineral food taking into account the raw materials and the analysis of the available water, calculation programs have been written (Calculations).

5) SOWING

5.1) Which strain of spirulina to use?

There are spirulina of different “races” (strains), although they all have common characteristics that distinguish them from other cyanobacteria. We recognize very quickly under the microscope or even with a magnifying glass of high magnification (25 times) if the spirulina are spiral or straight but it is less easy to say which strain it is because spirulina has a strong tendency to change size and shape (more or less tight spiral or "wavy" or straight). In the presence of straight forms there is a doubt: are they spirulina or Oscillatoria similar to straight spirulina and some of which are toxic? A trained eye cannot confuse a line with one of the common toxic Oscillatorias (foreign Cyanobacteria). Too high a percentage of straight lines leads to harvesting difficulties. So preferably take a 100% spiral seed, large size, of a beautiful green tending towards blue-green, filtering easily. Pure strains could be obtained at the Institut Pasteur where the "Lonar" is called PCC 8005, but Pasteur no longer offers this service. Almost all of our strains are actually "Arthrospira platensis" by scientific name. We call "Lonar-type spiral" the strains whose filaments are "pigtailed", such as "Lonar". We call "wavy spirals" (or "wavy" for short) strains whose filaments are in a stretched spiral, such as "Paracas". To facilitate the choice of strain, here are some useful elements:

- The Lonar-type spirals generally float more than the wavy and straight ones, which eventually allows their separation.
- Spirals filter better and their biomass less makes the ball easily on the filter, when the culture medium is pure enough.
- Spirals have a greater tendency to form floating green skins and lumps, especially at low pH and in the absence of ammonium (see § 7.9), which is a disadvantage.
- The dry matter content in the dewatered biomass ready for drying is higher in corrugated and straight than in Lonar-type spirals, which is an advantage.
- The biomass of Lonar-type spirals dries more easily.
- Corrugations have little tendency to become straight, at least under normal operating conditions.
- Corrugated ones resist pumping by centrifugal pump, whereas spiral ones break.
- The corrugated are more resistant to osmotic shock (we can sometimes wash the biomass with fresh water without the cells bursting, whereas with the corrugated it is rare).

There are no notable differences in composition or nutritional value between these strains, on the other hand the green color of the wavy ones is darker; some prefer the color and flavor of either strain, but that is a matter of personal taste.

The wavy and the straight have common characters, but the wavy do not suffer from the suspicion of not being "real" spirulina.

All in all, our practical preference is for "wavy", although "spiral" looks better under the microscope.

5.2) Sowing from a large quantity of seed

To inoculate, simply transfer a certain volume of culture from another pond in production into new culture medium until the color turns green (the " Secchi disk " should no longer be visible 5 cm from the the surface). Preferably sow in the evening. The volume to be transferred can be reduced by taking concentrated supernatant or by harvesting spirulina without wringing it (disperse it well in a little culture medium

before pouring it into the basin, to avoid leaving lumps, which is not easy with spiral strains: use for example a paint mixer propeller connected to a drill).

To successfully start a culture, it is always beneficial to start as concentrated as possible in spirulina. This is why we start with the minimum level of liquid (for example 5 to 10 cm) if the availability of semen is limited, and/or we use the “variable geometry basin” technique. A concentrated seeded culture (Secchi < 3 for example) is much less likely to be invaded by chlorella or to suffer from the entrainment of spirulina in the calcareous sludge (when working with hard water).

If the beginning culture is too dilute (“Secchi” greater than 5 cm), you must shade, otherwise you risk the death of the spirulina by photooxidation in the sun. Care must also be taken to avoid mineral deposits which carry spirulina with them (to do this, if necessary, filter the new medium before inoculating it and maintain agitation overnight if possible). If the initial level is the normal level, and if the new medium is based on sodium bicarbonate, do not inoculate too concentrated either, otherwise it will be necessary to harvest before the pH has reached the minimum level of 9.6 recommended (see 7.13) ; but it is easy to start with a culture medium at pH 9.6 or higher by mixing sodium bicarbonate with sodium carbonate or soda (see Appendix 12 and Appendix 13). Another advantage of a high initial pH is the reduction of the initial tendency to form lumps with spiral strains, an advantage that can be decisive when you have little seed: you must not lose any lumps! On the other hand, it is certain that there is an interest in not subjecting the seed of spirulina to a shock of pH: we have sometimes seen a beginner culture die following a shock of pH of 2 units (from 10 to 8): our recommendation is to limit the delta Ph to 1 unit.

It is allowed to store a few days and transport a very concentrated semen (3 to 4 g/l for example, no more), provided that it is stirred and aerated at least from time to time, otherwise it will ferment and smell bad. . At 2 g/l, transport can last ten days. Note that a floating layer taken with care can contain 5 to 10 g/l. In a highly concentrated semen, the pH drops and a mercaptured odor (“sauerkraut” odor) develops over time.
After inoculation with a spirulina that has "suffered" in storage, the new basin may foam excessively, but this normally disappears in one to two days.

The seed keeps better at low temperature, around 10°C for example (reduced respiration). Fresh biomass, even pressed, can be used to seed a pond. This is important for easy transport in concentrated form and for immediately inoculating a large volume. Transport cold as much as possible, and limit the duration. Gradually dilute the concentrated semen, homogenizing it: there should be no remaining lumps (pass the mixture through a sieve to remove any remaining lumps).

5.3) From a small amount of seed

To implant a culture of spirulina in a site that lacks it, or to start again with a new strain, it is generally not possible to have a large quantity of culture to inoculate. Frequently we only have a half-filled bottle (to maintain enough oxygen). If we manage to obtain a pure strain, we will probably only have a few milliliters of culture at the start (NB the culture medium indicated by the Institut Pasteur in its documentation accompanying its strains

corresponded to the maintenance of strains and it differs from the culture medium for growth). You can also start from a single filament that you isolate yourself (see § 5.6).
Suppose that the starting point is 150 g of culture at 1 g/l of spirulina concentration and that the objective is to multiply the initial seed volume to seed a 1000 liter pond. It will be necessary to make at least 4 successive cultures, multiplying each time the volume by 5, which requires about three weeks in total (with a growth rate of 35%/day, possible with culture medium based on sodium bicarbonate). sodium). The first mini-culture will be done in a two-litre jar, the second in a 10-litre basin, the third in a 50-litre basin, the last in a temporary 1 m² plastic film mini basin (or several large basins).

If the initial concentration of each culture is lower than Secchi = 5 cm, it is necessary not only to shade but to agitate day and night (otherwise the spirulina can agglomerate, in particular on the edges, and no longer be able to disperse afterwards). It is possible to avoid this agglomeration by raising the pH, but this risks increasing the mineral sludge which can trap the spirulina. By means of which we still manage to start a culture starting from very low concentrations (Secchi = 15).

The continuous agitation of cultures in small containers (bottles, buckets, basins for example) is done by means of a small bubbling of air as in an aquarium and requires a high liquid height/ diameter ratio, equal to or greater than 1 , with if possible a conical bottom, the air supply tube emerging close to the bottom (there are aquarium compressors running on electric batteries). It is practical to simultaneously heat and light the small initial cultures in the laboratory by incandescent or halogen lamps placed at the right distance to automatically maintain around 35°C in the culture (do not light more than 16 hr/day).

The agitation of large volumes (> 100 liters) of diluted cultures can be done by means of a small aquarium pump, but it is advisable to pump only intermittently so as not to damage the spirulina, especially the Lonar, therefore to use a clock programmer. Wavy stumps are much less susceptible to pump damage.

To avoid the formation of lumps (especially with the spiral strains of the Lonar type and if there is no continuous agitation) at the start of sowing, the concentrated seed must be diluted very gradually, by adding small doses of new culture medium based on urea, for example with each shaking, keeping a high concentration of spirulina for the first two days. It is then advantageous to maintain a high spirulina concentration (0.3 g/l or more) and therefore to dilute the culture as little as possible with each increase in volume: gradual dilution (for example daily) is best. This can be done using a "variable geometry pool", expandable on the surface, easy to make with plastic film.

Each increase in volume (therefore surface) is done by dilution using new culture medium (preferably based on sodium bicarbonate). The new dilution medium – if it is based on urea as a nitrogen source – must contain **a high dose of urea (0.04 g/l)** or, if it is based on urine: 6 ml of urine/l. If the new medium is based on sodium bicarbonate, therefore pH=8, the pH of the culture remains around 9.6 during its extension phase.
This pH may not be sufficient to avoid spiral lumps: in this case, raise the pH by adding carbonate-based medium to pH = 10.3. Growth is accelerated during the "variable geometry basin" phase by keeping the depth low (5-10 cm).

NB:

1) A culture can die following a dilution, a lighting or a too strong heating or an excess of urea.

2) Raising the level of a pool must be done by adding culture medium. Adding undissolved salts directly to the pond can be very dangerous to the crop.

3) If a reserve of dilution culture medium is prepared in advance, keep it closed for a short time and in the dark so that it does not risk being contaminated by foreign algae.

4) Warning: a pH shock of 2 units is often fatal: when seeding, take care to minimize the pH differences between seed and pond.

5.4) Initial growth rate

Growth rate depends on several factors including pH. It is maximum at pH below 10, so it is advantageous to use sodium bicarbonate to quickly start a new culture. It is also in our interest to maximize the cultivation surface (therefore a shallow basin if possible). The method of progressive extension of the pond surface (“with variable geometry”) described in the previous § promotes rapid growth. The speed of establishment of a new crop is best characterized by calculating the growth rate in the initial phase of growth which precedes the harvest phase. This rate is expressed in % growth in weight per day (weight expressed in dry matter). Under favorable conditions, in a sodium bicarbonate-based medium, it can exceed 30%/day. From one gram of seed (expressed in dry spirulina), a rate of 20%/day makes it possible to obtain 20 m² of pond 15 cm deep ready for harvesting in 40 days, or 120 m² in 50 days.

NB One would be tempted to light the cultures 24 hours a day to increase the growth rate, but it is better not to subject the spirulina to more than 16 hours of illumination per day, even if one has artificial lighting. .

5.5) Seed reserve

Normally the pools themselves serve as a reserve if they remain in good health and without contaminants, but you have to plan for accidents and how to get through the possible bad season. It is also in our interest, when possible, to completely empty the basins and restart them from scratch to ensure the maintenance of a good quality of spirulina (without contaminants, without lines, filtering well). For this you need pure semen. It is therefore recommended to keep some pure strain "in the laboratory" (= in the house), at moderate or room temperature, under low light for about 12 hours a day (in the total absence of light, spirulina dies in a few days , for example in 2 days at 35°C), slightly agitated, and renewed (“replanted”) every 2 or 3 months: under these conditions, it keeps well whereas in too intensive culture it tends to mutate and can degenerate.
A plastic bottle is very suitable as a container. For agitation and aeration, the most practical is a small electric air compressor for aquariums, which can only be operated occasionally thanks to a timer (there are such compressors and timers that work on direct current). To both light and heat the culture, a 40 Watt bedside lamp is sufficient, directed horizontally towards the bottle, at the distance giving the correct temperature (< 30°C). To preserve larger quantities of semen, basins or aquariums are used, with more powerful lamps, incandescent or halogen; luminescent tubes heat up little and

suitable if the ambient temperature is high enough. Photo of a reserve of seed:

5.6) Selection and monoclonal culture

Planting from any seed results in a crop having the same potential contaminants as the seed. To be sure of having a pure (“monoclonal”) culture, it is theoretically necessary to start from a single filament selected and washed with sterile medium.

It is possible to separate an individual filament from a mixture of strains. Various techniques, based on a dilution of the original culture, can be used to carry out this separation, which remains a difficult operation for a non-specialist.

It is easier and quicker to take from a culture that is very little contaminated (by straight spirulina for example) a drop without contaminant: the selection is made by microscopic examination at low magnification, rejecting the contaminated drops if only by a single foreign filament and putting the pure drops in filtered culture medium (by rinsing the microscope slide with a wash bottle filled with filtered culture medium). We collect as many pure "drops" as we can in the time available: the more there are, the faster we will obtain a usable seed. It is prudent to carry out this selection operation regularly in order to maintain a pure safety stock without waiting for a percentage of contaminants (straight lines for example) that is too high to make the selection operation difficult.

5.7) Drift from one culture to another strain

It is common to see a variation in the shape and/or size of the spirulina filaments during cultivation. Towards the straight shape of course, but also towards other spiral shapes, in particular towards smaller or constricted shapes which pass preferentially into the filtrate. It would be illusory to seek to counter this drift by using finer mesh filtration cloths (which generally only slows down the evolution). The only radical countermeasure, apart from purging, is the direct non-recycling of filtrates: recycling must be done through the purification system (Purification).

6) SPIRULINA MINERAL FOOD

[NB Software exists to facilitate calculations of media and food]

Although the main food of spirulina is carbon, this chapter will only discuss non-carbon food, only mineral food. For carbon food see § 7.8. The initial culture medium allows spirulina to grow to a spirulina concentration close to 1 g/l (without nitrate) to 2 g/l (with nitrate), but it is better to return the nutrients absorbed by spirulina without waiting for the depletion of the medium or better still follow the content of elements in the medium, especially if the culture is contaminated by phormidium which consumes inputs on its side.

Add urea (and if applicable CO2 and/or sugar as carbon input) daily depending on the desired or expected harvest during the day, the other nutrients may only be added once a week, or even once a fortnight. Be sure to add the urea (and if necessary the sugar) early in the day, just after the harvest and respecting the rule given in NB c below (uretheo). The use of nitrate does not require the same precautions as urea, but the latter is less expensive and more effective, it reduces the formation of lumps (important especially in spirals of the Lonar type) and it reinforces the sometimes failing vigor of the spirulina (without ammonium, especially the wavy ones, may not be easily wrung out by pressing); moreover, urea does not bring salinity but brings “free” CO2. Ammonia can obviously be used instead of urea, but with even more precautions: there, the drip is practically necessary. On the other hand, ammonia has an advantage over urea, which only hydrolyzes gradually (too high a dose of urea can constitute a "time bomb" by producing ammonia). Ammonium bicarbonate is an interesting possibility to provide both nitrogen and "free" CO2 (double the urea), and even ammonium acetate which provides even more (quadruple the urea).

All the ingredients must be dissolved before being introduced into the culture and during the introduction the culture must be under agitation.

[Note : The addition of small amounts of acid products (phosphoric acid for example) in a medium containing sodium bicarbonate and sodium carbonate does not reduce its alkalinity but lowers its pH, i.e. transforms a part of the carbonate into bicarbonate without loss of CO2. This applies both to additions when preparing culture medium and when adding food to a culture. But if you prepare a mixture with a high acid content, there will be a loss of alkalinity and CO2, which is unfortunate. So put the acid directly into the basin.]

Based on the elemental composition of spirulina given in Annex 19 and the indications of § 4.1 on the culture medium , it is easy, but often tedious, to calculate the mineral food needs according to the products (fertilizers) available. . Account is taken of the chemical purity of the products, losses during production (photooxidation, consumption by parasites, chemical and physical losses) and during harvest. We do not take into account the contributions by the make-up water unless the evaporation is very strong and if the make-up water is very mineralized.
As an example that can be used quite commonly, here is a formula calculated for the case of non-ferruginous water and low hardness, for an average evaporation and for a rate of losses common in small farms:

Grams per kg of spirulina produced (counted in dry matter):

Urea = 300 g (270 recommended by FSF)
Monoammonium phosphate = 50 (30 recommended by FSF)
Dipotassium sulfate = 40 (30 recommended by FSF)
Magnesium sulfate heptahydrate= 30
Calcium chloride = 20 Chelated iron (13%
iron, powder) = 4 (10 recommended by FSF)
Trace elements solution (according to Appendix 26.2) = 50

(monoammonium phosphate is often replaced by phosphoric acid, in quantity depending on the concentration of this acid: for example 57 g of 75% acid replace 50 g of monoammonium phosphate)

(calcium chloride can be replaced by 30 g of calcium nitrate if this product is available, or by 13 g of slaked lime)

(The iron can be introduced in the form of 50 ml of chelated iron solution at 10 g of iron/l, for example 77 g of Ferfol/litre, or "nail syrup").

NB

a) The above food formula does not include nutrient requirements for any flushes of culture medium, which should therefore be added where appropriate. b) The iron dose above corresponds to 500 ppm of iron in spirulina;

it can be adjusted on demand, some doctors preferring a lower iron content, others 1000 or even 1500 ppm. For these high iron contents, the addition of a chelating agent (EDTA, citric acid, carambola or lemon juice) or the use of chelated iron (Ferfol or Fetrilon type) is preferable to iron sulphate alone. It has often been reported that the introduction of iron (not chelated) in the form of sulphate is much more effective drop by drop (and with continuous agitation), but this remark does not apply, or less, if one uses chelated iron. c) The theoretical dose of urea is 270 g/kg, but especially at low pH an excess is necessary if there is a tendency to form lumps, skins, etc.

Unused excess urea turns into nitrate or is lost to the atmosphere in the form of ammonia. It is better to stop the injection of urea as soon as an ammonia odor becomes perceptible on the crop or, if the ammonium can be measured, follow the rule given in Appendix 18 (NB b). Urea is the cheapest source of CO2 (apart from the air) and if the pool temperature is high enough we can consume up to 0.8 kg/kg of spirulina, the part not consumed by the spirulina transforming into nitrate (by consuming alkalinity, according to the equation: $CO(NH_2)_2 + 4\ O_2 + 2\ NaOH = 2\ NaNO_3 + 3\ H_2O + CO_2$); in fact, many nitrogen balances have shown us that more nitrate seems to be formed: for example, we measured on a pond fed with 600 g of urea / kg of spirulina a "fixation" of atmospheric nitrogen corresponding 6 times the nitrogen contained in the spirulina produced!. Normally it cannot be nitrogen fixation from the air since spirulina does not have heterocysts, but nitrogen fixation without heterocysts is known to be possible in certain cyanobacteria under certain conditions.

But in the total absence of urea and also without excess urea, no nitrate seems to be formed. Note that the nitrate formed can then serve as a source of nitrogen

by biological reduction by spirulina, with restoration of alkalinity: NaNO3 + 4H2 = NaOH + NH3 + 2H2O, but this does not occur as long as there is enough urea because spirulina prefers to use ammoniacal nitrogen rather than carrying out this work of reduction which is very costly in terms of energy (glucose). An accumulation of nitrate then occurs in the culture medium, where up to 5 to 10 g of NO3 ions per liter have been measured!
When nitrate is not too expensive, a mixed nitrogen diet (50% nitrate, 50% ammoniacal) is recommended, as is practiced successfully at the La Mé farm in Côte d'Ivoire: 140 g of urea + 500 g of potassium nitrate. This avoids the accumulation of nitrate in the medium, but does not avoid the increase in salinity and pH due to the potassium introduced.

Note that if nitrate is too expensive or unavailable, you can still manage to make a mixed urea/ nitrate diet using the nitrate accumulated in another tank fed with urea alone (but this requires mixing tanks). You can also use ammonium nitrate which is a common fertilizer but which presents the risk of being an explosive!

Ammonium bicarbonate NH4HCO3 is better than urea because it provides twice as much CO2; it is a potentially very cheap product since it is the required intermediary in the manufacture of Solvay soda ash, but it is not commercially available everywhere (Solvay-England sells it). It takes 2.6 times more ammonium bicarbonate by weight than urea. One can also use ammonium acetate which provides four times more carbon than urea.

See also below in NB j (phosphate) the possible effects of an excess of ammonium on the PO4/Mg/NH4 balance of the culture medium.

d) According to the quantity and analysis of the water brought to compensate evaporation, the doses of sulphates, magnesium, calcium and iron can be reduced or eliminated. If the water is very calcareous, it may be necessary to increase the dose of phosphate to compensate for the possible precipitation of calcium phosphate, following the recommendation given for the culture medium: compensate the Ca by half its weight in P; it is recommended to do a phosphate dosage in this case about once a month or when the crop seems to be withering. e) The use of certain agricultural fertilizers with slow dissolution (slow release) or low solubility, superphosphate, diammonium phosphate (see paragraph f below), potassium sulphate, is not recommended because they generally contain colored additives and /or odorants and oils that soil the culture medium, forming a greasy film on the surface of the pond (slowing down the absorption of carbon dioxide and the desorption of oxygen). Moreover, fertilizers of this type may contain heavy metals (in particular cadmium present in natural phosphates) which are dangerous because they are quickly absorbed by spirulina. These remarks do not apply to: urea, magnesium sulphate, potassium sulphate, potassium nitrate, Chilean nitrate, mono or diammonium phosphate, potassium chloride sold as soluble agricultural fertilizers, even granules. Agricultural iron sulphate is of questionable quality from a purity point of view (after dissolution it requires at least decantation or filtration). f) To use granulated diammonium phosphate as a source of phosphorus, if no other is available, F. Ayala proceeded as follows: in one liter of 0.5 N hydrochloric acid (50 ml of 33% concentrate, diluted in one liter of water) add 250 g of crushed phosphate and bring to the boil; removing the supernatant oily layer and recovering the decanted liquid; repeat a second time on the sludge; mix the two decanted liquids, i.e. approximately 1.5 liters containing

approximately 50 g of phosphorus in usable solution, corresponding to 5 kg of spirulina. Check that the spirulina produced from this phosphorus source meets the cadmium standard.

g) The contribution of trace elements by the traces contained in the make-up water and salts may not be enough. If the make-up water is too little mineralized, you can use unrefined salt (possibly plus a little clay and/or ash water) in order to provide trace elements, without forgetting to practice corresponding purges in case of excessive salinity. However, part of the nitrogen can also be supplied by nitrate from Chile known as "salitre potasico" (rich in trace elements) or else one can use concentrates of trace elements prepared from chemical products (see § 7.7 and Appendix 26). h) The supply of calcium (lime or better nitrate or calcium chloride) is only necessary if the make-up water does not contain enough, or if one wants a spirulina enriched with calcium like that of several industrial producers. i) The consumption of chloride is theoretically 7 g of NaCl/kg of spirulina, but it is practically useless to add any, except for the extraordinary longevity of the culture medium. It is strictly unnecessary to add any when using urine or seawater. j) When the medium simultaneously contains ammonium ions (NH_4),

magnesium (Mg) and phosphate (PO_4), which is the usual case at intermediate pHs, the concentrations of these ions are interdependent because the solubility of the mixed ammonium and magnesium phosphate – called struvite – is extremely low. To avoid imbalances, the ammonium concentration must be kept low. The ammonium concentration is automatically low if the urea is added in small fractions and if the pH is high (part of the ammonium is transformed into ammonia NH_3 under the effect of the high pH). It is recommended to maintain the Mg concentration approximately equal to the P concentration. In the absence of ammonium, magnesium phosphate itself is very insoluble.

h) To simplify the operation, we can be content to feed the spirulina (apart from the urea) only once a month but this leads to fairly strong fluctuations in the composition of the spirulina, in particular in iron. That is why it is recommended to feed rather weekly, or even daily. If urea is used, it should be added daily. The basis of the food to be provided is not the quantity harvested but that produced by photosynthesis (there is a significant difference if the concentration of spirulina varies significantly).

What if we don't have chemicals?

Just add 17 liters of urine (this is an average dose since the concentration of urine varies greatly depending on the subject and his diet) per kg of spirulina harvested, plus iron. Urine also provides carbon, which reduces the tendency of pH to rise and increases productivity by 2 g/m²/day in the absence of other carbon feed. This solution is only offered to respond to survival situations, or to provide spirulina intended for animal feed, or even for those who would prefer a truly “organic” spirulina. Be careful to distribute the dose regularly (as for urea) and to add the urine just after harvesting (in any case not in the evening) and only in good weather; at cruising speed, it is recommended to limit productivity to 7 g/d/m², therefore not to add sugar, and to maintain a fairly high liquid height (minimum 20 cm) and also a spirulina concentration of at least 0.4 g/l. For a

personal consumption of the spirulina produced, the sterilization of the urine (personal, the subject being in good health) is not a necessity (the author has never sterilized his urine, but spirulina reserved for personal use), but otherwise it seems indispensable, at least for psychological reasons. Sterilization can be carried out by adding 3.5 g of soda per liter 24 hours before use (increasing the pH to 12-13 insolubilizes some of the components: do not filter, so as not to lose these components, and homogenize before use). There are other ways to sterilize. Some say that in black African countries, the urine could contain certain organisms resistant to this type of sterilization by high pH: to be checked. See also other sterilization methods (Olivier, Galaret). In any case , *Schistosoma haematobium,* whose eggs contaminate the urine of people infected with bilharziasis, must be able to be eliminated by filtration of the urine on 30 µ cloth, before sterilization with soda. The other parasite that can be found in urine, *Trichomonas vaginalis,* is not eliminated by this filtration but it only survives 24 hours in urine. It should be noted that these treatments by sterilization with soda and filtration have not yet been validated with regard to the quality of the spirulina produced; in particular, we do not know if the treatment of urine with soda does not induce undesirable chemical transformations (in any case it does not prevent spirulina from thriving!). Also see NB a), b), e) and f) below.

A special application of using urine to make spirulina is the recycling of biological waste from astronauts in future space stations: spirulina is the best way to both convert CO2 back into oxygen and waste into food . This process is being studied in large laboratories in different parts of the world.

The production of "organic" spirulina is also possible without resorting to urine, using only "natural" products (see § 4.3) such as trona, magnesium sulphate by-product of salt marshes or extracted from residues ash water extraction and phosphoric acid extracted from bone meal, as well as the leaves of cheap edible vegetable species. Chilean nitrate was refused “organic” approval, while Ferfol is accepted. The green leaves of non-toxic species are a good source of nutrients (including carbon and iron), and our various use trials by direct in-crop dipping were positive, but had to be discontinued due to soiling excess in the environment (which would have required a biological purification system that we did not have).

Some experiment with various plant manures. Compost juice (“compost tea”) would be a good solution, but here too it seems necessary to have a biological purification system, if only to release the nutrients contained in the many microorganisms in this juice. . In summary making spirulina from only plants is possible but it is rather complicated.

NB

a) As urine does not contain iron, its use does not dispense with adding iron. b) The urine used must have a normal odor and color and come from donors who are healthy and not taking medications that can cause toxicity to Spirulina such as antibiotics. c) It is said that animal blood would be a good food for spirulina and that it can be used in relatively large doses (50 ml/l of culture medium). Beware of possible contamination, however. We never tried to use blood

and we don't want to. d) It
is perfectly possible to “mix” chemicals and natural products. e) The use of urine as sole fertilizer is especially suitable when the water is a little hard (20 mg of calcium/litre) but not too hard; indeed the contribution of calcium and magnesium by the urine is a little weak and a supplement coming from water is welcome, but also to take into account that the urine brings an excess of phosphorus too weak to compensate for a strong dose calcium. f) Dysentery spreads through faeces, not urine.

7) CULTURE MANAGEMENT AND MAINTENANCE

Contents:

7.1) Harvests

Harvesting is done in such a way as to maintain the concentration of spirulina at the desired level, for example between 0.3 and 0.7 g/l, not necessarily every day. If the medium is cloudy, take this into account when measuring the concentration with the Secchi disk. In the absence of crops, with sufficient nutrients, heat and light, the concentration of spirulina increases until the balance between photosynthesis and respiration, corresponding to approximately 250 g of spirulina/m² of pond.

It is not good for the culture to remain long without being harvested, at very high concentrations: it can even be a cause of death for it. Conversely, it is not good to lower the concentration below 0.4 g/l, in any case 0.3 g/l: productivity is higher at low concentrations but the culture is there

less stable, and spirulina is produced there with a lower phycocyanin content.

7.2) Agitation (see § 3.4)

Manual stirring: stir (at least!) 4 times a day, but the minimum frequency depends on the conditions and the strain; it increases with the intensity of light and flotation. In the middle of a very hot day without shade, the shaking of a strongly floating stump should be very frequent (at least twice an hour) or even continuous. However, there are conditions where it may be preferable to agitate less because then the upper layer of the crop, which is warmer, produces more.

If you have an electrical stirring mode that is safe for spirulina (for example air bubbling, propeller or paddle wheel), the stirring can be continuous (with a 15-minute/hour stoppage anyway). preference). With pumps, it is better not to stir a spiral strain (Lonar type) continuously, but only 15 or 30 minutes/hour. Continuous agitation by aquarium pumps or vortex pumps is possible with corrugated (Paracas) and some resistant spirals.

The night stirring can theoretically be stopped, but when possible two or three nocturnal stirrings are beneficial to reduce the risk of lumps and improve oxygenation of the environment. Continuous nocturnal agitation, when possible, clearly promotes self-purification of the environment and reduces the risk of anaerobic bacteria.

The productivity of an intensive culture strongly depends on the agitation, without us being able to really quantify this effect yet. Several experimenters report record productivity (up to 30, even 40 or 50 g/day/m²!) under excellent agitation conditions, generally in small tanks, in tubes or in the laboratory.

In the simulation program presented in the Calculation chapter, the following convention has been adopted to deal with this problem:

> For ordinary tanks, of which we have experience, the degree of agitation is defined by the average speed of movement of the culture, up to 30 cm/s), with a weak influence on productivity (see appendix A1 page 86)
>
> For basins with sophisticated agitation systems, the degree of agitation is still characterized by the speed, but it must be fixed above 30 cm/s; by convention the model then multiplies the speed by 8 (for example if we set the "speed" to 40, the model will apply 320), which leads to the very high productivity reported by some authors, but which we do not believe to be realistic In practice.

7.3) Change in pH

A good test of a crop's growth is its increase in pH. In the absence of carbon supplementation and if there are no mineral deficiencies, for an alkalinity close to 0.1 N, a liquid height close to 20 cm and a spirulina concentration close to 0.4 g /l, with high temperature and sunshine, the normal pH increase is around 0.1 unit/day in the pH range between 10 and 10.6.

However, in the presence of organic matter in the environment, these can

oxidize by releasing CO2, which counteracts the increase in pH, and can even, at the limit, cause a drop in pH. Another way to check that photosynthesis is active is to observe the evolution of oxygen at the surface of the pond in the absence of agitation.

7.4) Shading

In the absence of carbon supplementation the pH can rise to 11.5 and more, but spirulina cannot withstand a pH above 11.3 for long and it is even recommended to limit the pH to less than 10, 8. A half-shade is generally sufficient to maintain the pH below 11. If the agitation is good, an excessive rise in pH can be prevented without shading by maintaining a high stock of spirulina (> 150 g/m²), that is to say a spirulina concentration greater than approximately 0.7 g/l for a height of liquid of 20 cm, which can be called “self-shading”.

Shading is also necessary when the temperature of the crop is too low (< 10°C) in strong sunlight, otherwise the crop can easily die by photolysis. It is necessary as a precaution to maintain the illumination of the basin below a certain limit which depends on three simultaneous factors: the temperature, the concentration of spirulina and the concentration of dissolved oxygen. The lower the temperature and the concentration of spirulina and the higher the concentration of oxygen, the more light must be moderated to avoid or reduce the mortality of spirulina. Without being able to give precise figures, it is recommended to keep the oxygen below 20 ppm (by vigorous agitation) and the illumination below 30 klux, especially if the temperature is below 25°C and the spirulina concentration less than 0.3 g/litre.

There is another occasion where photolysis can strike: it is at very high temperatures. A destruction of spirulina was observed in a few hours at 39°C under an illumination of the order of 50 Klux. Under illumination of 6 Klux above 32°C, according to Zarrouk's thesis, there is no more to gain in productivity.
It is also necessary to shade to save water in the dry season, or if the temperature tends to exceed 38°C in the crop.
A culture under shade is easier to harvest and the quality of the spirulina is improved (richer in pigments), with a reduction in productivity which may remain modest.

7.5) Water level

Be sure to add water to the pool (preferably in the evening) to maintain the desired level. Do not add more than 10% of pond volume per day. If the make-up water is very calcareous, mineral sludge is produced in the pool and in the long run it is preferable to eliminate it, but at the same time experience shows that calcareous water has two advantages: it brings little bicarbonate and especially the precipitation of calcium carbonate helps to flocculate impurities such as EPS. Make-up water also contains soluble salts which gradually increase salinity (likewise the use of nitrate as a nitrogen source or sodium bicarbonate as a carbon source increases salinity); this may require purging to prevent the salinity from exceeding 30 to 50 g/l. But make-up water (except rainwater) also provides beneficial trace elements. If the evaporation is noticeable and if the make-up water is very calcareous, there is a risk of

co-precipitation of calcium phosphate: closely monitor the phosphate content of the medium and add phosphate if necessary.
In open basins, rain is beneficial as long as it remains moderate (for example 10% of the volume of the basin per day), but a sudden too strong dilution of the culture medium causes the spirulina to fall to the bottom. At the end of the rainy season, it is in your interest to keep the maximum level allowed by the basin (which will save water in the dry season). If the source of alkalinity is not rare, and/or if the rainfall is not excessive, all the rain that falls can be admitted into the basin, taking care to perform culture medium purges in time to prevent the basin to overflow; these purges are done by collecting without recycling the filtrate or by sucking the bottom to eliminate sludge, then by putting back into the basin the salts corresponding to the volume of culture medium eliminated. These purges maintain the quality of the culture medium and provide it with trace elements contained as impurities in the extra salts. If you do not have a concentrate of trace elements , you may have to perform purges for the sole purpose of introducing trace elements through water and salts! *If purges are allowed.*

A high water level (30 cm or even more) reduces overheating in very hot climates and is probably useful to facilitate self-purification of the culture medium (see purification).
A low level is interesting to reduce the expense of culture medium, but requires a very flat bottom (with a more hollow point to facilitate the collection of the float in the basin as well as the emptying), sufficient purges to maintain the quality of the medium and increased monitoring of pH, temperature and nutrient concentration so as not to exceed allowable limits.
In an open pool, if purges are not necessary to maintain the quality of the environment and if the edges are high enough, the level and alkalinity vary during the year: make sure that the minimum level is sufficient and to that the alkalinity remains sufficient (> 0.05) at the maximum level.

7.6) Iron

Spirulina is one of the richest foods in iron. It is therefore necessary to provide it with a lot of it, and in an assimilable form which is not obvious because of the high pH of the culture medium. If the spirulina is not dark green enough, it may be due to a lack of nitrogen, but also to a lack of iron. Even a very green spirulina can prove to be low in iron on analysis (for example 200 ppm). An insufficient iron concentration (for example <0.1 ppm) in the medium hinders the cutting of the spirulina trichomes which become very long and on the other hand slows down the proliferation of bacteria useful for cleaning the medium.
Sometimes, but rarely, there is enough iron in the salts and/or water used. There may even be too much iron in the water if it is ferruginous, a case rarely encountered.
The classic way to introduce iron is to prepare a 10 g/l iron solution as follows: in 1/2 liter of water put with 50 g of iron sulphate heptahydrate + 20 ml of concentrated hydrochloric acid or, better, 100 g of food grade citric acid (citric acid is a good iron chelator); complete to one litre. [NB The purity of iron sulphates sold to treat lawns is often inadequate: the solution must then be filtered or decanted or pure sulphate used].
The use of 100 ml of iron solution at 10 g/l per kg of spirulina produced corresponds to 1000 ppm of iron. In practice, 50 ml is generally sufficient. You can also soak 50 g of rusty nails in a liter of vinegar with the juice

4 lemons or carambolas; store in a non-hermetic container (release of hydrogen), shaking from time to time: after two weeks, a "nail syrup" with about 10 g/l of iron is obtained, which can be a source of "organic" iron ".

A chelating agent such as EDTA or citric acid makes the iron more assimilable by spirulina, but also makes the iron in spirulina more assimilable by the human body (see Bibliography: Manoharan). Lemon juice (containing citric acid) and especially star fruit juice have a chelating power for iron, as do certain aqueous extracts of topsoil or clay sterilized by tyndallization (heated for 10 minutes at 80°C twice at 24 hour intervals).

Commercial products containing chelated iron, such as Ferveg, Fetrilon 13 or Ferfol with 13% iron, chelated with EDTA, can also be used as an iron supplement. Sequestrene 100 SG with 6% iron chelated with EDDHA, reputed to be more effective than EDTA at high pH, has the disadvantage of strongly coloring the culture medium red and we do not use it. Note that Ferfol was approved "organic" in France (in 2009) but no longer seems to be.
Blood would also be a source of "biological" iron deemed to be very assimilable (at 9 g/l), but we have not tried it.

How much iron to take is a subject of discussion. An average dose of 500 ppm seems suitable. It is possible, if necessary, to obtain spirulina extremely rich in iron (up to 5000 ppm).

The more iron is added regularly, the more regular the iron content of spirulina will be. If the (chelated) iron is only added once a month, for example, the iron content of the spirulina just after the addition will be very high (for example 1000 ppm), whereas it will be low just before addition (eg 300 ppm).

Drip is obviously the best and it seems to be able to replace chelation (from the experience of Koudougou, Burkina Faso). Here is a suitable procedure: make a pre-dilution of the iron solution (100 ml in 10 liters of water), shake well and add slowly (if possible drop by drop) in the culture by shaking it very well (this agitation is essential).

An article by Puyfoulhoux B. *et al.* (2001) tends to prove that the bioavailability of iron from spirulina is equivalent to that of meat.

7.7) Trace elements

Instead of relying on make-up water and salts to provide the trace elements necessary for the growth of spirulina, it may be safer and even more economical to provide them with a ready-made concentrated solution (of very high cost). low relative to the kilo of spirulina). The addition of trace elements seems to be a positive factor in ensuring good harvestability and good productivity on a more regular basis, but it also improves the nutritional quality of the product.
The contribution of at least the major trace elements (boron, copper, manganese and especially zinc) seems recommended in the event of low rate of renewal of the environment over a long period. The risk of exceeding the maximum allowable dose for a trace element which is already present in a significant quantity in the water or the salts used is

low if the solution of trace elements is added in proportion to the crops, for example up to a quarter or half of the theoretical needs. It would be safer to add only what is missing in the culture medium, but that would require the use of analytical means beyond the reach of the artisan. There are different formulas of trace elements. The most cited is that of the middle Zarrouk (see Appendix 18) but it is unnecessarily complicated, while being incomplete...

The contribution of selenium is generally done by the sodium selenite, of delicate handling because very toxic, which we prefer to avoid (it would be practically necessary to work with a gas mask to introduce the product). Some have more courage.

Should cobalt be added? This is a topic of discussion related to the fact that vitamin B12 (cyanocobalamin, which contains cobalt) is abundant in spirulina, while some regulations limit the ingestion of this vitamin; vitamin B12 in spirulina is rich in “B12 analogues” which, according to some, should be wary of. Scientific clarifications on this subject are desirable. Jacques Falquet sums up very well the current state of knowledge on this important subject as follows: A variable (but high) proportion of vitamin B12 present in spirulina is in fact one

(or more) analogue devoid of B12 activity in humans

This proportion varies according to the spirulina analyzed; that of Hawaii would contain 36% of active B12

B12 analogues exist in many food products and are naturally detectable in human plasma

Vitamin B12 present in multi-vitamin tablets can spontaneously convert into non-assimilable analogues

The actual dangerousness of the various B12 analogues is currently unknown (no serious clinical studies)

The scientific literature does not report any cases of disorders related to B12 analogues of spirulina (more than 30 years of spirulina consumption in industrialized countries)

The population of Kanem (where spirulina is traditionally consumed) does not seem to be affected by any particular disorders (but pernicious anemia is fatal and its symptoms are "spectacular"). »

In any case, cobalt never seems to be deficient in the culture medium.
The “JP Jourdan” formula therefore omits cobalt and selenium.

There is a good consensus on the benefit of a greatly increased dose of zinc (the “JP Jourdan” formula provides only a small supplement). Another means of introducing zinc, proposed by J. Falquet, is to add 20 g of zinc sulphate heptahydrate to the 50 g of iron sulphate in the preparation of iron solution reported in the previous § (classic). A dose of 500 to 1000 ppm of zinc in spirulina would be suitable while high additions of zinc to the culture medium can cause serious problems; here is the opinion of Jacques Falquet on this subject (2009) : *“Our own tests lead us to think that it is not so easy to obtain such levels of zinc by enriching the culture medium: not only does this zinc precipitate (or in any case is not absorbed by the spirulina beyond a certain threshold) but it presents a certain toxicity for the spirulina itself. In fact, I think that spirulina with very high iron or*

zinc are obtained by post-harvest treatment: it should not be very difficult, since the spirulina biomass behaves like a real ion exchange resin. It would therefore suffice to disperse the harvested biomass (and washed with salt water to lower the pH) in a solution of a suitable metallic salt and leave to incubate for a few minutes. After new filtration, washing, pressing and drying, we would surely obtain the desired product »
If we do not have a reliable source of salts or zinc oxide, we can try to manufacture some by attacking zinc metal with an acid , but there is the danger that zinc metal contains too much lead.

There is a little nickel in spirulina, but it is unclear whether this metal should be considered a beneficial trace element or if it is simply absorbed: it has not been included in the "JP Jourdan" formula due to possible risks of toxicity to humans.

What should be the purity of the salts possibly used to provide the trace elements? The "technical" quality is considered sufficient, given the small quantities used. No need to use “for analysis” type purities.

In countries where access to the necessary chemical products is impossible, the addition of trace elements can be waived, except for zinc, which deserves a lot of effort to obtain it.

7.8) How to increase productivity by carbon input

The main food of spirulina is carbon, the normal source of which is carbon dioxide. The simplest cultivation method, where the carbonaceous food comes from **the air** (which contains carbon dioxide, but extremely diluted), presents a modest productivity, but which, expressed in proteins, remains much higher than that of the best agricultural crops. or horticultural, and which, expressed in food calories, is equivalent to them, and this without consuming more water, or even much less. The absorption of atmospheric CO2 occurs night and day, independently of daily weather variations, which therefore does not influence the average productivity of these crops; the latter is also not affected by an exaggerated temperature at night (the pH drops due to nocturnal respiration, but without loss of CO2, which will be used later). In these crops, the pH is maintained at around 10.6 or less by adjusting the shade. Note that each year the CO2 content of the air increases (it reached 400 ppm in the northern hemisphere in 2014), which favors spirulina. The productivity obtained from the atmosphere peaks at around 4-5 g/d/m² if the absorption surface is limited to the pond surface, but it is possible to improve it by increasing the contact surface between crops and atmosphere, for example by making waves but above all by adding an absorption column to the basin: this column, filled with Raschig rings or others, is watered with culture at a pH of, say, 10.5 and fed at its base with atmospheric air while the culture leaves the bottom of the column at pH for example 10.4 and returns to the basin. But it is more than likely that such a column will be more expensive than an additional basin surface giving the same increase in production (to be studied case by case).

If the pool atmosphere communicates with a **source of CO2** in the air, such as fermenting compost, barn, clean gas combustion, or

a source of carbonated water, the pH of the pond in good weather will be lower and productivity will increase significantly. The case of combustion gases is treated quantitatively in the simulation program in the Appendix.

But it is also possible to increase productivity in good weather, to increase it for example to 12 or 15 g/day/m², if the agitation is sufficient, by injecting **pure carbon dioxide** directly into the crop to lower its pH at 10. CO2 consumption is around 1.9 kg/kg of spirulina (theory = 1.71). The gas is another way to inject the gas is to introduce it into a venturi at the outlet of a pump placed in the basin and to make the emulsion travel a length of pipe sufficient for the gas to be completely absorbed before return of the liquid to the basin.

If you do not have carbon dioxide in cylinders but an alcoholic **fermentation** near the spirulina basin, it is quite easy to capture the pure carbon dioxide produced by the fermentation, but its pressure will be very low and it will be necessary to suction by the gas emulsifier.

A Canadian website describes in detail how to supply a horticultural greenhouse with CO2: http://www.omafra.gov.on.ca./french/crops/facts/00-078.htm. You can burn propane or biogas in the atmosphere of the greenhouse, but because of the aeration the performance will be less good than with pure CO2 injected directly into the liquid.

Instead of carbon dioxide , **sodium bicarbonate can be used,** but then it will be necessary to carry out purges to maintain the salinity at an acceptable level (density close to 1015 g/l) and to add the elements of the culture medium (other than bicarbonate of sodium) corresponding to the purged volume. It takes about 2 to 6 kg of sodium bicarbonate per kg of spirulina, depending on the desired productivity. This method is very convenient; in particular, it avoids having to monitor the pH. The purges provided for in § 7.5 (level) count towards the total purges to be carried out. The purging procedure can be simplified by including in the food of the spirulina the salts lost in the purge: it is then sufficient to replace the purged volume with the same volume of water; the food formula provided by the calculation programs in Appendices A27 and A30 is established on this basis. The practice of purges requires precautions with respect to the environment (see § 4.5 in Purification).

We know that productivity is an inverse function of pH, all other things being equal.
We also know that photosynthesis consumes CO2 and raises the pH.
We therefore add sodium bicarbonate to compensate for the CO2 consumed: 2

$$NaHCO3 = Na2CO3 + CO2 + H2O$$

In doing so, carbonate is accumulated and the salinity of the medium is increased and there comes a time when the medium must be purged by replacing it with new medium, and then the salinity and pH conditions are maintained by these purges.
Note that these purges are at the pH that we want to maintain in the pool. For example, if you want pH 10, the purge will contain as much sodium bicarbonate as carbonate. We understand that the more we want to work at a low pH, the more sodium bicarbonate we will consume. The higher the productivity, therefore, the higher the consumption of sodium bicarbonate (and the quantity of mineral waste) will be.

Let us illustrate this fact by a small calculation for a process at pH 10 which corresponds to maximum productivity (below 10 productivity is no longer improved), pH where there is no absorption of atmospheric CO2 (which greatly simplifies the calculation):

We know that at this pH the culture medium contains 7 moles of CO2 for 10 moles of sodium hydroxide in the form of sodium bicarbonate + sodium carbonate. In stable operation, there is no accumulation of sodium hydroxide in the medium (the purges balancing the contributions), therefore an entry/exit molar balance around the pool gives:

Input: 1 sodium bicarbonate (= 84 grams) = 1 CO2 +1 NaOH

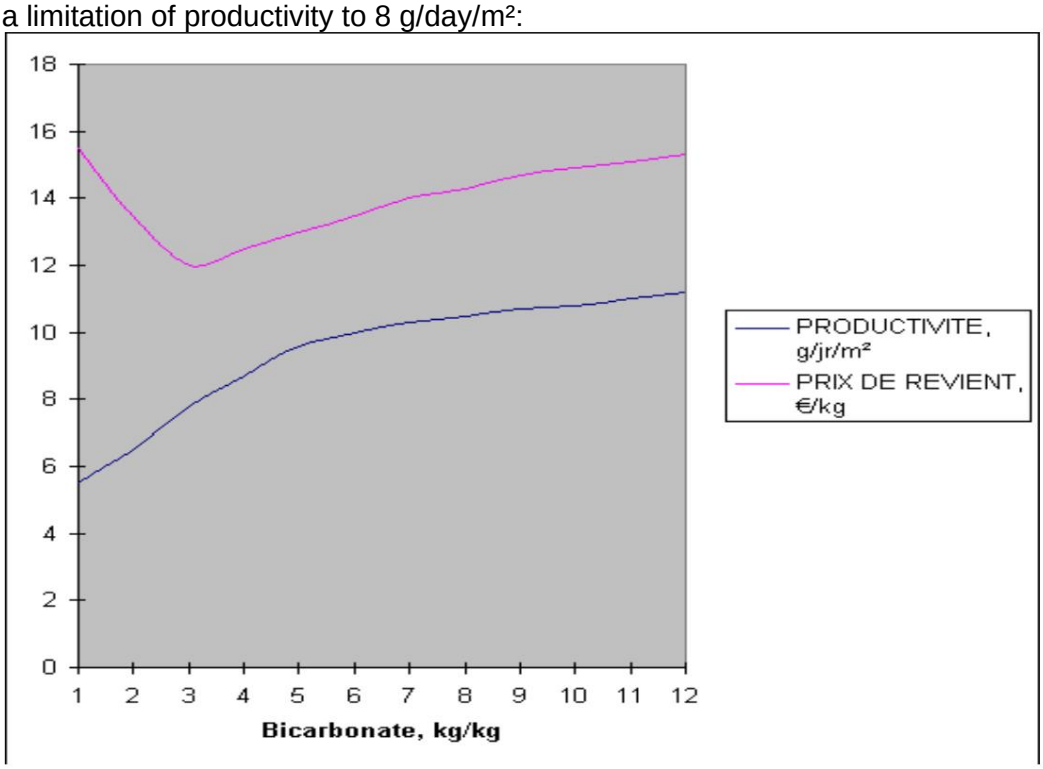
Purge outlet: 0.7 CO2 + 1 NaOH

CO2 leaving with the spirulina produced: 1 – 0.7 = 0.3 CO2, hence spirulina produced = 0.3 x 44/1.8 = 7.33 grams (in fact 1 mole of CO2 weighs 44 g and it need 1.8 g of CO2/g of spirulina)

Hence consumption of sodium bicarbonate: 84/7.33 = **11.5** g/g of spirulina

By using the simulation software (see CALCULATIONS), we can establish the relationship between productivity and sodium bicarbonate consumption, and find the economic optimum:

For example, the graph below was established for Koudougou with a sodium bicarbonate price of €0.5/kg.
It shows a clear optimum cost price at 3 kg of sodium bicarbonate/kg, with a limitation of productivity to 8 g/day/m²:



It is necessary to see the flexibility of market which we have: if the market demands more spirulina we can push the fires while waiting for new basins to be built. Conversely, if there is overcapacity, the supply of sodium bicarbonate can be lowered or even eliminated (but in this case, if there is no shade, the pH will settle above 11 in good weather, which the culture does not really appreciate).

If the evaporation is significant and if the make-up water is **very calcareous,** calcium carbonate precipitates and is found in the sludge, and this has the effect of reducing purges and reducing the consumption of sodium bicarbonate, by means of an increase in the quantity of mineral sludge which will have to be disposed of; in this case there is a risk of co-precipitation of calcium phosphate: closely monitor the phosphate content in the medium and add phosphate if necessary.

The proximity of a **natural sodic lake** offers an interesting possibility: that of sending the purges there and drawing from it what to replace them. In general, sodium lakes are at a pH close to equilibrium with the air, i.e. close to 10. Pumping water from the lake into the culture at pH 10.5 therefore brings it CO2 . The lake water must be filtered (for example through a sand filter) before being allowed into the culture, so as not to risk contaminating it. If its composition is not correct for spirulina, it should be corrected with the necessary additions (usually it will be urea and iron) and diluted if its salinity is too high. The purges recycled to the lake are biologically purified there by a natural process. The fact of having practically free CO2 makes it possible to make significant carbon contributions and to push the productivity in good weather easily to 12 g/day /m² (by pumping in the lake of the order of 3000 liters per kg of production , for a salinity of the order of 13 g/l).

Sugar constitutes **another** possibility of introducing carbonaceous food (see Jourdan (1996) in the bibliography). Its theoretical consumption, in the absence of other carbon sources, is 1.11 kg/kg. The weight of sugar that a pond is capable of oxidizing during the day is of the same order as its production of spirulina: this is the dose not to be exceeded anyway. Add the sugar in the morning, on sunny days only, so as not to cause fermentation odors, poor conversion efficiency of sugar into CO2 and excessive production of floating sludge (see § 7.15: sludge), especially if the medium contains other organic matter. In order for sugar to ferment producing CO2, it is often necessary for the pH to be below 10.8 (but I have seen sugar at least once rapidly lower the pH of a culture that had reached 11.1). If the ferments have been sterilized by too high a pH, reseed the culture with a "levain" taken from another basin. Start “sweetening” as soon as the pH reaches 10.4; it takes two days to see the effect; then adjust the sugar supply to maintain the pH around 10.4; an average dose of 0.6 kg/kg of spirulina is generally sufficient, in good weather. In fact, it is recommended not to exceed the dose of 6 g of sugar/m²/day in good weather (and even preferably 3) if we want to avoid undesirable side effects such as excessive turbidity of the culture medium and harvesting difficulties which can go as far as the impossibility of dewatering the biomass by pressing, especially at the start of the sugaring period. These difficulties may arise

a lack of nitrogen (causing an overproduction of exopolysaccharides) due to the consumption of nitrogen by the ferments; at the beginning of sugaring, it is therefore good to increase the urea. The protein content of spirulina obtained with sugar is strictly identical to that of CO2 production.

Pure sugar must be able to be replaced with profit by crushed sugar cane, at a rate of 7 kg/kg of sugar (soak the cane for a day or more in the basin then remove it) or by cane juice, but we have not tried these methods. Do not use molasses, too impure; on the other hand honey or pure glucose would be excellent if they were less expensive. Sugar can also be provided by various products containing it such as whey (do not exceed the dose of 4 liters per kg of spirulina, because whey is rich in nitrogen).

The sugar can also be replaced by leaves of fresh plants: green leaves soaked in the culture (preferably in a net) undergo an attack by the basic medium which dissolves in a few days all their elements except cellulose, which constitutes a means of feeding spirulina with carbon and also with mineral elements. The leaves must be of plant species chosen for their non-toxicity and their ease of “dissolution”; preferably choose plants that are edible but not very popular and therefore inexpensive, such as nettle, amaranth or lamb's quarters. It should be noted that sugar and leaves in high doses cause an increase in the turbidity of the medium, which must be taken into account when measuring the concentration by the Secchi disc. Such a culture is less “clean”: more sludge, slower filtration, and greater risk of pathogenic microbes that have become resistant to high pH. If an installation for the purification of the filtrates before recycling is available, this drawback should disappear (but this has not yet been tried).

Replacing sugar with glucose theoretically reduces the disadvantages of sugar. Glucose is indeed reputed to be directly assimilated by spirulina or it can be directly oxidized by the oxygen of photosynthesis: the ferments would become useless, resulting in a “cleaner” culture and better filtering, and the possibility of working at pH > 10.8 if desired. The only time we wanted to use pure commercial glucose at a dose of 1 kg/kg it actually behaved pretty much like sugar; after 15 days the pH was well maintained at 10 but the turbidity of the culture medium had risen to black Secchi = 6 cm (filtration remaining easy). This turbidity disappears a few days after the reduction or elimination of the addition of glucose. It seems that glucose boosts the health of spirulina. It also allows culture in heterotrophy, without light.

We must mention the non-negligible contribution of CO2 from **urea,** which is even the cheapest source of CO2. See § 6, NB c for essential precautions for use. Remember that in case of spirulina food through **urine,** it provides additional carbon equivalent to 2 g of spirulina / day / m². The sludge at the bottom of the pond itself is gradually oxidized (especially if the precaution is taken to brush the bottom and edges of the pond daily), thus contributing to bringing in, or rather recycling, CO2. Finally, let us mention that it is perfectly possible to mix the different carbon sources.

In general, it is recommended not to seek to maintain

record productivity, because they increase the soiling rate of the culture medium and, it seems, the frequency of mutations; at low productivity the medium has more possibility of self-purification. But the various hazards and in particular those related to the weather and the often weak agitation mean that the average productivity generally does not exceed 7 g/day/m² over a production season in the South of France.

7.9) Exopolysaccharide (EPS)

Spirulina secretes a sulfated exopolysaccharide (a species of alginate). Hypothesis: the low molecular weight EPS is gradually released into the culture medium where it first dissolves and then gradually polymerizes into larger and larger micelles, then into yellow-brown skins or lumps of variable size, microscopic (visible under a microscope after staining the medium with India ink, the EPS does not stain) or even visible to the naked eye; when the medium concentrates in EPS, its solubility decreases and it forms like an EPS sheath on the outer surface of the spirulina. EPS clumps or skins can clog filter pores and significantly slow filtration; slightly denser than the culture medium, they can settle at the bottom of the basin in the form of sludge, then finally detach themselves from it by taking on fermentation gas bubbles and floating. The collection sieve stops sufficiently large clumps of EPS. The normal production of EPS at low pH and under strong light is around 30% of that of spirulina, but EPS still seems to form at very high pH; if there is a nitrogen deficiency, photosynthesis produces exclusively EPS (Cornet JF, 1992). Even in the presence of nitrates, ammonium deficiency appears to favor the formation of EPS, if the light and temperature conditions are insufficient for the reduction of nitrates. In the presence of ammonium, proteinogenesis is slowed down by insufficient temperature, but less than with nitrates alone. Iron deficiency also seems to interfere with proteinogenesis and therefore promote EPS.

According to the Melissa 2004 report, page 199, an ammoniacal nitrogen concentration greater than 65 ppm with an illumination greater than 33 W/m² (very low level!) favors the formation of EPS and the formation of lumps; in fact at Cédric Lelièvre in July 2005 lumps were forming in a culture with 2.5 g of KNO3 + 80 mg of ammonium, in good sunlight. To fight against excess EPS and lumps you need ammonium, but not too much (a dose of 3 to 15 ppm is suitable) and avoid that the pH is below 10.2. The ideal would be to supply urea (or ammonia) drop by drop. But it has often been found that the abrupt addition of ammonia to a culture suffering from an excess of EPS (difficult filtration and/or pressing) is a quick way to improve the state of this culture. The quantities of ammonia at 22°Bé (i.e. 20.5% NH3) allowed depend strongly on the pH: 0.25 ml at pH 10 and 0.17 ml/litre at pH equal to or > 10.3 (to give a concentration of 30 ppm of free NH3 in a medium containing none at the start).

To better fight against EPS, we tend to use an excess of urea or ammonium, which is oxidized to nitrate. After a few months, we can then measure levels of 5 to 10 g of nitrates per liter in the environment! It is better to provide nitrogen by half in ammoniacal (urea) and nitrate form, without excess: this is what is practiced successfully at the La Mé farm in Côte d'Ivoire. Since nitrate is more expensive and sometimes not available, one can try to just reduce the excess urea.

It is obvious that a strong production of EPS is troublesome, not only because it is a loss of yield, but because it soils the culture medium and leads to harvesting difficulties.

The EPS is biodegradable more or less quickly depending on the circumstances, which limits the quantity that is found in the spirulina harvested. A 60% protein spirulina would contain 30% EPS (Melissa Report 1996, page 90).
The biodegradation of EPS is favored by the practice of daily brushing of the bottom and sides of the pool.

Addition of calcium ions causing precipitation of calcium carbonate allows some removal of EPS by flocculation.

The presence of a certain quantity of EPS seems to facilitate harvesting. With a spiral strain, the excess of EPS sometimes leads to the flocculation of spirulina with the formation of skins or floating green lumps. The latter, during harvesting, are easily retained by the sieve on which they gather in agglomerates immediately forming a "ball": if there is no floating sludge at the same time, they can be added to the harvested biomass by sieving using the extruder by replacing its die with a sieve; the quality of the spirulina thus harvested is a little worse than normal (an analysis carried out in June 1999 on the dried product gave 52% protein and a little too many aerobic microorganisms). One could fear that the formation of lumps increases the % of lines: experience, during a huge production of lumps (October 1999) has shown us that it does not.
The increase in pH and temperature, the addition of iron (if there is a deficiency) and above all the addition of urea or ammonia effectively combat these skins and lumps; follow the rule: "higher urea if there are green lumps or floating skins, lower the urea if there is a smell of ammonia". Sudden dilution and/or a sudden decrease in pH can also cause spiral spirulina to flocculate into floating green lumps.

An excess of EPS leads to sticky spirulina capable of clogging the pores of the filters, and to the impossibility of wringing the biomass by pressing, whereas a lack of EPS seems to lead to an easily wringable biomass.

EPS skins can be confused with clumps of foreign algae such as highly toxic microcystis, hence the need for toxicity testing in case of doubt, although we have never seen any cases of proven toxicity.

Publications seem to show that the polysaccharides (endo and/or exo) of spirulina have interesting therapeutic properties: awaiting confirmation.

7.10) Anomalies

In the event of poor growth when everything is otherwise fine, it is a good idea to check the phosphate content of the filtrate and, if it is low, to add phosphate; and if you don't have a phosphate test, you can try to add phosphate to revive growth. This mainly applies if the water used is very calcareous, because the

calcium phosphate tends to precipitate.

If a crop turns khaki yellow-brown without photosynthesis stopping, there is definitely a lack of nitrogen. Excess light, especially when cold or in the absence of agitation, or even at too low a concentration of spirulina, or maintaining a pH > 11.3 over a long period produces discoloration, then progressive destruction. spirulina. If too many spirulina have been broken, or destroyed, the culture medium becomes dirty (turbid, foaming yellow, or a little viscous, or "white" like diluted milk, or on the contrary brown, or smelly), ferments (release of bubbles even at night) and/or filtration and/or pressing during harvests become difficult, if not impossible. In general, the culture can heal on its own in one to three weeks, preferably at "rest" in mild light and temperature conditions, provided that it is not deficient (in nitrogen and iron in particular) . The practice of medium purges can aid in culture recovery; reseeding is particularly effective. If the restart does not take place, the environment has probably become toxic for spirulina: drain. A complete emptying from time to time is a powerful, but expensive, means to avoid culture anomalies.

If the culture contains a lot of spirulina broken into small fragments, this may be due to an excess of light (especially in the morning) or too sudden shaking, or even a lack of potassium. Abnormally long spirulina can be a sign of a lack of iron, unless it is a very weakly growing crop.

The spirulina of certain strains (spiral for example) usually float strongly on the surface of the culture medium, while others (wavy, straight) remain more readily in the mass of the culture (but still float normally). If the spirulina fall to the bottom of the pond, this is often a sign that they are undernourished in nitrogen or iron; a sudden change in pH or salinity can also cause the spirulina to fall to the bottom, for example a heavy rain which doubles the volume of water. A very low temperature has the same effect. The spirulina at the bottom of the basin are in great danger of dying and turning into brown organic sludge: to increase their chances of survival, they must be resuspended as often as possible. Similarly, the upper part of the floating layer is in danger of death by photolysis (browning or bleaching) in the event of too strong and too prolonged sunshine without sufficient agitation.

The spiral spirulina tend quite often to agglomerate in green lumps when the production of EPS is abundant; these lumps float if they are very rich in spirulina, unlike the brown sludge of EPS. But if the proportion of spirulina in the lumps is low compared to the EPS (colored lumps pulling towards brown), they no longer float and can remain in midwater and hinder the harvest by quickly clogging the sieve.

It may happen that the spirulina itself (including the corrugated type) flocculates into mini green lumps (with little EPS) under the effect of very fine mineral particles such as calcium carbonate in the process of precipitation or of an excess of certain ions. A dilution of the medium can then prove to be beneficial.

To counter the tendency to lumps, it is also prudent to shake the basin 2 or 3 times during the night.

Sludge rises to the surface, and floats temporarily during active photosynthesis, especially when the bottom is stirred, but normally it falls back to the bottom before the next morning. They can be removed by sieving (dip net or net). The nocturnal flotation of this sludge is due to the anaerobic fermentation of a layer of sludge that is too thick and lacking in oxygen (hypoxia, anoxia), a situation which requires several days to recover (shaking the sludge more frequently, and/or remove the majority). The recommended remedy is to transfer the basin to another and clean it. The sludge is a mixture of insoluble minerals (carbonates and/or phosphates), decomposition products of dead spirulina (containing chlorophyll A and especially carotenoids which give the sludge a characteristic brown color), EPS and biodegrading microorganisms ; there are also apparently colorless filaments , much smaller in diameter than spirulina (rated at 2.5 microns), but generally longer in length. Observation under high magnification with a phase-contrast microscope makes it possible to distinguish cells in these filaments, which appear green; it is a cyanobacterium of the genus *Phormidium,* potentially toxic although toxicity has never been detected on samples containing these filaments with the brine shrimp test. The appearance of these "colorless" filaments occurs very quickly in agglomerates containing residues of dead spirulina, and this even in fresh water: if you put spirulina in fresh water, it does not survive long and breaks down into brown sludge made up of “balls” of these tightly packed colorless filaments.

Note that the agitation of the sludge can cause a rise of sludge containing captive spirulina and a lot of Phormidium. Flotation can be due to captive spirulina or to equally captive bubbles, so that this sludge falls with difficulty to the bottom of the basin.

The color of the sludge in the basins sometimes tends towards pink, but it is generally brown, the color of carotene.

Needle-shaped crystals are also frequently found in the sludge, often grouped together in bundles: these are mixed ammonium and magnesium phosphate, soluble in an acid medium; it happens that these crystals are drawn into the floating layer of spirulina and harvested with it, but they will redissolve under the effect of stomach acidity. To prevent the formation of these crystals, excessive doses of phosphate, magnesium and/or ammonium must be avoided.

A bad odor generally corresponds to a bad state or an insufficient harvest, or an anaerobic fermentation or an excessive addition of urea, sugar or urine. A moderate smell of ammonia, corresponding to 20-30 ppm of ammonia in the environment, is not serious but alerts to a possible imminent danger. The use of sugar as a carbon supply sometimes causes odors of ferments or yeasts that are not really unpleasant. A culture of spirulina in good health and at the ideal temperature sometimes gives off a characteristic and pleasant aromatic odor, verging on geranium or rose.

7.11) Contamination by small animals

Unless the pool is completely protected, it is inevitable that insects or sometimes small animals (snakes, lizards, frogs, mice, snails), leaves and other plant debris fall into the pool. They can be removed with a net, but if left, which is not recommended, they will eventually be "digested" by the growing medium and serve as food for the spirulina.

On the other hand, some worms and insects are able to live in the culture medium as parasites. This is the case with Ephydra fly larvae (a small brown fly that walks on water), mosquito larvae, zooplankton (rotifers, especially brachyonus, cyanophages and amoebae capable of eating spirulina), which settle and live for some time in the pool: to hasten their disappearance, the pH can be temporarily raised to pH 12 then maintained at this pH overnight, by re-acidifying in the morning to pH 10; but this pH shock also kills some of the spirulina: the culture must then be put into convalescence (shaded). This pH shock is hardly effective on amoebas. But sometimes all it takes is a sudden increase in salinity of 3 g/l to make the invaders disappear (especially the larvae). You can also raise the temperature to 40°C (with peaks at 44°C). The addition of a high dose of ammonia, for example 100 ppm, kills larvae and amoebae but also some of the spirulina... Finally, the best way to eliminate the larvae is to eliminate them physically by harvesting them using a 300 µ mesh trap placed across the culture stream.

The disappearance of the amoebas generally occurs naturally in a few days of good weather with good temperature and rapid growth of the spirulina; maintaining a concentration of spirulina that is not too high and good agitation promotes the disappearance of amoebas. In fact, amoebas only seem to coexist with spirulina when the latter are weakened or in weak or no growth.
For example, in a sample of a culture in good condition, we can see the appearance of amoebas after 24 hours of storage in the laboratory.

Similarly, rotifers cannot in principle invade a healthy crop.

In the event of infestation by larvae or rotifers, harvesting remains possible because they are stopped by the sieve (adjust the mesh of the sieve if necessary: for brachyonus a mesh of 120 µ is required); one can try to eliminate the larvae and nymphs as much as possible with a sieve, or place the basin in a sealed greenhouse or mosquito net.
The infestation by larvae depends on the place, the climate. It may only be transitory. Some years it does not happen. In a greenhouse, the risk of infestation is reduced or eliminated (the ventilation openings and the doors of the greenhouse must be equipped with mosquito nets for this). We have never had rotifers at Mialet, but our Indian colleagues have had them quite often. Note that rotifers are not toxic and do not eat spiral spirulina in good health, but on the other hand develop rapidly in the event of poor health of the spirulina and end up invading the culture, giving it a reddish color. Rotifers are very often present in open-air crops, in small numbers, and contribute to

eliminate chlorella and also straight spirulina.
Ripley Fox explains that any amoebas that may be present in a culture have an almost zero probability of being toxic. As a precaution, however, it is recommended not to consume fresh biomass from a culture containing amoebas. When drying at 65°C they are killed anyway.
NB:
1) the male mosquitoes from the spirulina ponds would be sterilized by the high pH of the culture (according to an Indian study) and the ponds would then constitute a means of biological control against mosquitoes; this information is however called into question by the fact that mosquitoes proliferated in Lake Nakuru before the introduction of tilapias precisely to fight them, when this lake was full of spirulina but probably cohabiting with other algae... 2) the zooplankton and the larvae that we saw living with spirulina were not toxic to humans. 3) mosquito larvae and rotifers eat straight spirulina, but not Lonar-type spirals.

7.12) Contamination by lines or foreign algae

A) Straight

“Straight spirulina” appear frequently in cultures. They resemble the Oscillatoria cyanobacteria, of which there are toxic varieties (see paragraph B below), but we have verified that the "straight lines" that we have had so far are indeed spirulina *(Arthrospira),* moreover of normal composition, not only by using dimensional and morphological criteria, shade of color, etc., and by checking their gamma-linolenic acid content (much higher than that of Oscillatoria) and their alpha-linolenic acid content (very present in the majority cyanobacteria and absent in *Arthrospira),* but also according to a study of "genetic fingerprints" by the University of Geneva (see Bibliography: **Manen** downloadable from this link : http://ijs.sgmjournals.org/cgi/ reprint/52/3/861.

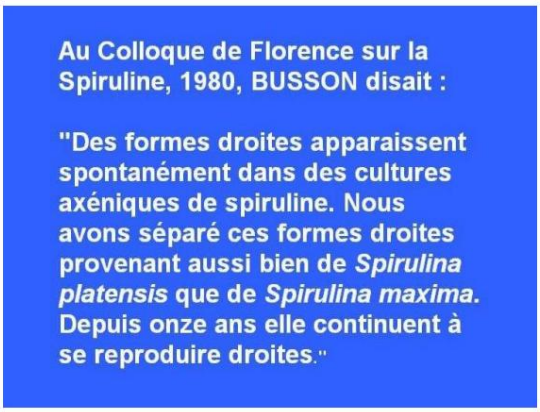
Straight spirulina generally float less, or slower, than spiral ones, one can try to counter their proliferation by not harvesting the floating layer but by harvesting the homogeneous culture and managing the culture to reduce the growth rate of the straight ones. Good agitation avoids the photolysis of the spirals, the most floating and therefore the most exposed to the sun, so it reduces the proliferation of straight lines. When there are few straight lines, the floating layer can contain them all and can therefore be harvested; but beyond a certain % of lines, this is no longer the case. In case of advanced infestation, one can try a reduction of the agitation and a massive reseeding in floating spirals.

In 2011, the presence of floating lines was observed in a Paracas XXL strain…

Since the spirals have a marked tendency to agglomerate into lumps under certain conditions (low pH, low temperature, absence of ammonium), it could be feared that the formation of lumps would increase the % of straight lines: experience has shown us, during of a huge production of lumps in October 1999, than it was

not the case.

The straight lines are genetically true spirulina but they have drawbacks, often including a notorious difficulty in harvesting. The problem is not new since almost 40 years ago Félix Busson was already fighting with him:



The "Little News of Spirulina" of November 2002 said:

" ***Straight spirulina defeated ?***

An e-mail from Jean-Denis N'Gobo, from Bangui, of 4/11/02 gives very good news: "that's it, we only have spirals in our 3 basins"

A phone call from Pierre Ancel on 8/11/02 tells us that in Koudougou (Burkina Faso) Lonar type spiral strain spirulina is also 100% spiral now.

We know that in Madurai (India) the rights have disappeared since 2001 and in Mialet since 2000.

But there are still sites suffering from rights, and we are still looking for remedies that will allow the control of rights for sure…"

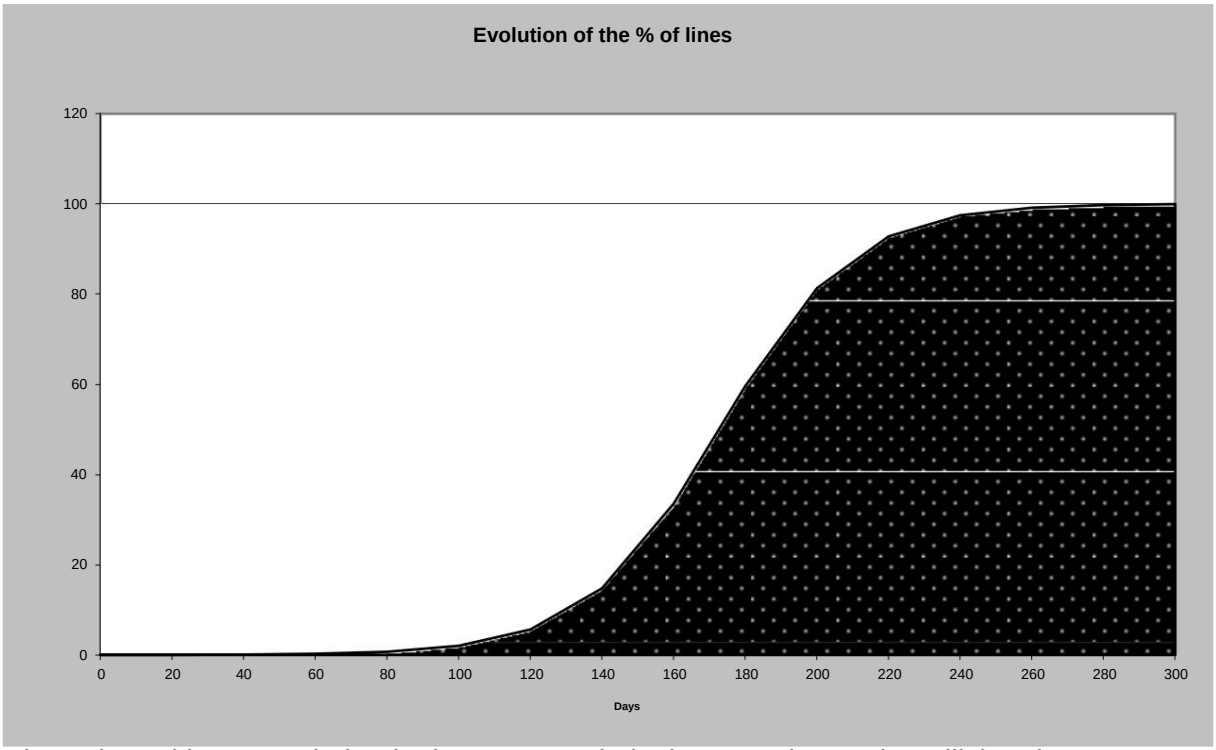
Not all lines are a problem: there are the "long" ones which hardly interfere with the harvest and there are the "endemic", non-virulent ones which coexist with the Paracas without invading them. Those that we fear above all are the short virulent ones, that is to say whose growth rate is clearly higher than that of the spirals. A small software (DRIMPR) makes it possible to simulate the way in which these lines can suddenly "explode" in a few weeks; in the example below, we start from a culture containing 1 straight line for 10,000 spirals at time zero:

Data: 1)

Basin depth, cm = 10 2) Initial %
of lines = .01 (1/10,000)
3) Rate of injection of spirals, g/m²/day = 0 4) %
of lines in injection of spirals = 0 5) (% of lines in
crop)/(% in crop), fraction = 1 6) Mutation rate of spirals,
fraction/day = 0 7) Respiration rate of lines, fraction/day = 0

8) Concentration, g/l = .3
9) Productivity of spirals, g/d/m² = 8 10)
Productivity of lines, g/d/m² = 10

RESULTS :



The only real known solution is rigorous prophylaxis: emptying and sterilizing the infested basin and restarting with a strain guaranteed without rights, such as those from the Institut Pasteur or from Jacques Falquet in Geneva at the time when he supplied strains. But that does not guarantee the reappearance of lines.

One can wonder why in nature do we generally not find straight lines (in fact there are a few)? A possible explanation: the lines do not float, or less, they fall to the bottom of the lake and die for lack of oxygen and light. Another possible explanation: insect larvae or rotifers preferentially eat straight lines. In cultures in Koudougou (Burkina Faso) and Pahou (Benin), among other places, a disappearance of the lines has been observed at the same time as a proliferation of larvae, and in Maduraï (India) at the same time as a proliferation of rotifers.

If this hypothesis is true, it would be an argument for not putting the ponds in greenhouses, since in greenhouses there are no, or fewer, larvae. But moreover, greenhouses do not favor straight lines since many greenhouse basins operate from year to year without being invaded by straight lines (even if there are, of course, also greenhouse basins full of straight lines). A too weak agitation exposes the spirals more, because of their strong flotation, to photolysis thus indirectly favors the lines: in other words, the lines are satisfied with a weaker agitation than the spirals; but a very effective agitation will not prevent the virulent rights from dominating, if they are present in the culture.

Regarding the possible "advantages" of straight lines: it is true that some have the

potential to grow faster than spirals, but this does not necessarily translate into increased productivity: productivity is logically the same when carbon is the limiting factor in the diet (carbon supply from atmospheric air only). On the other hand, virulent lines, i.e. capable, as in the example above, of completely invading a crop, allow greater, even very significant production, if they are fed with artificial carbon (CO2, bicarbonate of sodium) … on condition of being able to filter them and wring them out, which is by no means guaranteed, we have had the sad experience of this.

This is an opportunity to recall the misadventure we experienced at the Imade Company (Motril, Spain), which had laboriously selected a particularly virulent strain of spirulina (short line), which received the name M1 and wide publicity in the local press; it grew so fast that the larvae, however extremely abundant, could not consume them all, so that the concentration of spirulina was very high. This strain turned out to be unharvestable and inescapable by our methods, and had to be abandoned…. Those of us who knew this sad affair hoped that the disappearance of the M1 would be total and remained marked by the phobia of the rights. Would they be wrong? We must not be sectarian: who knows if a somewhat “high tech” technology will not one day make it possible to harvest and wring correctly “M1”? The mechanical rotary drum harvester by Robert Nogier (Saint Paulet de Caisson, Gard) is a step in the right direction, although still insufficient. Vibrating filters, both for harvesting and for dewatering (vacuum), are undoubtedly a solution.

[A Spanish grower would continue to use this M1 strain, surely going to great lengths].
Preserving easy harvesting conditions with the usual small artisanal means seems preferable to us. On the other hand the biomass of straight lines is often too difficult or impossible to wring out by simple pressing and must then be washed and drained before drying, and this drying must often be done only by the "Indian" method described in the chapter drying (spreading in thin layer on plastic film). One facilitates the filtration and the pressing of the rights by mixing them with 20% of spiralées or Paracas; Philippe Calamand collected straight lines by pre-coating Paracas on his filter before filtering the straight lines.

We must relate here an experience during a chlorella elimination operation: the biomass harvested from the contaminated basin (at pH > 10) was washed with new medium (at pH 8.2) and immediately reseeded in this medium, but the pH shock was too strong and the new culture died a day later. However, a few filaments survived this shock treatment and the culture started again, but, and this is interesting to note, absolutely without a line. The starting culture was a Paracas containing 0.5% straight (non-virulent) apparently more sensitive to pH shock.

In this old affair of the rights, we must remain very humble and recognize that our ignorance is still great!

Two additional disadvantages of straight lines should be noted:

- their fresh biomass is difficult to consume because it is presented as a

a bit viscous and stringy mass instead of the nice easy to cut and spreadable "cheese" normal.

- it must be checked that the lines are indeed Arthrospira and not foreign cyanobacteria such as potentially toxic Oscillatoria.

B) Foreign

As long as the spirulina is actively growing, as long as it is well nourished, harvested, agitated and at pH > 9.5, of a beautiful dark green and the medium is regularly purged or purified, no species of micro-algae competitor usually fails to invade the pond. However, a straight filamentous cyanobacteria is practically endemic in spirulina ponds; it is probably a *Phormidium* whose diameter is less than that of *Arthrospira.* This organism does not show toxicity in the artemia test, generally lives in conglomerates or non-floating mats. Its favorite place is the sludge at the bottom of basins, but it seeks light and willingly colonizes the blades of water wheels and the edges of basins at the air/water boundary (or the walls of aquariums). It obviously consumes inputs.

The appearance of chlorella (edible single-celled green algae) at the end of winter in the temperate zone is quite frequent, and may not be visible at the beginning.
It is prudent to have a culture sample examined (once or twice a year, for example) in a laboratory equipped with a good phase-contrast binocular microscope, and trained to recognize what is not *Arthrospira :* it can be simple chlorella or Oocystis (large chlorella), but also toxic cyanobacteria such as Oscillatoria agardhii (resembles a straight spirulina but of double cell length), Oscillatoria rubescens or Oscillatoria nigri-viridis (resembles spirulina straight but with a much larger diameter and length of cells and a different color), Anabaena flos-aquae (resembles a straight spirulina but with indentations at the level of the walls between cells), Anabaenopsis arnoldii (resembles a spiral spirulina but with heterocysts, swellings allowing it to fix nitrogen) or Microcystis aeruginosa (see Appendix A 22 to compare spirulina to these algae). ata tenius, non-toxic and too small to remain in pressed biomass can be seen under an ordinary microscope, possibly after tinting the sample with India ink. If the contaminating algae is eukaryotic (cells with a distinct nucleus), it is a green or brown algae, which are generally not toxic. A trained eye can easily distinguish the main toxic Oscillatoria from straight spirulina.

A simple biological toxicity test was proposed by R. Fox: if young brine shrimp larvae do not die after more than 6 hours in contact with a cyanobacterium culture extract, this would not be toxic. To have brine shrimp larvae (nauplius in scientific terms), just soak their eggs (which in scientific terms are called cysts, and which are sold in aquarium stores and can be kept in the refrigerator) for two days in the salt water at 30 g/l at ordinary temperature. We put about 10 to 30% of spirulina culture to be tested in the culture of brine shrimp larvae, in a thin transparent container by

example a "mini-aquarium" made with two microscope slides. It is necessary beforehand to lyse (break the membrane) of the micro-algae to be tested because the possible toxins are especially inside (R.Fox verified that the toxins of a toxic Oscillatoria came out sufficiently even without breaking the membrane, but for more security it is better to break it). To break the membranes the normal means is sonication with ultrasound, but otherwise you can boil a suspension of the micro-algae for one to two minutes or freeze/thaw fresh spirulina several times). Recently we found on the market mini-aquariums of 10 cm x 15 cm for which the following short description was written:

There is a supplier of **mini-aquariums** to carry out brine shrimp toxicity tests, in silicone-glued glass measuring 15 x 10 cm.
The spacing between boards is two millimeters (if necessary, it can be laid horizontally without the water flowing out). The price is 5 € per unit (without the support) but the supplier does not take care of the shipping.
Here are the contact details of the supplier (near Angers):

''Best Practices'' guide test for performing a brine shrimp toxicity test:

- Hatch brine shrimp cysts (eggs) in salt water at 30 g/litre, at ordinary temperature and away from direct sunlight; it takes about 3 days of incubation (faster at 25°C than at 20°C). Some cysts are slow to hatch or fail to hatch; note that hatching cysts float and dead individuals no longer float.
[Aerated hatchers are commercially available, such as the ''Artemio'' kit from JBL].
- Prepare an extract of the cyanobacteria to be tested, by boiling the sample in water, for 1-3 minutes (but preferably by sonicating the sample suspended in water with ultrasound), then filtering through a filter coffee or centrifuge and let cool. Keep the extract cool until you use it.
- Fill a mini-aquarium to a quarter with the cooled extract. It is important that the mini aquarium is well cleaned before use.
- Add brine shrimp culture until you have about twenty live individuals (use a dropper or a fine-tipped pipette). Hatching cysts often mingle with free larvae: this explains why the number of larvae sometimes increases at the start of the experiment.

- Fill in the same way another mini-aquarium but without broth, which will serve as a blank (because there may be some mortality even without toxins, especially if the aeration is insufficient)

- Monitor the number of live brine shrimp over time, over one or two days. As living brine shrimp move quickly counting is a bit difficult and approximate, but it is the average trend that matters. The lighting conditions of the mini aquarium are important for easy reading.

Even with this advanced test, the brine shrimp test is neither quantitative nor guaranteed.

The presence of alpha-linolenic acid in a sample of spirulina indicates contamination: analysis very useful, but insufficient. It is still necessary from time to time to dose the toxins. Several laboratories are now equipped for this in France, at affordable prices. But a good microscopic observation can show that an analysis is not necessary.

In the event of infestation by chlorella, a non-toxic unicellular green micro-algae (for example following the use of unfiltered raw water, and/or too heavy harvests, or during the wintering of the pool), it is necessary to get rid of them, otherwise they will quickly take over if the spirulina continue to be harvested, then the harvests will be canceled out. To get rid of it, we can theoretically try to play on the fact that the chlorella settle at the bottom where, deprived of light, they will die: but this method remains difficult to apply because the general agitation of the pool must be stopped and replaced. by very moderate agitation, on the surface, but sufficient so that the spirulina themselves do not die by asphyxiation or photooxidation (shading is necessary); but chlorellae, because of their small size, tend to resuspend on the slightest agitation, making this method inapplicable in practice. On the other hand, one can easily play on the fact that the chlorella being very small passes through the filter: it is therefore possible to recover the spirulina by harvesting it and washing the biomass with an isotonic solution (for example from new medium), then reseeding the basin, this method has proven suitable if it is practiced with care, as was the case with Cédric Lelièvre in 2005 and with Etienne Boileau in 2006, but care must be taken not to expose the spirulina to too great a pH shock in the practitioner (a pH difference of 2 can be fatal, see next paragraph).

The elimination of chlorella from the filtrate can, theoretically, be done by several means: refiltration through a finer filter (sand filter for example), sterilization by UV or ultrasound. It should be noted that a test for the destruction of chlorella at pH 13.5 at 21°C (addition of 8 g of sodium hydroxide per litre) proved negative in the short term but obviously positive after a few days, and likewise a test at pH 12.

We must relate here an experience during a chlorella elimination operation: the biomass harvested from the contaminated basin (at pH > 10) was washed with new medium (at pH 8.2) and immediately reseeded in this medium, but the pH shock was too strong and the new culture died a day later. However, a few filaments survived this shock treatment and the culture started again, but, and this is interesting to note, absolutely without a line. The starting culture was a Paracas containing 0.5% straight lines (non-virulent). *Straight lines would therefore be more sensitive to the pH shock, at least this type of straight line.*

In April 2007, another method of eliminating chlorella was successfully tested in the Etienne Boileau basin in Montpellier: the

concentration of spirulina under agitation reduced to around 0.8 g/l and this until the chlorellae were suffocated, which ended up disappearing in a few days.

Repeated treatments with 17 ppm of ammonia would prevent the proliferation of chlorella in a culture of spirulina according to Vonshak (see Bibliography Vonshak 1997, page 91); the same reference indicates other means of preventing, in most cases, contamination by chlorella: working at a high alkalinity (0.2), with a limpid environment and a high temperature. These measures had no effect on Cédric Lelièvre, but on the other hand the fine filtration of his supply water (surface water) was positive in preventing the reappearance of chlorella.

Finally, rotifers are able to prevent the invasion of a spirulina culture by chlorella (see article by Mitchell and Richmond below from 1986):

The use of rotifers for the maintenance of monoalgal mass cultures of *Spirulina*

S. A. Mitchell [1], A.Richmond [2]

1Botany Department, UOFS, PO Box 339, Bloemfontein 9300, South Africa
2Microalgal Biotechnology Laboratory, The Institute for Desert Research, Ben Gurion University, Sede Boqer 84990, Israel

ABSTRACT

Zooplankton was successfully used for the biological control of unicellular algal contaminants in Spirulina mass cultures even under conditions adverse to the growth of Spirulina (maximal winter daily temperature of approximately 10°C and very low bicarbonate concentration). Brachionus plicatilis (Rotifera) was the most successful species of zooplankton used. The interrelationships between Spirulina, green unicellular contaminant, and B. Plicatilis were studied under various conditions. Two species of unicellular contaminant were used; Monoraphidium minutum was isolated from local cultures and Chlorella vulgaris, obtained from contaminated Spirulina cultures in Israel. The rotifer B.
Plicatilis successfully controlled the population size of both contaminants whether they were introduced in a single addition or as a daily dose. The biological control of the unicellular contaminants allows Spirulina to be cultured in a medium low in bicarbonate, thereby reducing the cost of the medium and increasing the quantity of CO2 that may be freely absorbed from the atmosphere at the optimal pH for Spirulina cultivation.

The presence of naviculas, diatoms (single-celled algae containing silica) brown in the form of a shuttle, is quite frequent in cultures of spirulina containing sufficient silicate ions: the addition of 50 to 100 ppm of calcium chloride combats it effectively by reducing the concentration of soluble silicate, because calcium silicate is insoluble.

It seems prudent to completely drain or sterilize the time basins

in time (for example every 2 years), and to restart the culture from a strain of guaranteed quality (monoclonal) to avoid the risks of a possible genetic "drift" of the cultured strain. However, this recommendation remains somewhat theoretical, and probably useless: the great genetic similarity of Arthrospira suggests that one can rely on simple "technical" criteria (filtrability, resistance, appearance, etc.) to estimate whether there is instead of renewing the strain [opinion transmitted by Jacques Falquet, Antenna Technologies on 02/25/2003].

7.13) Contamination by micro-organisms

In the culture medium, at the high pH (> 9.5) where we work, the majority of microbes dangerous to humans are normally inactivated within two days.
Be careful with cultures at pH < 9.5 (young cultures based on sodium bicarbonate, or too strong injection of CO2), which may not benefit from this protective effect.

It has been reported the risk that certain pathogenic microbes introduced into spirulina cultures (probably as a result of poor observance of hygiene rules) become resistant to high pH, this risk may be increased if sugar is used as a contribution. of carbon ; but it was never confirmed. The existence of African microbes or parasites that may be resistant to high pH has also been reported: here again, no real cases have been observed if normal rules of hygiene are followed.

Insufficient agitation (still too frequent a case) risks leading to areas in anaerobiosis and the proliferation of sulphite-reducing anaerobic microorganisms, the maximum standard of which is 100 per gram (at least in France) and of Clostridium perfringens.

The cultures also contain biodegrading bacteria adapted to the culture medium and which play a beneficial role, alongside zooplankton, by purifying the medium and recycling nutrients, while helping to eliminate oxygen and supply carbon dioxide. The proliferation of these good bacteria requires a sufficient concentration of iron (preferably chelated), for example 0.5 ppm, and a pH lower than 10.8.

Germs of molds are always present in the cultures because molds appear regularly on the float left a long time without agitation (as on the surface of the artisanal jams), and the bacteriological analysis detects commonly from 5 to 500 colonies/g, without that no standard has been imposed in most countries.

The use of sugar as a carbon supply, as well as the fact of not harvesting for a long time, cause an increase in the culture of the number of apparently colorless filamentous microorganisms, which interfere with filtration but are practically not found in the finished product (NB these apparently colorless filaments seem to come from the muds where they are present in large numbers). We now know that it is a cyanobacterium, a Phormidium or Jaaginéma.

Bacteriological verification analysis should be done on the finished product of

once in a while (once or twice a year?). Due to the risk of post-harvest contamination, pasteurization of the finished product may be necessary but should be avoided if possible.

Please note: in some countries, water used for cleaning, rinsing, etc. may be contaminated, this can be a source of contamination for the harvested product.
In this case, the systematic use of bleach is suggested for all cleaning, with final rinsing with chlorinated water (minimum 1 ppm of free active chlorine: see following §; i.e. approximately 1 drop of bleach (sold by the litre, 2.8% active chlorine) in a liter of rinsing water).

It is reassuring to know that the microbes disappear in two months of storage of well-dried and well-packaged spirulina.

7.14) Chemical poisoning

Detergents and sugars are not toxic at 100 ppm.
A large excess of urea or ammonia causes the death of spirulina, the culture medium becoming "milky", with yellow or greenish foam and abundant sludge; but in general there are enough surviving spirulina (otherwise you can reseed) to regenerate the culture spontaneously in about ten days if you take the precaution of shading.

In a series of experiments, it was found that a dose of 8 ppm of active chlorine provided by bleach (sodium hypochlorite) kills spirulina in their culture medium at pH < 9, but that they resist 4ppm; at pH 10.6 they resisted a dose of 12 ppm (but the effect of chlorine varies according to the chlorine demand of the medium). Algaecide doses generally recommended for water with a neutral pH are between 0.5 and 1 mg of active chlorine per litre.

NB: commercial bleach concentrated in cartons has 11% active chlorine, ordinary bleach sold by the liter has 2.8%. You should know that the algaecidal power of hypochlorite is much stronger at low pH than at high pH. Thiosulfate ($Na_2S_2O_3$, pm = 158) can be used to neutralize active chlorine: theoretically 4.5 g of thiosulfate are needed per g of active chlorine, depending on the reaction: $2\ Na_2S_2O_3 + Cl_2 = 2\ NaCl + Na_2S_4O_6$ (dithionate)
Thiosulfate is often sold as the pentahydrate (ww = 248), in which case 7 g/g is needed. It is recommended to use an excess of thiosulphate, as a precaution.

7.15) Lack of oxygen (hypoxia)

If oxygen can be considered a poison for spirulina when it is in strong supersaturation during active photosynthesis, this is not the case in the absence of light since spirulina then needs oxygen to breathe, as other aerobic microorganisms present. The oxygen content of the culture medium in equilibrium with atmospheric air is given by the following approximate formula: mg/l or ppm of oxygen = 0.616 x (atmospheric pressure in mmHg) x (1 – 0.0009 x altitude in m.)/(31.64 + T°C) – 0.035 x (salinity in g/l), or for example 8 ppm at 25°C.

At the peak of the active photosynthesis period, the oxygen content of the culture medium can greatly exceed saturation and rise to more than 30 ppm. But the respiration of spirulina consumes 1.2 g of oxygen per g of "burnt" spirulina, i.e. easily 3 g of oxygen/m²/night, and oxygen is also consumed by other microorganisms, especially if the medium contains sugar and other biodegradable products; in this way the oxygen level in the medium drops rapidly after the cessation of photosynthesis, especially if the spirulina concentration is high.
As Jacques Falquet has shown, anoxia is easily reached in the presence of 100 ppm of sugar, even by stirring at night. Oxygen from the air dissolves in the medium as soon as it is below its equilibrium concentration, but this effect is negligible if there is no agitation. The absorption of oxygen from the air is evaluated, in g/hour/m², by the very approximate formula taken from the fish farming experiment = 0.3 x (stirring power, W/m²) x (concentration in oxygen at equilibrium – current concentration, in ppm), or for example for a continuously stirred pool with 1 W/m² and containing 200 l/m² at 5 ppm of oxygen: 11 g of oxygen/m²/night. It is therefore not surprising that the bottom of a pool that has not been stirred overnight lacks oxygen and that the sludge undergoes anaerobic fermentation with the formation of bubbles of insoluble gas (methane) causing the sludge to rise to the surface. To combat this situation, one can agitate the sludge deposit with a broom and maintain the agitation of the culture at night, but the most effective is to regularly remove the excess sludge from the bottom of the basin. This sludge is removed either by transferring the culture to another basin, or by sucking the bottom by pump or siphon. There is a commercial sludge vacuum cleaner, but you can make one quite easily with an aquarium pump attached to the end of a broom handle. The mixture of sludge and removed culture medium can be collected in a basin to decant the sludge and recycle the majority of the culture medium.

Spirulina does not seem to suffer from anoxia for a few hours per night. Amos Richmond showed that respiration was very weak in very concentrated cultures, therefore in floating layers. We know that we can keep a culture alive by giving it only a small bubble of air during the night, allowing only minimal respiration. We also know that in the early days of the existence of spirulina on earth, there was still no oxygen in the atmosphere, and yet spirulina crossed this era victoriously: it is likely that oxygen that they produced during the day they still had traces of them, dissolved in their living environment, sufficient traces at night to survive.

7.16) Diseases

It happens, very rarely, that spirulina present deformations, or a blister, or else yellow excretions at one end or on one side of the filaments, suggesting a bursting of the wall with effusion of the contents of the cells (so-called spirulina gutted"). It is possible that this results from an attack by cyanophage viruses. In practice, these anomalies disappear on their own after a few days of walking under normal conditions; it rarely results in the death of the culture.

Photos of "stripped" spirulina seen under a microscope, Don Bosco School of Agriculture

in Linares (Chile), 1998: 

Keeping spirulina lit 24 hours a day produces deformed, irregular filaments.
Spirulina needs at least 8 hours at night.

7.17) Heavy metals

Spirulina very easily absorbs the heavy metals present in the culture medium. Some are toxic to humans (mercury, lead, cadmium). You will find in Appendix 17 the maximum levels of heavy metals authorized in France in spirulina.

7.18) Pool cleaning

It is a good idea to clean the ponds about every 3 months, or before the bottom sludge is thick enough to ferment and form floating sludge.
It is in fact better to at least partially eliminate the sludge, from time to time, by suction at the bottom and settling in a separate tank: this practice, together with maintaining night aeration (by agitation), a moderate pH (< 10.5) and careful daily brushing of the sides, bottom and folds of the pelvis, promotes self-purification of the environment.
Brushing alone, without removing the sludge, is less effective and can cause sludge to rise. To be effective, brushing must be imperatively daily (even on Sundays) and complete, and begin from the beginning of the culture; it is therefore a major penalty that many do not accept.

The best method for complete (eg annual) cleaning of a pond is to temporarily transfer most of the contents of the pond to a nearby pond, then drain the sludge, and brush the edges and bottom, rinsing.
Pay attention to the corners (folds of the plastic film in the corners). There is often a white deposit encrusted on the film: this is a mineral deposit, which can be removed by brushing on dilute hydrochloric acid, which has the advantage of sterilizing at the same time (it is used especially during strain changes).

7.19) Purification of the culture medium

After 2 to 6 months of culture (depending on the level of productivity and cleaning care), without purging, the culture medium, new and perfectly clear at the start, becomes more or less cloudy and colored yellow-brown, the filtration rate drops and the pressing of the biomass becomes difficult. The practice of regular purges or the total replacement of the medium solves this problem but this can disturb the environment, and cost too much in products.
Experience has shown that a “spent” environment can be partially regenerated by simple decantation in a deep, non-agitated basin, for a variable time depending on the degree of purification desired. It is likely that part of the EPS will

biodegrades during this operation, but part of it settles at the bottom, in the form of a more or less colored deposit which can be sent to the compost.

It is possible to obtain a low turbidity in this way (black Secchi of more than 30 cm), on the other hand there remain dissolved organic products (the filtration test on 400 g is still good = 330 g filtered in one minute for example).

Before reusing the purified medium, it is a good idea to aerate it to eliminate the anaerobic bacteria present at the bottom of the settling tank.

If the medium sent to the purification basin contains spirulina, it does not matter: we can recover the floating layer. This can even be a complementary way to reduce the percentage of non-floating straight spirulina or chlorella.

But there is better than simple settling: filtration on sand filter (for example swimming pool or drip irrigation), which eliminates the majority of micro-algae.

And there is even better: the decanted and/or sand-filtered medium can be subjected to biological oxidation in the absence of light, with air injection (no inoculation with special bacteria is necessary, ambient bacteria are sufficient, provided however that the pH is moderate, preferably less than 10.5, and that the medium is sufficiently rich in nutrients for the growth of the purifying microorganisms), followed by a new settling or filtration to eliminate the residues (“activated sludge”).

By these means, it is possible to reduce the organic load (COD or BOD) and the coloring of the purified medium sufficiently so that its recycling makes it possible to never need to renew the culture medium; this has been practiced at the BIORIGIN spirulina farm in Ecuador for about ten years (see publication at the Colloquium of Embiez 2004).

Note the possibility of rapid and simple chemical purification of the used culture medium, for example by adding bleach (about 5% bleach to 2.6% active chlorine) which sterilizes and completely purifies the medium in a few minutes, but requires the neutralization of the excess active chlorine by adding sodium thiosulphate (Na2S2O3, 5 H2O) at a rate of 5 g/litre before recycling. However, chemical oxidation with bleach, or potassium permanganate, or hydrogen peroxide cannot be recommended until it has been verified that it does not induce the appearance of potentially dangerous compounds. for the consumer.
The oxidation with ozonated air is very effective and would not produce dangerous compounds, but it is necessary to have an ozonator and moreover the dissolution of the ozone in the culture medium to be treated is not so easy.

Purification by oxidation of the organic load, possibly followed by exposure to air, reduces the pH of the culture medium, down to around 10.

Another way of purification and recycling certainly works but will have to be checked before it is used. It would consist in letting the spent medium evaporate to dryness into "artificial natron", then in calcining the latter at high

temperature (800°C?) in the presence of air so as to obtain white ashes containing sodium carbonate and salt (plus remains of other minerals).

An additional advantage of the purification of the filtrates before their recycling is the disappearance of the small spirulina passing through the filter cloths (their direct recycling leads to a more or less rapid enrichment of the culture in small spirulina).

In 2013 two other purification methods were proposed: the addition of soft clay at 10 grams/m²/ week (mainly eliminates proteins), or skimming with simultaneous UV lamp sterilization. Attempts to clarify.

7.20) Sudden death of crops Many cases of more or less sudden death of ponds (especially in Paracas) are reported even in summer, without the origin of the disease being known with certainty yet. The hypothesis of a cyanophage virus is not proven. The photolysis hypothesis seems probable, even in summer in the South of France: it is necessary to admit a very strong photosensitivity of the strain to cold (even at 20°C) and perhaps to very high temperatures as well.

Philippe Calamand recommends as a remedy to add ammonia at a dose of 1 liter at 13% per 10 m3 of medium if the problem is spotted in time, a dose that can be tripled if the case is serious. Jeff Thévenet recommends treating with clay at a rate of 1 gram/litre as soon as symptoms appear.

The search for causes continues; in the current state of the investigation, we are tempted to blame the poor quality of the environment. Pure water and pure air seem necessary for the proper functioning of spirulina; you have to avoid non-organic neighbours, cities, industries, roads. We are entitled to make a correlation between the problems of many spirulina cultures located in wine-growing areas (slowdowns in growth, death of ponds, etc.) and the virulence of mildew, forcing winegrowers to treat with energetic products.

There can be several causes. Contamination by a cyanophage virus is one of them (under study).

Another hypothesis advanced, but which seems improbable, is the invasion of the culture by a competing cyanobacterium in fine straight filaments which is probably *Phormidium,* which can slow down the growth of spirulina and even cause them to die if its concentration becomes very high. This cyanobacterium originating in the sludge (on which it feeds in heterotrophy no doubt), it is necessary to hunt the sludge to avoid it. Another suggestion, easier to implement: work with a mixed strain and a high concentration of spirulina (which goes hand in hand with a reduction in the depth of culture): this will promote the dominance of at least one of the types of spirulina on foreign cyanobacteria, and in addition we will better protect against photolysis.

A remark: the contamination of a culture is all the more probable that the surface of culture is large, that it is distributed in only one basin or between a great number exchanging between them the strain. It may be necessary to adopt the good practices of large spirulina farms, namely: - Only start a pool from a pure strain, itself obtained from a single

filament in a sterile and axenic medium, taking the draconian precautions usual in biological laboratories. It means giving up easy and instant reseeding from a complacent neighbor!

- Do not admit any visitor or employee to the farm who is not dressed from head to toe in the required protective clothing and who has not gone through a sterilizing footbath...

8. HARVEST

It is better to harvest in the morning because the protein content of spirulina is generally higher there than in the evening, but also for other reasons: excessive heat afterwards, need to put the harvest to dry as soon as possible (especially in the event of solar drying if the good weather is not guaranteed in the afternoon). Filtration under full sun is strongly discouraged because the biomass on the edges of the filter quickly turns brown and soils the filtration cloth. In cloudy weather this obligation to harvest early in the morning is obviously less imperative, and in good weather you can always shade the filter. Whatever the weather, if you operate outdoors, you must cover the filter to prevent the harvested biomass from degrading and becoming dirty.

When possible, it is very advantageous to set up a harvesting station sheltered from the sun and dust, preferably in a building. This is the general case of filtrations on Rampelt automatic filters.

8.1) Filtration

Harvesting consists of filtering part of the culture through a fine cloth (25 to 50 µ mesh), recycling the filtrate in the basin, directly or, better, through a purification system (sand filter, settling, biological oxidation or skimming). The culture is sent to the filter through a 300 µ mesh sieve intended to intercept foreign bodies such as insects, larvae, leaves, sludge or lumps of spirulina. A finer mesh sieve may be necessary to stop any rotifers (the mesh opening is chosen so as not to stop too many spirulina).

The filter cloth can simply be placed on a large sieve with 10 cm high edges or a large colander, but a bag or a tube (see end of this §) may be preferred.
Screen printing frames (canvas very stretched over a frame, like a drum skin) can also be used as filters, but are too expensive and fragile, without offering any decisive advantage. It is useful, but not mandatory, that the filtration cloth be stretched flat (in the case of a bag, it is the weight of the liquid which stretches the cloth, otherwise one edge of the cloth is lifted by hand if it is is possible) to facilitate its unclogging with a shovel with a straight edge and also to pick up the biomass if it sticks. In the case of tube filtration, we do not unclog.

You can pump the culture (pump of a type that does not break spirulina! check under a microscope), or siphon it or let it flow by gravity if the filter is below the level of the basin; for manual harvesting, basins with preferably straight sides are used; in any case, care must be taken not to stir the bottom of the basin too much, so as not to suspend the sludge from the bottom during sampling. Although the sieve stops the most visible sludge, fine particles are however almost always drawn into the harvested spirulina: they are deposited in the tissue, especially at the place of arrival of the culture to be filtered (if it there are a lot of them, the canvas, especially of fine mesh, will clog fairly quickly by turning brown and it may then be necessary to clean it with a jet of water during harvest). Filtration is facilitated, when a layer of biomass has formed on the canvas, by scraping the canvas to

unclog: a plastic shovel is used for this and it is in your interest, when the filtration is slow, to take the contents out of the shovel and put it to drain separately.

For productions that are already a little large, it is advisable to use a high-flow pump, like a cellar pump (at least with the corrugated or "Paracas" type stump that is not very sensitive to breakage by pump), placing the sieve at suction or on the discharge, and by sending a tangential jet onto the horizontal fabric, which automatically unclogs this fabric. You can also place a stainless steel plate or a plastic shovel on the filtration fabric to make the jet horizontal.

After stopping the sending of culture on the filter (avoid lowering the concentration of spirulina below 0.4 g / liter), it is allowed to drain, then the green paste obtained, called "biomass", is collected. The spirulina biomass containing less than 75% of straight spirulina and coming from a culture in good condition, with a pH and ammonium content not too high, is easily filtered and drains easily by pressing. Sometimes the biomass drained on the filter collects easily by rolling it on itself to form a ball (like making a snowball) or a cylinder; this biomass does not stick to the plastic. Other types of biomass do not form a ball and stick to the plastic but can be wrung out easily. On the contrary, biomasses too rich in straight lines, or coming from an "old" or "tired" culture due to too much sun or too rapid growth, or too rich in dissolved organic matter (including sugars) give a sticky "cream" , which must be picked up with a ladle or a plastic shovel, and which, ultimately, cannot be wrung out by pressing.

Photo: A beautiful "ball" (biomass rich in Lonar-type spiral spirulina):

You can also leave the biomass in a hanging bag to finish draining. The drained biomass contains 8 to 12% of dry matter for a culture medium of usual salinity.

As described in § 7.9 (eps), any spirulina lumps retained on the sieve

can be recovered.

A polyamide (Nylon) or polyester (Tergal) monofilament filter cloth is preferable to a cotton cloth because it facilitates the detachment of the harvested biomass and is then easier to wash. Monofilament fabrics said to be for industrial use are preferred, but it is possible to make do with suitably chosen (and much less expensive) nylon, terylene or silk clothing fabrics. The lifespan of a synthetic fabric filter cloth will be shorter the longer it is left exposed to the sun. The fabrics end up puncturing or tearing. It is possible to use a cotton fabric (sheet) provided you choose it well and provided that the biomass is of "good" quality (not sticky) otherwise it passes through the cotton fabric.

Do not hesitate to wash the fabrics in the washing machine from time to time to unclog the pores. It may also be a good idea to iron the synthetic fabric sheets, with an iron that is not too hot, to eliminate the creases that form over time and hinder filtration. Do not abuse bleach which accelerates the aging of fabrics.

Manual harvesting of the floating layer (when it forms) using a basin with a straight edge is tempting because it allows obtaining a spirulina concentrate at around 3 – 6 g/l, therefore with (relative to the dry weight) approximately ten times less of the clogging products possibly found in the medium, and it is fast. If several basins are available, it is advantageous to place the filter on a basin other than that from which the floating layer is collected, so as not to disturb it by the filtrate. As the Lonar-type spiral spirulina float faster than the wavy and straight ones, the floating layer should only be harvested in the case of a 100% spiral culture or with total flotation, otherwise the culture would be enriched with non-floating spirulina (straight by example) which would end up suddenly taking over: monitor the evolution of the % of the different shapes – especially straight lines – in the culture over time. It happens that even wavy and straight lines float completely (this happens especially in the dark when there is little oxygen, for example in a closed or very poorly ventilated container, with about 1 ppm of dissolved oxygen). It would certainly be interesting to use the following device to harvest 100% floating spirulina (but we have not tried it): transfer the culture to a deep tank where total flotation would occur, then inject water into the background to recover the floating layer by overflow.

If the flotation is not complete (you can see this from the appearance of the medium under the floating layer), do not harvest the floating layer alone; homogenize the culture before harvesting (only let the sludge settle for 5 minutes), and harvest preferably at the pump. To reduce the concentration of straight lines or prevent it from growing, one can harvest by pumping near the bottom, where there is the greatest concentration of straight lines.

There is a case where the floating layer can be harvested without hesitation: that of a culture of wavy spirulina (Paracas) tending to turn into spirals (Lonar) while we prefer to keep as many wavy as possible. There is also the case where the lines would be floating (noted in 2011)!

Pictures :

Filtration in a 6 m² pond in Mialet, 1998:

Filtration at the Agro-Piscicultural Cooperative of N'dress, Bangui (RCA), 1995 :

A reduction in the size of spirulina can be caused by a very rapid growth rate or too high a salinity or a pH or too high a light, or come from the strain (in Lonar-type spiral spirulina the turns can become so tight that they touch each other). In this case, use a fine mesh cloth (25 to 35 microns), otherwise there will be significant leaks of spirulina through the cloth, especially during unclogging, resulting in poor filtration performance and selection resulting in enriching the basin in increasingly small spirulina. A fine mesh canvas is suitable in all cases, it is therefore recommended but it is twice as expensive; it is practically essential in the case of 100% spiral strains in the middle of summer. A certain percentage of straight or wavy facilitates filtration and can avoid the need for very fine mesh.

It may be advantageous to refilter a filtrate containing too many small spirulina or spirulina debris, through a very fine mesh filter (5µ but preferably 1µ, or a

sand), this to prevent them from accumulating in the crop. This precaution applies to both spirals and straight lines.

For fairly easy filtration even with straight lines, it is good to have at least a quarter of spiral (or wavy) shapes, preferably large. At 10% spirals and pH 11, or 4% and pH 10, filtration is still possible but more difficult. Filterability can be assessed by carrying out a simple test described in Appendix A6.1. If all the spirulina are straight in shape, clogging is so rapid that filtration may be considered impossible. If the biomass becomes usually infiltrable, do not hesitate to change the strain.

The gravity filtration speed varies according to the type of filter, the concentration of the culture, and the movements imparted to the cloth or to the biomass to unclog. A filtration rate considered to be good provides about 300 g (of dry spirulina)/hour/m² of filtering surface.

To accelerate filtration, the vacuum produced by a household vacuum cleaner (see filtration) or pressure can be used.

The vibrations imparted to the filter accelerate the filtration, which is easily explained by the unclogging effect but also by the rheological properties of the EPS dissolved in the culture medium (the viscosity decreases when the speed of movement increases).

Pressure filtration is done in tubes made of filtration fabric, 5 to 6 cm in diameter, fed by gravity or by pump and closed with a clip or a knot. These tubes can be arranged horizontally in the basin itself, but preferably they are suspended vertically above the basin or in the collection room. A ramp of several tubes with a length of 1 meter is practical. The sieve is placed upstream of the suction of the pump. It is important that the pressure in the tube does not exceed 1 m of water column, otherwise the mesh of the fabric may increase under the effect of the excess pressure and the filtration performance will suffer.

The choice between flat filters, bags or tubes is a matter of personal taste, but if the filtration station is not protected from dirt (dust, insects) the tube should be preferred since it protects the biomass .

8.2) Washing and Spinning (spinning and pressing are synonymous here)

Some producers want to neutralize with acidified water and/or wash their biomass with fresh water before wringing and drying it, at the risk of losing part of it by bursting the cells.

While some spirulina can withstand washing in fresh water, others discolor or burst on contact and can only be washed in salt water or with new culture medium at the same salinity (or more exactly at the same ionic strength) as the harvested pool. Indeed, spirulina brought into contact with an environment of salinity different from their original environment react almost instantaneously by absorbing or losing water to put themselves in osmotic equilibrium with the environment, which can cause their wall to burst. Corrugated (Paracas) are more resistant to bursting than spiral (Lonar). Washing also risks causing microbial contamination: on the one hand if the water used is not pure, on the other hand because the drop in pH makes the biomass more fermentable during storage or

drying.

At the Nayalgué farm (Burkina Faso), since systematic washing with salt water at 5 g/litre was adopted, a clear improvement in the organoleptic quality of the dried product has been observed.

But in general it is recommended to wash the biomass only if you have to harvest a dirty or smelly crop, or one that is really too rich in nitrates, or if dewatering is impossible, or even to produce biomass for diets without salt to be consumed fresh. It should also be noted that spirulina washed with fresh water is very bland in taste.

Biomass from a culture in good condition does not need to be neutralized or washed, only wrung out. It should however be noted that it may exceptionally be necessary to at least partially rinse the biomass to reduce its nitrate content (the nitrates may come either from the nitrate introduced as an input or from the oxidation of the excess ammonium, or from the fixation nitrogen); normally this is not necessary even if the nitrate ion content of the medium is 1200 ppm as in the new Zarrouk medium. Rinsing can also be useful if cyanotoxins are present in the culture. The rinse liquid should be at the same pH as the culture to be filtered.

Draining can be done with a wringer or on a vacuum filter (water pump or vacuum pump), but more simply by pressure as follows: the drained biomass is placed in a cloth of the same type as that used for the filtration, lined on the outside with a strong cotton canvas – the two canvases being folded over the biomass – and it is pressed between two mats or grooved boards: the major part of the free water is expressed by the pressure (0, 2 kg/cm² is enough but you can go up to 1 kg/cm²). The press may be just a pile of weights, but a top screw press is convenient and cleaner, especially if it's made of stainless steel, like the one shown in the following photo:

Photo: Biomass pressing using a fruit juice press, Mialet, 1998:

:

You can also use a car jack to exert pressure, or a weight-and-lever cheese press. By increasing the pressure slowly and stopping it in time (before or as soon as

the juice begins to be a little green), the loss of spirulina through the canvas is reduced to almost nothing; in the case of biomass of good quality and rich in spiral spirulina, we obtain very good results (pressed biomass with a very firm consistency) without taking many precautions, but all the biomass would still end up passing through the canvas if the pressure was excessively increased; in the case of biomass that is more "fragile" or too rich in straight lines, the juice flows green more easily, the pressed biomass is soft and sticky and if you press too much the spirulina risks being reduced to a mush too soft to be able to be then extruded. Even a biomass of excellent quality can give a soft pressed biomass if the pressure has been too strong or brutal: if a car jack is used this danger is real and it is recommended to be very careful (a dynamometric indicator would be useful). It is good to observe the flow rate of the pressing juice for guidance.

Spinning/pressing must be done without delay and it is above all necessary to avoid that the biomass suffers from the heat while waiting.

Do not press too much biomass at the same time, even if it is not "fragile": do not load more than 8 cm of biomass per layer (but it is possible to superimpose several layers separated by a spacer allowing free flow of liquid). Under the lower layer, put several spacers to facilitate the flow of juice. The pressing lasts at least 15 minutes, because it takes time for the liquid to travel through the very fine interstices or capillaries between the compressed spirulina.

The pressing juice is preferably not recycled to the basin, especially if it is cloudy (but if a purification system is available, it can be recycled to purification). Pressing green lumps always results in a “milky” juice. During pressing, some of the exopolysaccharides lining the outer face of the spirulina come off and end up in the pressing juice, even if the latter is neither cloudy nor colored (this can easily be seen by carrying out the standard filtration test on the medium of culture and on the pressing juice, the latter generally giving a less good result).

A spirulina poor in straight lines (less than 50%), coming from a young culture, and properly wrung out, has a very firm, non-sticky consistency and gives a clean slice with a knife, and its pH is 7 to 9 (depending on the degree of pressing; in fact 9 seems preferable for drying and conservation, so do not press absolutely thoroughly). The dewatered spiral spirulina usually contains around 20% dry matter (more for the wavy ones and even more for the straight ones) if it comes from a culture with normal salinity (10-13 g/l) and if there is no no washing or if the washing was done with water at the same salinity (or rather at the same “ionic strength”) as the culture medium. A Zarrouk-type medium or washing water with a salinity of 20 g/l gives a % dryness increased by 5 points, salt water at 30 g/l gives a % dryness increased by 10 points (and a saltier taste), on the other hand, washing with fresh water will often give a % of dryness reduced by 5 points (and with a “bland” taste). Culture media based on ash or potassium bicarbonate give, at equal salinity (but lower ionic strength since the atomic weight of potassium is greater than that of sodium), a lower dryness content. Corrugated spirulina give pressed biomass that is richer in dry matter (about 2.5 points above Lonar-type spirals); however, their % of dryness peaks at 33% from a salinity of 44 g/l (in NaCl). Clearly distinguish six independent factors governing the % dryness of the pressed product: the strain, the shape of the filaments within the same strain, the salinity of the culture medium, the quantity of biomass drained at once, the pressure applied (or vacuum, or centrifugal force) and pressing time.

If the biomass is "fragile" or very rich in straight lines, do not press more than 2 cm of initial thickness and only apply moderate and progressive pressure (or vacuum or centrifugal force) and let it act longer (for example 30 minutes): this is generally effective and makes it possible to obtain an extrudable biomass.

The advantage of dewatering by vacuum or by wringer is to allow the treatment of low-concentration drained biomass, for example at 7% dry matter, almost liquid, whereas pressing is difficult or even impossible to implement in this case there.

In some cases, especially when there are 90 to 100% straight lines, the biomass cannot be wrung into an extrudable biomass (the spaghetti, even if they can be formed, "melt" on drying), but it can be washed, then spread with a spatula in a thin layer (1 mm) on a polyethylene film stretched horizontally for rapid drying in the sun or on an oven tray with side ventilation. This method must be used with caution, especially if the washing is done with fresh water: the drying must be very rapid because the biomass washed with fresh water ferments quickly and it is recommended to carry out more frequent bacteriological analyzes on the dried product thus obtained. The success of this type of drying depends strongly on the thickness of the spread layer of biomass: if the distribution is not well done, the thickest parts will dry poorly, take on a bad smell, and cannot be used for drying. human food. This drying method, which I call the "Indian method" (because it was widely practiced in the state of Tamil Nadu in South India), can also be applied to biomass which has been properly pressed but which remain too soft to be extruded (in this case, no washing, but possibly a small re-dilution to facilitate spreading in a thin layer). This drying method gives scales or flakes of spirulina with a very attractive appearance, preferred by some consumers, but whose apparent density is very low. It has the disadvantage that the drying rack (plastic film) is quite difficult to clean when it is no longer new, whereas the mosquito nets or grilles do not need cleaning or can be washed instantly with a water jet. . Finally, the flakes obtained by this method have an annoying tendency to become electrically charged as well as the plastic film, which then attract each other mutually.

It should be noted that a “soft” pressed biomass, practically impossible to extrude, generally remains good to consume fresh.

Biomass that is not wrung out and not washed with water, or not enough, turns brown quickly in the sun.

The dewatered or pressed biomass must be cooled as soon as possible so that it does not spoil. Even if it must be dried, it is in your best interest to put it in the fridge while waiting for the extrusion, otherwise unpleasant odors can be released during the extrusion. If it must be eaten fresh, it is in your interest to cool it to almost 0°C as quickly as possible if you want to keep it long enough (up to 15 days for example, which is possible at least in winter in France) .

In 2009 it was found that biomass kept at 2 – 3°C for one or two days squeezes more easily, and that pressed biomass stored at this temperature for the same time extrudes better.

8.3) Washing tools (see also hygiene)

It is in our interest to rinse as soon as possible, or at least to soak, the tools, fabrics,

containers, instruments that have been in contact with spirulina; otherwise, if the spirulina dries before cleaning, it becomes very difficult to clean and excessive washing water consumption may result. Filtration and pressing cloths must be washed and dried after use to maintain their effectiveness and prevent them from picking up odors; Caution: for them to last longer, do not expose them to the sun for too long.

Washing the filter and press cloths in the washing machine with detergent is practical and recommended at least occasionally.

9) DRYING

Drying is the only safe way to long-term store and distribute spirulina without a cold chain.

In industry, spirulina is conventionally dried by "atomization" (spray-drying), in a stream of combustion gases at high temperature but for a very short time. For this, the filaments must first be reduced to a paste to break their membrane: it is in fact the crushed spirulina juice that is dried. Unless the drying gas is very low in oxygen, spray-drying runs a high risk of altering the product, at least from the odor and taste point of view.

In artisanal production, on the contrary, it is the whole filaments of spirulina that are dried: the drying time is longer, but the interior of the cells is not subjected to direct contact with hot gases.

If the pressed spirulina cannot be dried immediately, it must be kept in a closed container in the very cold refrigerator and not for too long (otherwise it gives off an unpleasant odor during extrusion); be careful not to freeze it, and to avoid the fallout of drops of condensed water on the biomass being stored. In a cold room at 1°C the biomass can be kept for up to a week. The washed biomass cannot be stored, even in the refrigerator (unless it has been washed with isotonic salt water and at pH > 9.6).

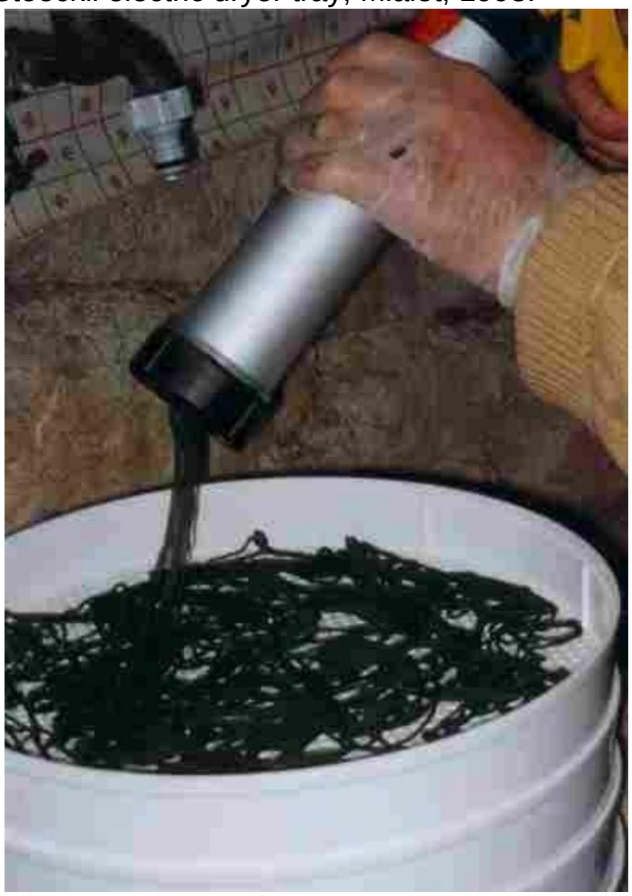
9.1) Extrusion

Drying should be fast enough for the product to dry without fermenting. The biomass resulting from the pressing is first distributed by extrusion in "spaghetti" on a plate formed of a frame lined with a nylon or better stainless steel mosquito net (mesh 1 mm) or on a plastic grid (mesh of the order of 5 mm). If the biomass is too fluid, it is spread in a thin layer on a polyethylene film (“Indian” method). Then the biomass is dried in the sun, or, much better, in an air current with low relative humidity and high water absorption capacity (indirect solar, or electric, or gas dryer, or dehumidifier), until so that it is no longer soft at all, easily detaches from the support, and grinds easily.

Spaghetti extrusion can be done using a cake decorator, or with a common kitchen tool in India (“idiyapam maker” in Tamil Nadu) and in the Far East. Orient (made of a box with a bottom pierced with small holes and a piston, or using a gun

professional silicone glue such as Sika, modified (50 mm PVC cap pierced with 2 mm holes), or with a sausage stuffer, etc. Choose a model that does not include any aluminum part in contact with the biomass. For already large productions, it would be advantageous to use a stainless steel “push” (device used by butchers), operated by a crank with gear, equipped with a stainless steel or plexiglass die. By placing the spaghetti on the support (drying tray) avoid forming large heaps of biomass, which would not dry quickly enough. If the biomass is very firm, it can be extruded with a die with larger holes (which is less painful) than if it lacks firmness. If you want to obtain very straight spaghetti, the thickness of the plug in which the holes are drilled must be three times the diameter of the holes (use a drill press). In the case of use of the 300 ml SIKA gun, we have noticed that it is necessary to plug the slot in the piston with a little self-adhesive plastic to avoid an excessive bypass of biomass behind the piston.

Photo: Extrusion of pressed biomass, with Sika silicone gun (professional, manual “pocket” type), on a Stoeckli electric dryer tray, Mialet, 1998:



9.2) Drying

You can dry in the shade simply in a stream of air at room temperature, under a mosquito net (it is enough that the air is at a temperature clearly above its dew point); it's the same principle as drying laundry on the line or dishes on the dresser: we were able to dry very well in a laboratory hood equipped with a powerful fan and a 0.2 µ filter (stopping bacteria), without heating. But the final water content of the product appearing dry can be disappointing; it essentially depends on the temperature and humidity of the air, the ventilation rate and the drying time.

Generally, the dehydration must be completed in a hot air dryer or with a dehumidifier.

We easily dried the spirulina in a metal cabinet equipped with a dehumidifier and a fan that recirculated the air through the drying trays. The dehumidifier must be able to lower the relative humidity of the air to 30%. This device, which can be called a "thermodynamic dryer", makes it possible to completely eliminate the humidity of the ambient air and dust. It even allows, if you want, to replace the air with a neutral or oxygen-depleted gas to reduce the oxidation of the spirulina during drying (preservation of beta-carotene). The only problem is that the cabinet must be cooled to avoid exceeding 42°C inside. In a hot and humid climate, this may require the use of an air conditioner, unless it is dry overnight. We obtain 40-50 g of dry spirulina per hour with a power of 350 Watt (excluding air conditioner).

Drying in full sun outdoors is the fastest and least expensive, but it has drawbacks: the product is exposed to dust and animals (it must at least be protected by a mosquito net), and it risks turning blue in surface by destruction of chlorophyll by ultraviolet rays; after grinding this bluing is no longer perceptible, but an alteration in taste remains noticeable. This type of drying, which has been practiced for a long time in Madurai (India), is successful if the conditions are good for very rapid drying: if it is rapid enough or if the sunlight is sufficiently low in ultraviolet rays, the alteration of the color and taste may be imperceptible.

Plans for more elaborate solar dryers are given in Appendix 27. An improved solar dryer includes an air heating part separated from the area where the spirulina is located, sheltered from light, rain and insects; the air must circulate there with a good flow, preferably caused by a fan. The thermosiphon (chimney effect) is only suitable if there is little "spaghetti" on the trays. The influence of ventilation on drying is essential. With a strong air flow, up to 3 cm of "spaghetti" can be stacked on the trays. If an axial motor fan is used to blow air through nearby plates, take into account that the flow rate is often very low near the center of the fan: it is advisable to interpose an empty intermediate plate serving as a distributor of airflow.

If the air is heated to lower its degree of relative humidity, the temperature must be limited to 80°C. In fact, drying is often done at a lower temperature (generally 65°C or even 40°C) with good results, but if there are concerns about the bacteriological quality of the product, it is possible to briefly increase to 80°C to "pasteurize" it without, it seems, its content of sensitive constituents such as phycocyanin, gamma-linolenic acid or beta-carotene decreasing too much. Phycocyanin is particularly heat-sensitive: to preserve it, it should not exceed 42°C.

The drying time varies according to the thickness of fresh biomass on each plate, but also according to the number of superimposed plates, the % of dryness in the biomass, the strain (the spirals dry a little faster), the temperature and the air humidity and, of course, airflow: in practice this is usually around 4 hours, but it is perfectly possible to dry in an hour if desired. In case of bad weather, if a solar dryer is used, an electric or gas heater can be added, or the drying can be finished (or done entirely) in an electric or gas dryer or even in an oven. at low temperature and airy.

An electric dryer for fruit and vegetables, such as the Swiss brand Stoeckli, with a power of 450 Watt, with trays 30 cm in diameter, has an average drying capacity of 20 g (counted in dry matter) per hour. Its aeration flow tends to be a little low and that's a shame.

If you don't have electricity, you can use a gas-heated dryer (butane or methane from the digester) – an outline of which is shown in Appendix 27. It is very important to provide the burner with a safety device.

The temperature of the biomass being dried in a dryer with superposed trays without air recycling (case of Stöckli) remains theoretically close to the dew point temperature of the air, whatever the dry temperature of the latter, as long as there is free water on the surface of the biomass; practically the surface temperature is established halfway between this dew temperature and the air temperature, at the start of drying, then rises gradually to reach the dry temperature of the air at the end of drying. The temperature at the core of the product rises gradually from its initial temperature to the temperature of the air. It is desirable to minimize the time when the wet product is in the vicinity of 37° C., the temperature most favorable to fermentation. It is also necessary to avoid heating the still moist product (at the heart of the spaghetti) to more than 60°C, which could “cook” by decomposing (changes in color). The first plate receives the air at its maximum temperature but with a minimum dew temperature (usually around 20°C), while the upper plates receive air that is still hot but laden with humidity, therefore at a high dew temperature and d especially as the air flow is low. We can see the advantage of limiting the number of superimposed trays and not hindering the air flow (keep the filters or mosquito nets protecting the air inlet and outlet of the device clean and unobstructed). In practice, with Stoeckli dryers, which have a low air flow, we absolutely limit the number of trays to 5 and their individual load to 2 kg of fresh biomass per m² of tray (i.e. 150 g/tray). If too much fresh biomass is loaded in relation to the airflow, or if the fan does not work well, or if the biomass is too soft, or if the weather is too humid, or if the thermostat is set too low , the drying is not done quickly enough, the spirulina begins to deteriorate before it is dry, it releases an abnormal smell (“propionic” or “butyric”) and sometimes the “spaghetti” flatten (“melt”) , remain like soft plastic and do not come off the tray: in these cases, it is better to reserve the product for animal feed or simply throw it in the compost. A badly dried spirulina is generally too soft to be crushed, which is an indication, but be careful: it happens that we do not notice that a deterioration has taken place because the product can still seem dry and well. green on the surface while it is soft and blackish on the inside, or it may have changed color and ended up drying all the same; it is therefore important to check the quality of the dry product according to its smell and taste and to prick it with the tip of a knife to check if it is hard and green at the core. The release of odor at the beginning of drying does not necessarily mean that the dry product is of poor quality or bad taste.

A good quality biomass, pressed very firm, dries without the cylinders of the spaghetti becoming deformed: they remain cylindrical, but obviously of reduced diameter (shrink). If they deform, it is because the biomass tends to “melt”. If it is too soft it "melts" downright and spreads.

Often we prefer to dry spirulina in two stages, especially when the air is humid: drying at low temperature (40-50°C) but at high air flow (air speed of 1 m/s)

allowing a high load (20 kg of fresh biomass per m², in 5 trays), duration 2.5 to 3 hours for a final water content of 15 – 20%, and a relative humidity of the outgoing air of 10 to 20 %, followed by drying at low air flow (for example in a Stoeckli dryer) but at higher temperature (65-80°C), ensuring both a certain pasteurization and the extraction of water up to to 4% water in one hour (or even much less).

The maximum load which has been indicated above is for a pressed biomass of good quality (firm); if it is soft, the load must be reduced to 10 or even 5 kg/m².

The dryer of the first stage was made around a 50 W fan with a diameter of 30 cm corresponding to that of the Stoeckli trays which are placed on it. This fan draws air through a dust filter (synthetic wadding sold to line kitchen hoods) which is heated by an electric fan heater (power 1 to 2 kW).

The first stage is sufficient when the ambient air is very dry (drying to less than 9% water content in 4 hours). The 2nd can be done in a rotary drum dryer, the pre-dried spaghetti being enclosed in a canvas bag: it is a simple system that works to the satisfaction of several French producers.

Another method of drying, giving flakes of a beautiful appearance, is that where extrusion is replaced by spreading in a thin layer with a spatula on a plastic film (long practiced in Madurai in India). This is hardly practical, but it is the only way to dry a biomass rich in straight lines that are not suitable for extrusion.

End of drying test: see following § 9.3 test. ___

To establish the curve of the % of water in the product as a function of the drying time, it suffices to measure the gross weight of the trays being dried, the tare of the trays, the weight of biomass to be dried, the dry net weight and to know the % of water at the beginning or at the end of drying.

If the drying has been insufficient, it is possible to complete it either by passing it through the dryer again at 65-80°C (putting the product on plates if it has already been ground), or preferably by enclosing it in an airtight container in the company of a desiccant sachet (silica gel or molecular sieves). These desiccants can be regenerated by passing through the oven.

We must insist on the advantage of filtering fresh air before it enters the dryer to eliminate dust and greatly reduce the risk of anomalies in the bacterial load of the dry product. A 5 µ aperture filter already does a good job, but requires a high pressure fan.

This risk of airborne contamination is eliminated if a thermodynamic dryer (with dehumidification by heat pump) is used. These drying devices have a bright future in the drying of spirulina in areas with high humidity (such a dryer has already been used since 1999 in Adzopé in Côte d'Ivoire at SAP La Mé). They also have the advantage of allowing drying in an inert atmosphere.

Note also the possibility of vacuum drying. This process certainly has a future on condition that the heating temperature is limited to 40°C so as not to destroy vitamins, enzymes and phycocyanin. The absence of oxygen avoids the degradation of the components

oxidizable like carotenes. It is believed that the biomass disintegrates under the effect of the water vapor bubbles, which makes it possible to process blocks of biomass, even frozen ones.

9.3) Grinding and drying test

Properly dried spirulina is crunchy, detaches itself from the drying medium and can be easily pounded or ground in a coffee grinder into a more or less fine powder according to individual taste. A well-suited manual shredder is the Sfinx or Corona brand, which is widely used in many African and Latin American countries. An electric coffee grinder works well too.

The apparent density of extruded, dried and ground spirulina is 0.5 to 0.66 kg/litre depending on the fineness (the density of dry spirulina itself is close to 1). Some prefer not to grind the dry spirulina to keep its "texture" in sticks which is more reminiscent of "algae", but then its apparent density is much lower and there is more risk of piercing the packaging.

Spirulina must contain less than 9% water to keep well. Measuring the water content is very easy with the following device (see Appendix 6.2.6) : put the product to be tested (about 200 g) in a “Tupperware” type container of about one litre, with a transparent lid to allow the reading of the hygrometer placed (taped) inside. A sufficiently dry product must give a % relative humidity at equilibrium below 45 (around 25°C). For the measurement to be accurate, the measurement assembly must be in equilibrium not only with respect to humidity but also temperature, which may require a fairly long time (1 to 2 hours). The official method is steaming at 104°C in a ventilated thermostatic oven, until constant weight.

To make spirulina tablets without additives, its humidity must be adjusted to around 7%.

9.4) Packaging

Dry spirulina can be kept for a long time without losing too much of its qualities provided it is stored in well-filled and sealed bags, away from light, air and strong heat. A storage time of more than 2 months causes natural sterilization.

Multilayer, heat-sealable aluminized plastic bags are very suitable, but it is preferable to create a vacuum in the bag while heat-sealing it (commercial devices exist for this): in this case the product can be kept for 5 years. If it cannot be vacuum sealed, the absorption of oxygen remaining in the properly sealed bag will often (but not always) cause it to 'vacuum' spontaneously within a few days if the container is properly sealed; this absorption of oxygen is accompanied by the destruction of at least some of the oxidizable components of spirulina, such as beta carotene. It sometimes happens that the sachet swells instead of being put under vacuum: a plausible explanation would be the release of CO2 by acidification of the remains of bicarbonate (acidification by migration of the interior of the cells which is very acid).

Even better than vacuum is storage under an inert atmosphere (nitrogen).

If the product must be used quickly (less than 3 months), packaging in non-metallic plastic bags is possible.

Warning: rodents willingly pierce these spirulina sachets. They must be kept in a safe place, for example in a metal canteen.

9.5) Bacteriological quality control

Drying at low temperature (40 to 50°C) has the advantage of better preserving the nutritional quality of the product and already gives a generally correct product from a bacteriological point of view, especially after storage for two months. No dangerous microorganism can survive for long in a product with less than 9% water corresponding to a water activity of less than 0.5 (< 50% relative humidity in the air at equilibrium with the product at 25°C). Spirulina does not normally contain spores because of the pH of the culture medium. Microbiological quality improves on storage. Storage in vacuum-sealed bags allows a posteriori verification of the quality of drying: if a vacuum forms, the product was correct; if the bag seems swollen (this may take a few months), it means that there is fermentation or enzymatic evolution or acidification of traces of residual bicarbonate, or simply that the powder has become denser (packed).

If in doubt about the bacteriological quality or the degree of drying of the dried spirulina, it is possible to heat it to 120°C in an oven or a solar sterilizer. But dry heat does not sterilize well or destroy bacteria spores or any toxins that may be present. This is why it is still necessary to work while respecting at least the classic rules of hygiene (do not touch the product with your hands, work away from the ground, with instruments and containers made of stainless steel or plastic, etc.), and it It is a good idea to have the conformity of the product checked from time to time in relation to the bacteriological standards in force.

10) CONSUMPTION

10.1) Human food

Spirulina does not replace high-calorie foods such as cassava, rice, wheat, potato or corn, but it is an ideal ingredient of the protein sauce that accompanies the African "ball", for example, providing not only its proteins, but many other elements very favorable to the good health of all and in particular of the small children.

A thousand mixtures and recipes can be invented to consume spirulina raw or cooked, fresh or dry, in a pleasant way. It is no exaggeration to claim to be able to make very good gastronomy based on quality spirulina, especially fresh.

Spaghetti-dried spirulina is generally preferred by consumers to the industrial spray-dried product, both for its physical consistency and its smell. The presentation in the form of unground or slightly ground spaghetti (only broken) is generally very popular, but costs more in packaging.

If you are on anti-coagulants, there are precautions to take (see Appendix 5 page 172)

10.1.1) Fresh biomass

Good quality fresh biomass can be consumed directly after pressing or it can be canned (frozen, salted, sweetened). Fresh, it can be kept in the state for two to a few days in the refrigerator, depending on the speed at which it was cooled and according to its storage temperature in the refrigerator, and according to the season, but it can only be kept if it is not was not washed after filtration. Before consuming a spirulina stored in the refrigerator, check that there is no odor. Although the best time to harvest is in the morning, it is possible to delay the harvest a little until a more convenient time for cooking or eating if you do not have a refrigerator. During storage in a refrigerator, in an unclosed container, it happens that, due to surface evaporation, residual salts migrate to the surface of the product, giving it a bitter taste: in this case, remove the "crust". The best mode of storage in the refrigerator is in the form of sausages (without contact with air) which avoids any risk of superficial evaporation and fallout of drops of condensed water on the biomass. In a temperate climate, fresh spirulina harvested in winter can be kept for a long time in the refrigerator at 3°C: 10 to 15 days for example.

In the freezing option, take care not to freeze too large unit masses that it would be impossible to divide during use: it is better to make very practical "ice cubes" (you can use the classic ice cube trays) or even better "tablets" (like chocolate). To make these tablets, you can use the method developed by Marc Pilard in Quissac: put the fresh spirulina in a polyethylene freezer bag and spread it with a rolling pin in a thin uniform layer 2 to 3 mm thick. then "crossed out" into a grid of squares or rectangles of the desired size. The very rapid freezing thus made possible means that during thawing the phycocyanin does not come out (the spirulina cells are not pierced by the ice crystals). For ease of storage and use, the squares can be separated (simply by breaking along the stripes) and then stored (preferably vacuum packed):

The biomass of certain strains (spirals in general) does not require any precaution: no appearance of blue juice during defrosting.

11) HYGIENE

The industrial production of a food product that meets the standards requires compliance with draconian hygiene rules in terms of equipment, personnel and packaging:

- food-grade plastic, glass or stainless steel equipment - wearing gloves, masks, hairnets - air filtration - sterilization of tools, product and packaging.

Such precautions will seem beyond the reach of family or small-scale farms, but they must at least strive to work as cleanly as possible. The level of hygiene to be respected is similar to that which is usual in the kitchen and family or community dishes in the region. Here are some common sense recommendations: - Wash your hands before working with spirulina - Check that there is no spirulina left in the recesses of the equipment after cleaning (for example on the edges of the filtration frames, or in the extruder).

- Use preferably white utensils.
- Avoid contact of dry spirulina remains with fresh biomass.
- Avoid contact of utensils with the ground or cement which are breeding grounds for microbes.
- Never touch spirulina with bare fingers, even dry, to avoid the risk of contaminating it with staphylococci aureus.
- Keep rodents away (there are ultrasonic devices for this) and flies.
- Cover containers containing biomass to prevent it from getting dirty.

An artisanal spirulina can be of very good quality. But if it is produced and above all dried and handled in an environment rich in "domestic" microbes, it can only be consumed by people accustomed to this environment: there is no question of marketing it in town or on the international market, except to sterilize it. and/or to analyze it to check that it complies with the standards in force. The Chinese often sterilize their spirulina by irradiation, but this method is not recommended because it destroys vitamins and produces free radicals; anyway, it is not within the reach of craftsmen.

Please note: in some countries, water used for cleaning, rinsing, etc. may be contaminated, this can be a source of contamination for the harvested product. In this case it is suggested the systematic use of bleached water for all cleaning, with final rinsing with chlorinated water (min 1 ppm free chlorine).

12) FINAL RECOMMENDATIONS

After his long apprenticeship in very varied spirulina cultivation conditions, the author would like to underline those which he considers the easiest for the craftsman, and which boil down to not trying to perform feats of productivity or of cost price.

Practically this means:

- protect your ponds with a shaded greenhouse, - use the air as the main carbon source, - resist the temptation to harvest the floating layer,

- use trace elements and chelated iron,
- if you have to be away, only leave your crops in safe hands.

In addition, especially for beginners who have not yet acquired the fingertips:

- maintain your spirulina concentration high enough (Secchi between 2 and 3 max) - brush the bottom and sides of your pool daily - practice a fairly high purge rate (> 1%/ day) - lightly load your dryer (< 5 kg of fresh biomass/m² of section)

Don't forget to check the calibration of your thermometers and other instruments!

SUMMARY OF APPENDICES

A1) Influence of temperature, light, alkalinity, salinity and pH on spirulina photosynthesis

We can assume that the maximum rate of photosynthesis, in a well-agitated pond, and under the best conditions of temperature, light, alkalinity, salinity and pH, is close to 1.8 g/hour/m² of pond.

This speed can also vary depending on the strain of spirulina and the possible presence of catalysts.

In the simulation programs given in CALCUL, it is assumed that the photosynthesis function is directly proportional to functions of temperature, light, salinity, pH and degree of agitation:

Photosynthesis rate = kxf(T) xf(klux) xf(salinity) xf(pH) xf(agitation)

This hypothesis has no real scientific basis, but it facilitates the calculations and it gives results that are often close to the reality on the ground.
Here are some examples of these functions, which are largely inspired by Zarrouk's thesis (taking also into account experimental results).
[Note that Zarrouk's results date from the 1960s. Thirty years later Vonshak (Vonshak1997) was able to use much more sophisticated methods to study the photosynthesis of spirulina and deduce that spirulina was often "photoinhibited", more or less less depending on temperature, salinity, concentration, agitation and strain.
We remain here with the Zarrouk model, imperfect but simpler]:

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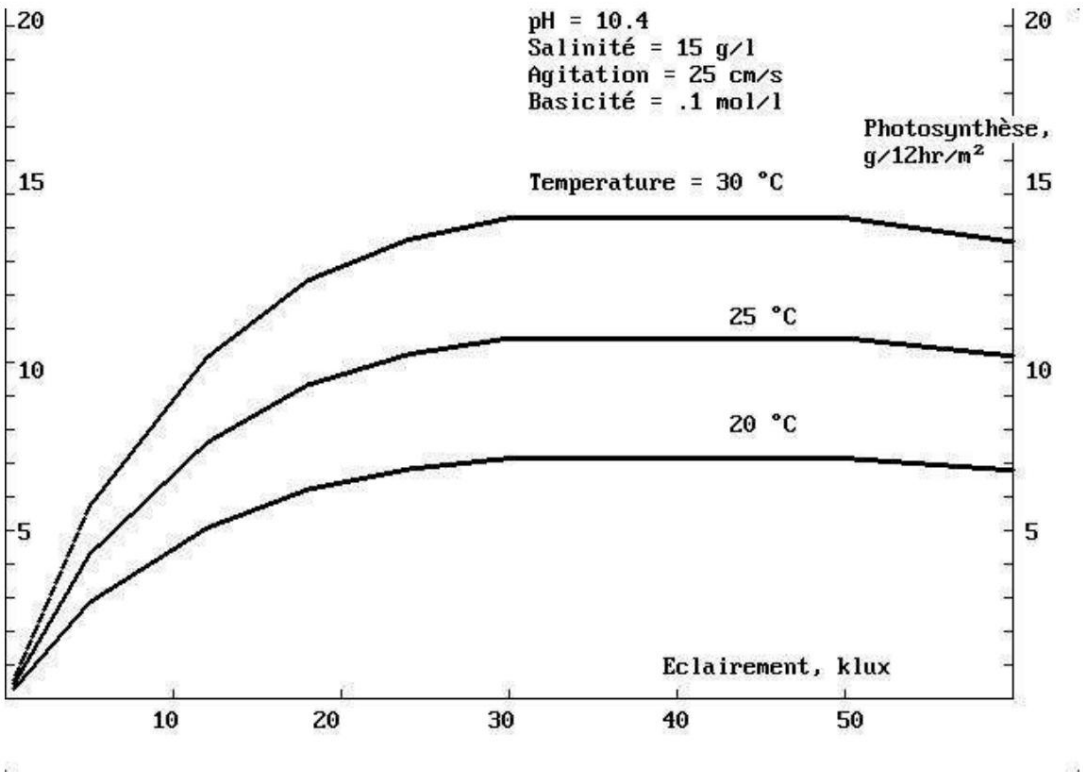
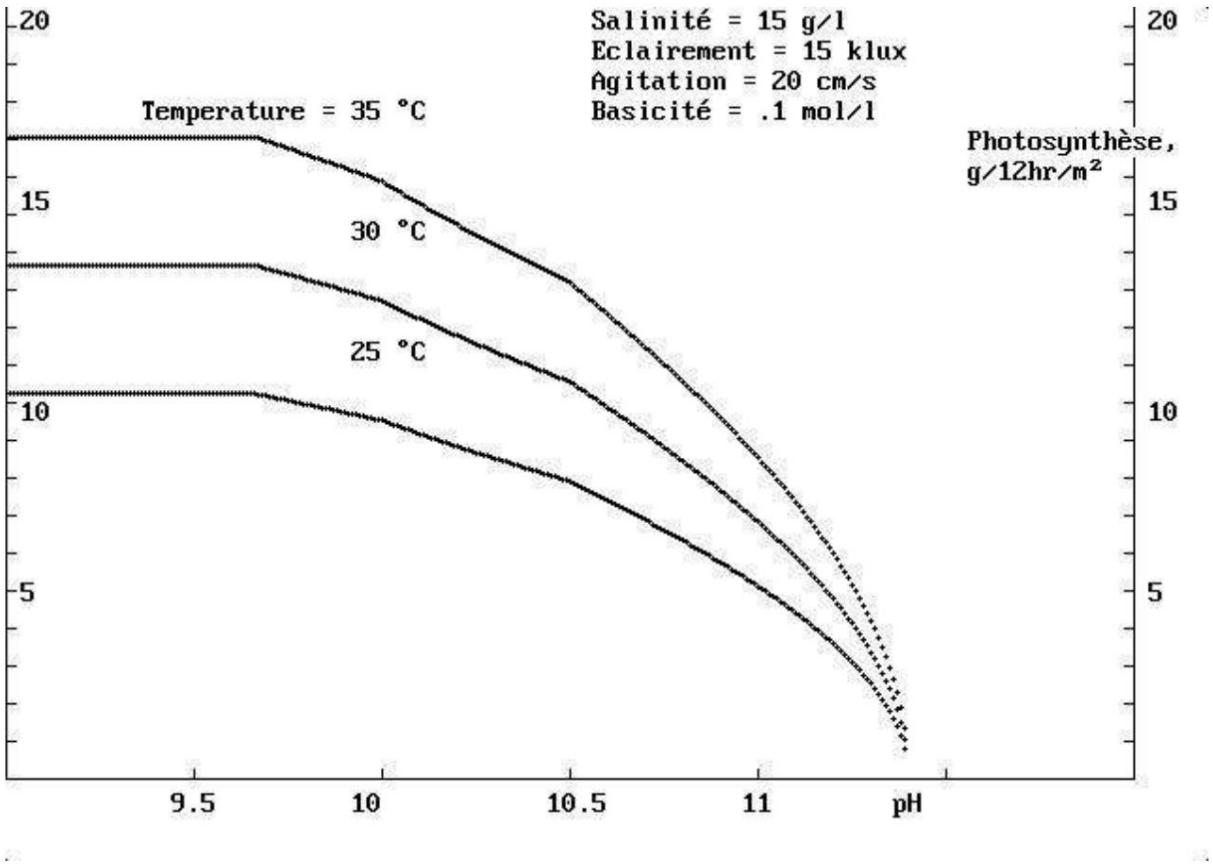
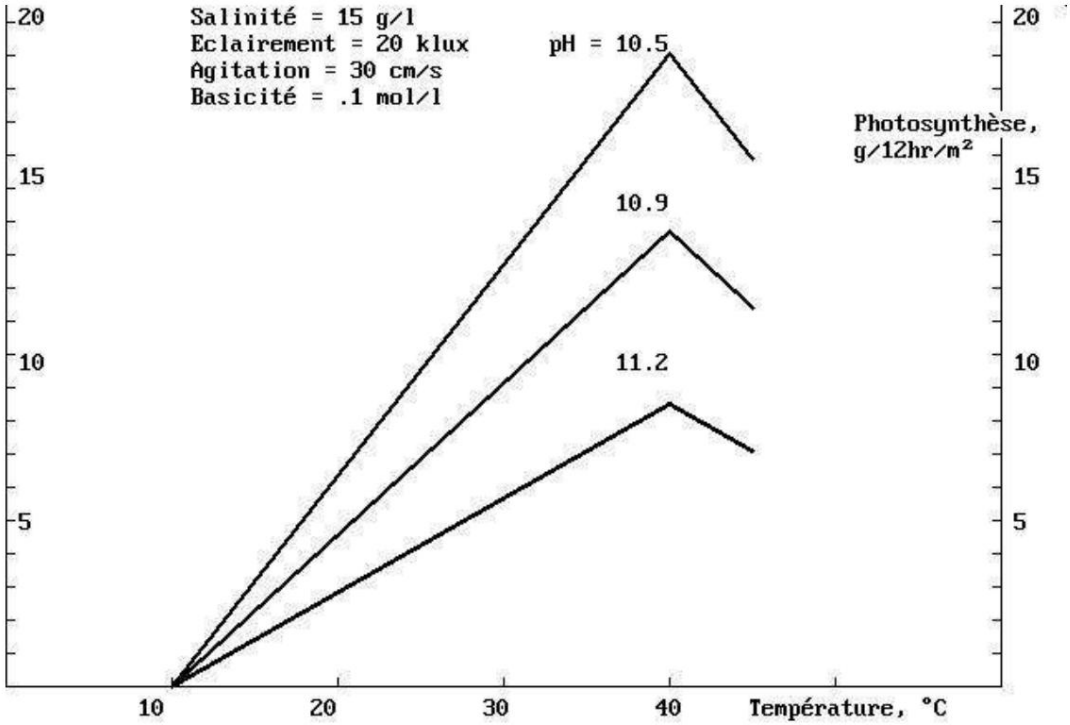


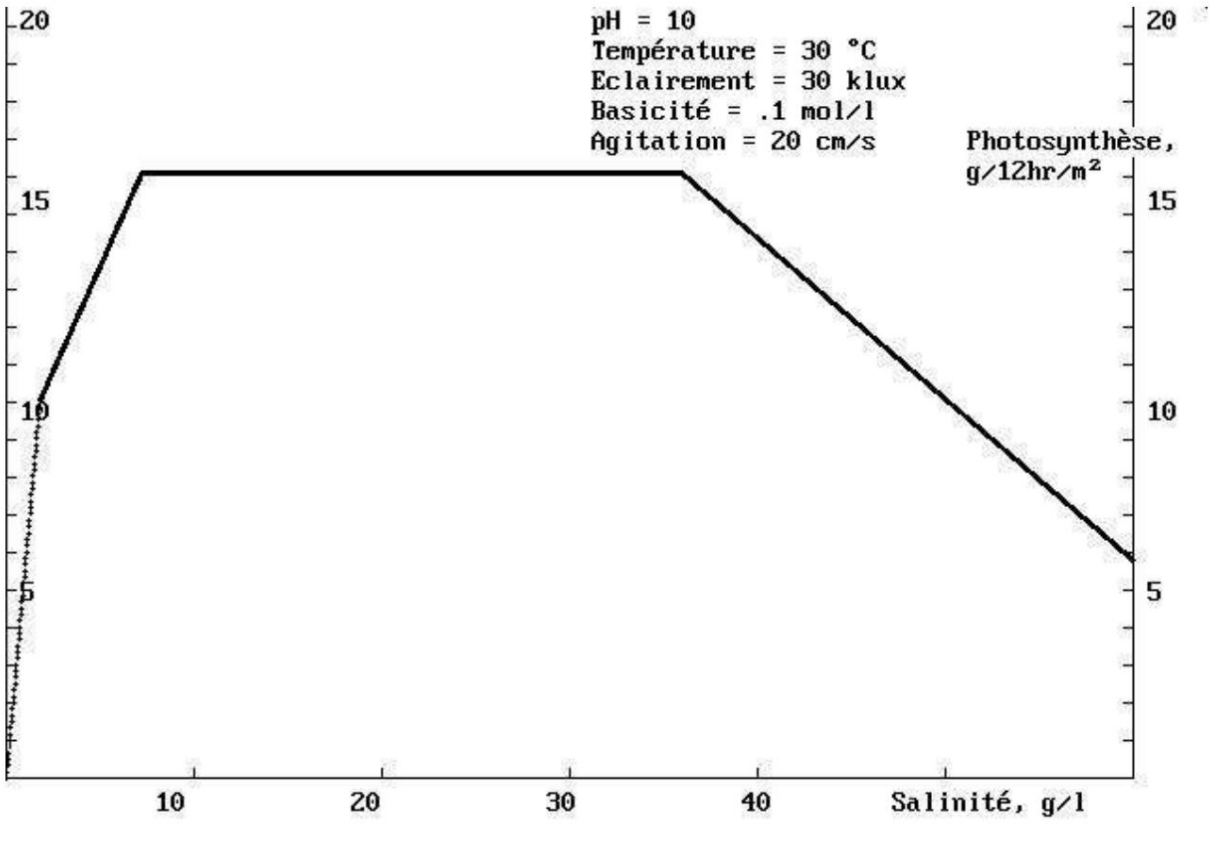
Photosynthesis rate of spirulina as a function of illumination according to Zarrouk's thesis, Fig. 3 (BIBLIOGRAPHY Zarrouk)



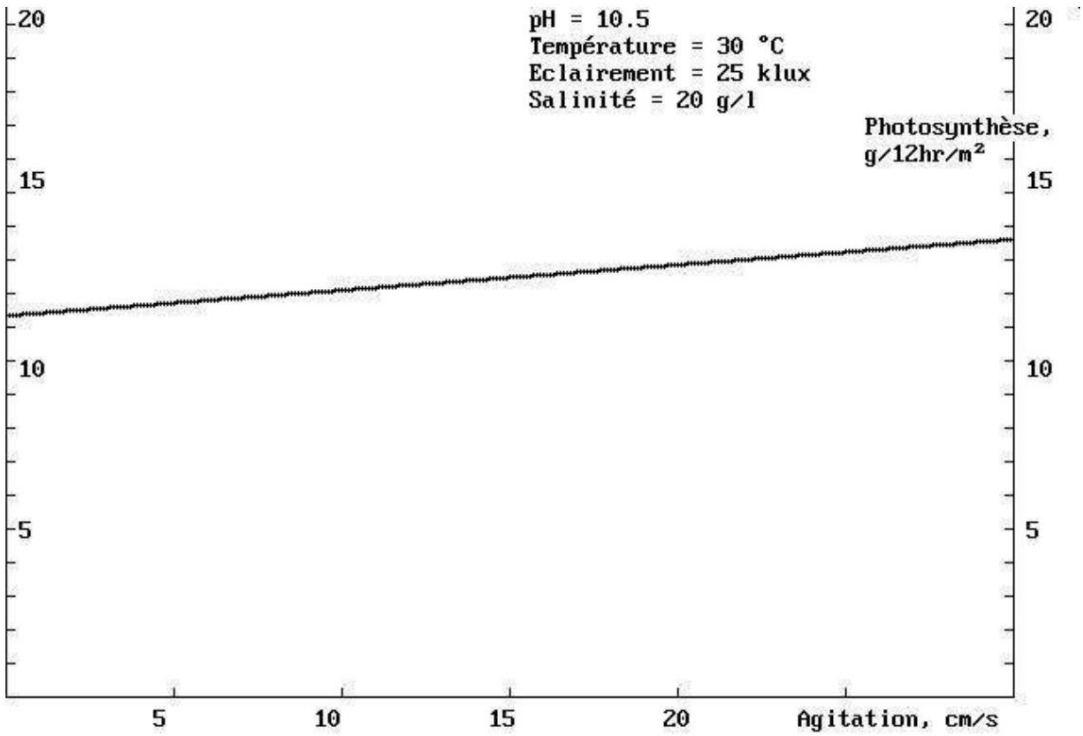
Photosynthesis rate of spirulina as a function of pH according to Zarrouk's thesis, Fig. 20



Speed of photosynthesis of spirulina as a function of the temperature of the culture according to Zarrouk's thesis, Fig 19.



Speed of photosynthesis of spirulina according to the salinity of the medium according to Zarrouk's thesis, Table IV



Speed of photosynthesis as a function of agitation (more or less imaginary function, in which the pH also intervenes, valid for usual agitation systems up to a speed of 30 cm/s; it is possible that with improved systems we can significantly increase the speed of photosynthesis, but we do not yet have the experience: beyond 30 cm/s the function is provided to take this effect into account, but without any quantified experimental basis)

A2) Measurement of the spirulina concentration

The "Secchi disc" (instrument consisting of a rod 30 cm long, graduated in centimeters, carrying a white disc at its lower end) allows an approximate measurement, quite subjective, which depends on the subject, the lighting, the angle, the size of the disc and the turbidity of the culture medium ("turbidity" = disorder + coloration) and to a large extent the morphology of the spirulina filaments, which depends in part on the salinity of the medium. The data below have been established for salinities close to 12 g/litre.

Before measuring, shake to homogenize, then let the sludge settle for a few minutes, but do not allow a floating layer to form! We note the depth, in centimeters, where it just becomes impossible to distinguish the disc.

Everyone should determine their own depth-concentration-turbidity correlation, under standard conditions: filter a known volume through filter paper (previously oven-dried and weighed), press gently and oven-dry, then weigh. The two tables below were established by the author with a white disk 3 cm in diameter under a lighting of 4000 lux (shadow not too dark).

Turbidity is measured on filtrate without spirulina, with a black disk (see A6.1.2 Turbidity).

SECCHI FOR SPIRAL STRAIN

Zero turbidity (>30 cm)

1.0 cm = 1.05 g/l 1.5 cm = 0.75 2.0 cm = 0.55 2.5 cm = 0.43 3.0 cm = 0.34 4.0cm=0.24 5.0cm=0.19 8.0cm=0.10

Turbidity 12 cm

2.0 cm = 0.5 g/l 3.0 cm = 0.3 4.0 cm = 0.21 5.0 cm = 0.16

Turbidity = 6 cm

1.0 cm = 0.75 g/l 2.0 cm = 0.35 3.0 cm = 0.19 4.0 cm = 0.10 5.0 cm = 0.05

SECCHI FOR WAVY STRAIN

Zero turbidity (>30 cm)

1.0 cm = 1.0 g/l 1.5 cm = 0.55 2.0 cm = 0.40 3.0 cm = 0.24 4.0 cm = 0.16 5.0cm=0.11 8.0cm=0.06

Turbidity = 6 cm

1.0 cm = 0.85 g/l 1.5 cm = 0.50 2.0 cm = 0.35 3.0 cm = 0.20 4.0 cm = 0.10 5.0 cm = 0.05

Turbidity = 4 cm

1.0 cm = 0.70 g/l 1.5 cm = 0.36 2.0 cm = 0.20 2.5 cm = 0.11 3.0 cm = 0.06

~~NB 1~~) Jacques Falquet, of Antenna Technology, has developed an “electronic Secchi” whose response is independent of light and operator, but not of other factors. But he was not successful (too complex?)

2) The use of an instrument to measure the concentration of spirulina generally becomes unnecessary when the operator has acquired sufficient experience. He knows how to judge the concentration by the appearance of the culture.

3) The spirulina concentration can also be measured with a spectrophotometer at the wavelength of 560 nm as Zarrouk did in his thesis: he had found that 1 unit of optical density corresponds to 0.7 g of spirulina per litre.

4) Failure to take into account the correction for turbidity can lead to overestimating

seriously the concentration and the productivity (if calculated from the measured concentrations).

A3) Measurement of the salinity of the culture medium with a hydrometer

It is accepted that the presence of spirulina in a culture medium does not modify its density.

A hydrometer is used for densities (specific weights) greater than 1 (like those sold in aquarium shops or to measure the density of urine). The reading is done at the lower level of the meniscus. Wait until the micro air bubbles are eliminated before taking the reading.

Density DT at temperature T°C and density D20 at 20°C are related by the formula:

D20 = DT + 0.325 x (T – 20), g/l

The salinity SAL and D20 are related by the following approximate formulas for culture medium based on ash salts or sodium bicarbonate:

if D20 is greater than 1007.6:

SAL = 1.250 x (D20 – 1007.6) + 10. g/litre or

else by:

SAL = 1.041 x (D20 – 998), g/litre

NB There are other, more modern instruments for measuring salinity: the conductivity meter and the refractometer. The following approximate equivalence is noted: 1 g/l = 2000 ÿS/cm.

A4) Measurement of the pH of a culture medium

Only a good quality and well-calibrated pH meter makes it possible to follow the fine evolution of the pH of a culture and to possibly adjust the progress of the culture very close to the maximum authorized pH of 11.2.

The pH varies with temperature. The pH measured at T°C must be increased by K x (T – 25) to obtain the value at the standard temperature of 25°C, the coefficient K depending on the electrode and the medium. In practice K varies in the range 0.006 to 0.018.

Some pH meters are equipped with a more robust milliVolt scale than the pH scale.
It is used to calculate the pH from the indication in mV using the theoretical formula:

pH at T°C = (K1 – mV) x K2 /(273 + T)

where K1 and K2 are two electrode-dependent constants (glass electrode) determined by calibration from pH standard solutions. This formula can be written, for T = 25°C:

pH = A – mV/B

where A is the pH for 0 mV and B is the slope in mV/pH unit. Usual values are for example A = 7 and B = 50. The value of the mV measured practically does not depend on the temperature, which is fortunate because it dispenses with making a temperature correction: it suffices to apply the formula to the reference temperature.

To prolong the life of a pH meter, keep it away from moisture. To prolong the life of its electrode, keep the sensitive end of the electrode in

a saturated solution of potassium chloride in distilled water, at a temperature above 15°C, and rinse it carefully before and after the measurements, with clean water and if possible demineralised. If mold settles in the KCl solution, it is better to renew it.

The fragility and the limited lifetime of the electrodes, and their high cost, make it difficult to use a professional pH meter in many situations. A cheap pH meter, "pen type", recalibrated frequently, can help, but its life may be short. pH papers are not accurate enough.

It is very important not to neglect to calibrate your pH meter from time to time.

The pH standard solutions sold on the market are expensive, but it is possible to save them by using the following approximate standard solutions (store those with average pH away from light to prevent algae from developing spontaneously) whose indicated pHs correspond to 25°C:

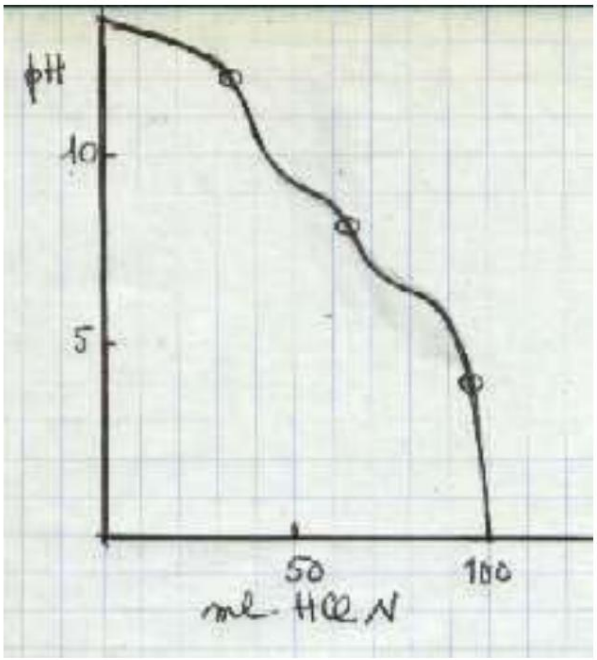
- N hydrochloric acid (36.5 g/l): **pH 0 ;** N/10: **pH 1 ;** N/100: **pH 2** - lemon juice: **pH 2.3** - "6 degree" vinegar (6% acetic acid, density 1.01): **pH 2.8** - aqueous solution at 5.8 g/l of monoammonium phosphate: **pH 4** - tomato juice: **pH 4** - aqueous solution of 5.8 g/l of monoammonium phosphate + 11 g/l of sodium bicarbonate: **pH 7** - sodium bicarbonate N/10 (8.4 g /l): **pH 8.3** - aqueous solution containing 5.3 g/l of sodium carbonate + 4.2 g/l of sodium bicarbonate (or 1.4 g/l of sodium hydroxide + 5.46 g/l of sodium bicarbonate) at equilibrium with the atmosphere (keep in contact with the outside atmosphere, do not block the container, add water to compensate for evaporation): **pH 9.8** (varies slightly depending on CO2 content of the air and altitude) - sodium carbonate N/10 (10.6 g/l): **pH 11.6** - sodium hydroxide N/100: **pH 12 ;** N/10: **pH 13 ;** N (40 g/l): **pH 14**

NB With experience it is possible to do without a pH meter to conduct a culture of spirulina, especially if one cultivates under shade or with the addition of sodium bicarbonate or sugar.

A5) Measurement of alkalinity (alcalimetry)

A sample of the culture medium or ash water to be studied is gradually neutralized with a strong acid of known normality (for example 159 g of concentrated hydrochloric acid at 23% HCl + 857 g of demineralized water = 1 liter = 1016 g of "N" acid, ie Normal, at one gram-molecule/litre) until pH = 4. Let V be the volume of sample and V' the volume of N acid used. The alkalinity is equal to V'/V, moles/litre. NB: the drop in pH is very sudden below 5. If the title of the acid is not exactly N, correct V' proportionally. NB: The concentration indicated on the bottles of acid sold in supermarkets is sometimes lower than the reality (by 9% for example).

Example: alkalimetry on 200 ml of partially carbonated ash water:



On this graph, at pH 4, we read V' = 96, hence total alkalinity 96/200 = 0.48 N (ie 0.48 mole of base/ litre). At pH 12 we read V' = 33 from which free potash 33/200 = 0.165 N.
At pH 8 we read V' = 62 from which potassium carbonate = (62 – 33)/200 = 0.145 mol/l.
The inflection at pH 8 corresponds to the carbonate/sodium bicarbonate transition (here there was no sodium bicarbonate in the sample, the one being measured comes from the acidification of the carbonate). What is commonly called "alkalinity" or "basicity" corresponds to the total alkalinity measured at pH 4. In practice, weights are measured rather than volumes and the weight correction corresponding to the CO2 released is neglected. This can lead to an error of 5% by excess on the alkalinity of an average culture medium.
NB If you do not have a pH meter, you can use a colored indicator that changes around pH = 4, such as methyl orange (10 drops of 1% aqueous solution for 100 ml of sample to be studied) which changes from orange to red, or paper to “Congo red”.

Caution: The "concentrated" hydrochloric acid sold in some countries is only 20% HCl.

A6) Easy to perform quality tests

A6.1) Culture test

A6.1.1) Filterability test

To characterize the rate of filtration, a standard test has been established. Measure 400 g of culture to be tested and quickly pour it into a coffee filter holder lined with white filter paper such as “Grand Jury” or Carrefour N° 4 or equivalent. Note the filtered weight in one minute. A weight greater than 250 g corresponds to correct filtration. Do not neglect the effect of temperature or the nature of the paper on this test. It is recommended to establish your own scale of values with the type of paper available. It is interesting to redo the test on the filtrate obtained, which gives an indication of the share of resistance to filtration due to the biomass and that due to the impurities of the medium.

In the case of very lightly soiled media, the comparison must be refined, taking new medium as a reference, and comparing with the weight of new filtered medium (there remains approximately 10 g of liquid in the filter paper, the filter support and measuring containers, so the best possible result is 390 g).

A6.1.2) Measurement of the turbidity of the culture medium

It is done in the shade on the filtrate obtained during the filtration test (A6.1.1), like a Secchi disc concentration measurement . A black Secchi disk is preferable if the staining is weak. Wait for the foam and micro air bubbles to disappear before taking the reading. Warning: poorly filtering spirulina tend to pass through the filter paper, turning the filtrate green; in this case it may be preferable to refilter the filtrate on double paper to eliminate the spirulina before measuring the true turbidity of the medium.

It can be seen that the initial degradation of a medium is detected much more finely by the turbidity than by the filtration test. Thus a filtrate with a turbidity of 25 cm can very well go hand in hand with a filtered weight practically equal to 100% of the reference. While the turbidity of a new medium is much higher than 35 cm, “like water”.

A6.1.3) Measurement of the washability of the biomass

After the filterability test (§ A6.1.1), pour 400 ml of fresh water into the filter, diluting the biomass and note the volume filtered in one minute. If the biomass is of the "washable" type (its cells do not burst upon contact with fresh water), this volume remains close to that of the filterability test. Confirm by microscopic examination of the washed biomass.

A6.2) Tests on spirulina

A6.2.1) pH test

It is easy to get an idea of the quality of the washing or dewatering of the biomass, either by taking the pH of the pressed biomass (which must be between 7 and 9), or by measuring the pH of a suspension 4% dry spirulina in water. When a spirulina has been dried at a fairly high temperature (60 to 65°C) and is rehydrated, its cells burst and the pH drops, sometimes to 5. The pH obtained is all the lower as the spirulina is well drained. This low pH would be due to the internal acidity of the cells and/or the beginning fermentation.

A6.2.2) Estimation of pigments

In the pH test of the previous § the pigments are released and it is possible to see them and judge their concentration. The blue is sometimes slow to come out (wait 24 hours for safety, shaking occasionally). Sometimes it is necessary before the test to heat the powder for a few minutes at 65°C to better burst the cells. On the other hand, it is necessary to centrifuge or at least decant the solid residues because they may still be rich in phycocyanins. To assess the concentration of phycocyanins (blue pigment), place a drop of decanted solution on a very flat and horizontal blotting paper or filter paper: a very clear chromatogram is obtained; the coloration and area of the blue spot is an indication of the phycocyanin concentration. But this test does not differentiate allo-phycocyanin from C-phycocyanin: for it to give a more or less valid indication, the stain must be close to that obtained with a reference spirulina whose C content is known. -phycocyanin. This test is not quantitative, but let's at least work under comparable conditions.

To assess the concentration of carotenoids (therefore beta-carotene which represents approximately half of the carotenoids), mix dry powdered spirulina with 4 times its weight of 90° alcohol (methylated spirits) or acetone, shake, cover and wait 5 hours: the carotenoids go into solution, and their more or less strong yellow-brown color is an approximate measure of their concentration. Shake, decant the remains of powder and use the filter paper stain system to appreciate it.

Warning: the coloring of the stains is labile (it fades little by little by oxidation or decomposition in the light).

A6.2.3) Color test

The green color of good quality spirulina is easy to spot. Reference samples may be in stock for comparison. The shade of green depends on the strain (the spiral is less dark than the wavy) and the treatment (pressing, extrusion, centrifugation).

A6.2.4) Simplified colorimetric determination of phycocyanin

A more accurate method for measuring pigment content is colorimetry. Start with the same "pH test" standard solution as in Appendix A6.2.1. Let C% be the concentration of dry spirulina soaked in water around 4%. Leave to decant and take the blue solution, centrifuge it if you have a laboratory centrifuge. Take the centrifuged or decanted solution: about 0.5 to 1 ml. Dilute this sample by a factor of approximately 100 with water. Let DIL be this dilution factor, in volume. Measure the optical density (OD) at 615 nanometer (nm) wavelength, OD615, and at 652 nm, OD652, using a colorimeter or spectrophotometer (11 mm optical path cell). Calculate the % by weight of phycocyanin by the formula:

1.873 x (OD615 – 0.474 x DO652) x DIL /C

A correct value is: > 10% of dry spirulina.
We can also calculate the % of allophycocyanin by the formula:

1.1965 x (DO652 – 0.208 x DO615) x DIL/C

NB1/ The OD is equal to the logarithm (base 10) of the incident light/transmitted light ratio or of the ratio 100 / (% transmission) or 100/ (100 - % absorption).
NB2/ If the cuvette used has an optical path of 10 mm the above formulas become respectively: **2.0603 x (DO615 – 0.474 x DO652) x DIL /C 1.3162 x (DO652 – 0.208 x DO615) x DIL/ VS**

A6.2.5) Simplified colorimetric determination of carotenoids

Add 25% acetone or, failing that, 90° alcohol, to a suspension of the "pH test" type above, and keep it in the refrigerator for 24 hours. Let C be the concentration of spirulina in this suspension. Decant, and if possible centrifuge, and take P ml of the solution (approximately 0.5 ml). Dilute with acetone or alcohol. Let DIL be the volume dilution factor. Measure the optical density at 450 nm. Let DO450 be this density. The concentration of carotenoids in spirulina is obtained by the formula:

Where **0.357 x OD450 x DIL/C, mg/g** with **11mm pathlength 0.3929 x OD450 x DIL/C** with 10mm pathlength

A correct value is 2.5 mg/g. Beta-carotene represents about half of the carotenoids.

NB The OD is equal to the logarithm (base 10) of the incident light/transmitted light ratio or of the ratio 100/(% transmission) or 100/(100 - % absorption).

A6.2.6) Dosage of moisture in dry spirulina (% water)

Put the spirulina to be tested (about 200 g, no need to weigh) in a container like "Tupperware" (two liters maximum), well sealed and sufficiently transparent

to be able to read the digital hygrometer placed (taped) under the lid. Follow the evolution of the % relative humidity (% RH) of the air in the container until equilibrium (about 2 hours): if this % is less than 45, the spirulina complies with the standard (< 9% water). For the measurement to be accurate, the measurement assembly must be in equilibrium not only with respect to humidity but also with temperature around 25°C.

In the area that interests us (%RH between 10 and 60), the % of water in spirulina is equal to 1 + (%HR)/6 according to our measurements and according to Lembi (BIBLIOGRAPHY).

A7-1) Absorption of atmospheric carbon dioxide by the culture medium

We measured the absorption rate of CO2 from the air by following the decrease in pH of the culture medium without spirulina, with weak and intermittent agitation. Knowing the surface exposed to air, the alkali concentration, the volume per m² and the correspondence between pH and C (C = CO2/base molar ratio: (see Appendix 11), it is easy to deduce the speed of absorption of CO2 as a function of pH We find increasing values of 0 for the pH corresponding to equilibrium with the air (around pH 9.8), at the equivalent of approximately 4.5 g of spirulina/ day/m² towards pH 11.

The theory says that the rate of absorption is proportional to the coefficient of absorption and the difference in the vapor pressures of CO2 in the air and on the liquid. The vapor pressure of CO2 over a sodium carbonate/bicarbonate solution is given in the literature. Kohl and Riesenfield (1960) give in “Gas Purification” by Kohl (BIBLIOGRAPHY) on page 117, a formula having as variables the temperature, the alkalinity and the ratio c (moles of CO2/mole of base), in mmHg:

$$pCO2 = 68.5 \times b1.29x (2c - 1)^2 / [(1 - c) \times (333 - 1.8\ xt) \times (0.0487 - 0.0006\ xt)]$$

where :

b = alkalinity of the absorbing medium, gmoles of strong base/litre
c = CO2/base molar ratio corresponding to the pH of the medium t
= temperature of the medium, °C

The absorption of CO2, expressed in g of spirulina/day/m² (assuming 1.8 kg of CO2 per kg of spirulina) is then calculated by the formula:

$$0.772 \times ka \times [0.00076 \times vpm \times (1 - alt/10000) - pCO2]$$

where :

ka = absorption coefficient,
gmoles of CO2 absorbed/hour/m²/atmosphere
vpm = CO2 content in the air, volume ppm alt =
altitude, meters 0.772 = (44 x 24)/(1.8 x 760)

The average ka value resulting from direct and indirect absorption measurements (productivity of spirulina basins supplied with carbon only from the air) is around 23. Our direct measurements carried out in 1991 in basins gave ka = 25.

In August 1999 a 6 m² basin was filled with 1000 liters of culture medium based on N/10 sodium hydroxide and stirred like a normal culture. Its pH fell from 12.44 to 10.68 in 16 days, which corresponds to ka = 24. So ka = 20 gives a large margin of safety.

The value of vpm is 400 in 2014 in the northern hemisphere, a little lower in the south.

A7-2) Analysis of CO2 in the air

The formula above (§ A7-1) giving pCO2 makes it possible to measure the CO2 content of the air with very simple equipment, whereas an infrared analyzer costs 4000 €. Simply bubble a small flow of air (mini aquarium compressor) through a diffuser at the bottom of a test tube containing a solution of sodium bicarbonate at 8.4 g/l (alkalinity 0.1 N), and measure the pH at equilibrium. The result depends on the temperature of the solution.
This method is obviously unsuitable for sudden changes in the CO2 content of the air, because of the inertia of the solution. To reduce this inertia, it is advantageous to reduce the volume of solution and to finely divide the bubbling gas.

For long-term measurements, keep the test tube protected from light to prevent it from turning green and add distilled water to maintain the level if there is evaporation (if the temperature of the solution is below the dew temperature of the air analyzed, the solution will gradually dilute: in this case, sodium bicarbonate must be added to maintain its alkalinity at 0.1 N).

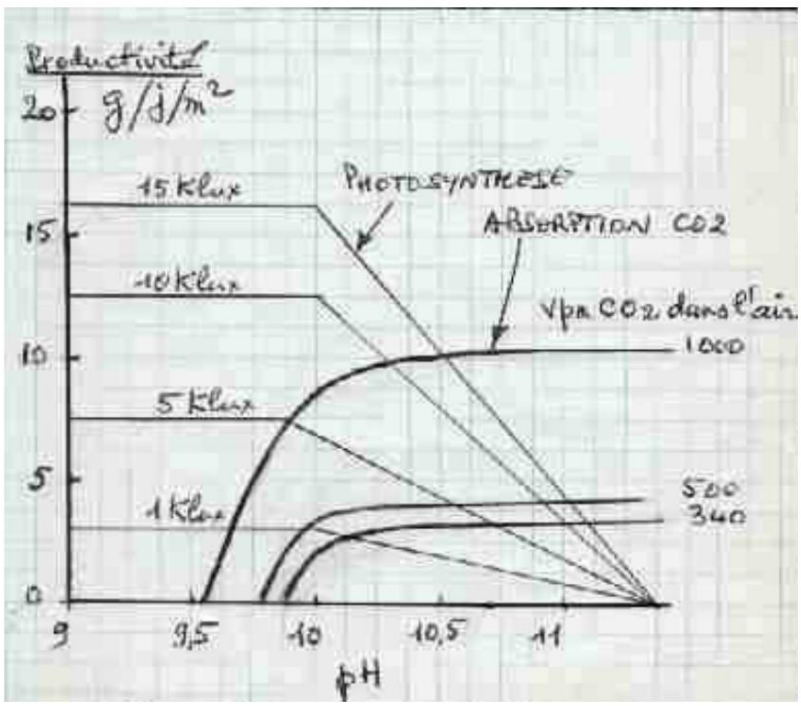
A small program (see CALCULATION) makes it very easy to calculate the CO2 content of the air (in vpm = volumes per million) according to the temperature and the pH of the solution at equilibrium. The program provides a pH/vpm table for each desired temperature.

NB In 2011 Conrad sells a “CO2-meter” for only €189 including VAT, whose characteristics are more than enough: accuracy 25 vpm, range 0 – 3000 vpm of CO2 in the air; this device is called Air Control 3000, brand TFA, manufacturer reference 5020-1016, Conrad reference 101365-62. This invalidates the self-constructed device described above, which nevertheless remains valid and is free.

A7-3) pH of a culture medium in equilibrium with the atmosphere

It is easy to calculate this pH by combining the two equations given in § A7-1 (absorption). To facilitate this calculation a small program (see CALCULATION) was written.

A8) Photosynthesis/CO2 Absorption Interaction



The graph above shows examples of variations in the rate of absorption of CO2 from the air as a function of the CO2 content of the air and the pH of the culture medium, calculated using the formula given in the Appendix A7 (formula CO2), and expressed as spirulina equivalent at a rate of 1.8 g/g of spirulina, for the following conditions: altitude = 0, temperature = 30°C, ka = 18 and alkalinity = 0.1 N. We see that there is little to be gained by working at pH > 10.3. On this same graph have been reported examples of variation in the rate of photosynthesis, expressed in the same unit as the absorption of CO2 (in productivity of spirulina), as a function of pH, for a given luminosity, in the absence of other limiting factors. These examples are given for illustrative purposes only without precise value of the parameters other than the pH, simply to show the mechanism of the interaction.
Photosynthesis/absorption of CO2. If we follow one of these photosynthetic speed curves starting from the minimum pH, we see that this speed decreases beyond pH 10. Simultaneously the CO2 absorption speed increases and there comes a time when the two speeds are equal (the two curves intersect): from there, the pH can no longer continue to increase; this point of balance corresponds to the rate of photosynthesis under an atmosphere having the CO2 content indicated. The corresponding equilibrium pH is all the higher as the photosynthesis conditions (light, agitation) are better and the CO2 content of the air is lower.

A9) Productivity according to shading, example calculated by Simulation model, with purge rate 1% and with addition of 18 g CO2/day/m²:

0% shade = 50% shade	14.3 g/day/m²
= 75% shade = 80%	13.3 g/day/m²
shade =	9.9 g/day/m²
	8.4 g/day/m²

We see the weak influence of the shading rate up to around 50%.

A10) Water consumption according to shading, example calculated by Simulation model, with purge rate 1% and with addition of 18 g CO2/day/m²:

0% shade = 65% shade	732 liters of water/kg of spirulina
= 75% shade = 80%	**556** liters of water/kg of spirulina
shade =	623 liters of water/kg of spirulina
	698 liters of water/kg of spirulina

There is a minimum water consumption around 65% shade.

A11) Correspondence between pH and molar ratio C = CO2/base (soda or potash)

This relationship is of great importance for many calculations concerning the cultivation of spirulina. It has been established experimentally in the usual range of alkalinity (around 0.1). It also depends slightly on the value of the alkalinity.

A small calculation program reproduces this relation (see CALCULATION).

A12) Mixtures of sodium carbonate and bicarbonate

- To obtain an aqueous solution having the following characteristics: molar ratio CO2/strong base = C and alkalinity b moles/litre, the following products can be dissolved in one liter of water:

Sodium carbonate = 106 xb (1 – C), grams

+ Sodium bicarbonate = 84 xbx (2C – 1), grams

By combining this relationship with that of Appendix A11, it is possible to calculate the carbonate + sodium bicarbonate mixtures giving a desired pH for a given alkalinity.

- To go from a solution characterized by Ci and b to a solution characterized by V and b, we can add to one liter of the first (V – Ci) / (1 – V) = E, liter of water and: 84 x E xb, grams of sodium bicarbonate.

Warning : purchased sodium carbonate may be a mixture of sodium carbonate and bicarbonate (either by natural bicarbonation of stored carbonate under certain conditions, or because it is natron or trona); before using carbonate check its bicarbonate content by taking the pH of a 5 g/l solution (which should be close to 8 - 8.5.

A13) Neutralization of ash water with sodium bicarbonate

The aqueous extract of good quality ashes generally has a very high pH when it has just been made, up to 13. Before using it as a base of culture medium it is necessary to wait a long time (for example 15 days) so that its pH drops sufficiently by absorption of CO2 from the air.

An artifice to make such extracts instantly usable is to dissolve pure CO2 or sodium bicarbonate in them. The amount of sodium bicarbonate (“bicarb”) to add to lower the pH to 10.5 can be calculated by either of the following formulas:

bicarb = 187 x (0.55 – C) xb, g/l
bicarb = 1.83 x S – 234 x C x S / (56 + 26 x C), g/l

formulas where:

C = CO2/base molar ratio, determined from pH (see Annex 11) b = alkaline normality of ash water, moles/l
S = alkaline salinity (potash + potash carbonate) of the ash water, g/l
= bx (56 + 26 x C)

NB The alkaline salinity S can be calculated approximately from the total salinity determined by the density (in general the alkaline salinity represents 2/3 of the total salinity), but it is more precise to determine it by alkalimetry from C and B.

Example: Ash water has a density of 1.013 and a pH of 12.45 at 25°C, i.e. a total salinity of 18 g/l and C = 0.4; the alkalimetry gives b = 0.2 or an alkaline salinity S = 13.3; sodium bicarbonate to add = 5.6 g/l.

Application to caustic soda solutions:

Obtaining culture media based on caustic soda can be considered as a special case of the neutralization of ash water (which is a solution of caustic potash). This case can be useful when the carbonate is rarer than the soda, to obtain media with an average pH. Examples of mixtures of soda and sodium bicarbonate for b = 0.1 moles/litre:

6.1 g of sodium bicarbonate + 1.2 g of soda per liter of water = pH 10.0 5.6 g of sodium bicarbonate + 1.4 of soda per liter of water = pH 10.2 5 g of sodium bicarbonate + 1.7 soda per liter of water = pH 10.5

NB The use of caustic soda requires the usual precautions for use for caustic products (gloves, goggles).

A14) Composition of various products

(NB: ppm = mg/litre or mg/kg)

Raw sea salt (unrefined) Analysis of La Salorge de Guérande salt:

Phosphorus: practically 0; potassium: 1 to 2 g/kg; sulphur: 3 to 7 g/kg; magnesium: 4 to 8 g/kg; calcium: 1 to 2 g/kg; copper: 2.5ppm; zinc 0.5 to 2 ppm; manganese: 4 to 8 ppm; iron 30 to 100 ppm.

wood ash

Dumon gives the following composition of the ash in g/kg: Phosphorus: 43; sulfur: 8; potassium 219; magnesium: 90; calcium 236; manganese: 50; iron: 14. Highly variable soluble content (from 1 to 25%).

Average analysis of soluble salts extracted from ashes, sold on Burkinabe markets: mixture of potassium carbonate and potassium bicarbonate (at 15% by weight of potassium bicarbonate) with 10% dipotassium sulphate, 0.1% phosphate and calcium and traces of magnesium.

The solubility of the magnesium and calcium contained in the ash depends a lot on the pH: almost zero at pH 13, it is notable at pH 10 (about 100 ppm of magnesium in the ash water, which is also very rich in sulphur: 1500ppm).

From http://www.woodash.net/chart.html :

Range in elemental composition of industrial fly ash and bottom ash samples Element Fly Ash 16.9 (8.6-22.4)		
		Bottom Ash
Boron		1.0 (0.7-1.3)
Potassium	19236-29526	12572-19634
Arsenic	1.0 (1.0-1.0)	1.0 (1-1.0)
Copper	53.5 (39.5-81.5)	31.3 (24.8-28.0)
Nickel	17.3 (14-24)	14.0 (13-15)
Cadmium	9.6 (3.0-21.1)	5.0 (2.0-13.1)
lead	11.0 (4-20)	7.7 (5-10)
Selenium	1.0 (1.0-1.0)	1.0 (1.0-1.0)
Cobalt	6.4 (5.3-8.7)	4.6 (4.2-4.9)
Mercury	0.01 (.01-0.01)	0.01 (0.01-0.02)
Zinc	886 (522-1529)	497 (348-896)
Chromium	26.0 (17.0-40.7)	20.3 (16.0-25.4)
Molybdenum	11.3 (7-18)	3.0 (1.0-6.0)

* Mean and (Range) taken from analysis of 7 ash samples
* All results expressed as mg/kg unless otherwise stated

Wood ash has a very variable composition depending on the species but also on the combustion temperature, potassium and boron being volatile above 1000°C, according to "Wood ash composition as a function of furnace temperature" , Mahendra K. Misra et al. In Biomass and Bioenergy Vol.4, No.2, pp 103-116 (1993), Pergamon Press (http://www.fpl.fs.fed.us/documnts/pdf1993/misra93a.pdf). This article gives (page 111) the following elementary compositions for ash obtained at 600°C, in g/kg of ash:

- **Pine :** Ca = 290.5; K = 162.4; Mg=70.3; S=10.7; P=8.4; M n = 40.4; Zn=3.6; Fe = 5.8; A1 = 4.7; Na=0.6; B=0.6; Cu = 0.4
- **Poplar :** Ca = 256.7; K=79.3; ;Mg=90.9; S=10.2; P=9.5; M n = 4.5; Zn = 0.4 ; Fe=3.2; Al=3.5; Na=2.3; If = 0.11; B=0.5; Cu = 0.3
- **White oak :** Ca = 313.5; K=102.5; Mg=75.7: S=12.1; P=5.6; M n = 1.4; Zn = 0.8; Fe=0.9; Al=<0.6; Na=<0.3; If = 1.3; B=0.4; Cu = 0.2

Waters River waters have on average the following typical contents (in ppm): iron = 0.1; Calcium = 40; magnesium = 14; sulfur = 6. Adding water to the pool then generally provides enough magnesium and sulfur.

The water from the well of the County of the Bread of Life in Arequipa, Peru has the following contents (in ppm): calcium = 72; magnesium = 16; sulfur = 50; potassium and phosphorus = negligible. If the evaporation is 2.4 mm/day, the additional water brings sulfur and magnesium, and of course calcium, for 20 g of spirulina/day/m².

The water from the well of the Foyer de Charité de Bangui (RCA) contains virtually no calcium, magnesium or iron. The same is true of water from the city of Linares, Chile.

Water from the well of the School of Agriculture in Catemu, Chile, contains 96 ppm of calcium, 34 of magnesium and 130 of sulphur.

Analysis of water from a spirulina lake near Tulear (Madagascar): salt = 35 g/l; bicarbonate + sodium carbonates (pH 10) = 16 g/l; sulfur (from sulphates) = 0.5 g/l; iron = 0.44 ppm; calcium = 6.5 ppm; magnesium = 80ppm; phosphorus = 3.6 ppm; nitrogen = 0.3 ppm (including 0.2 ammoniacal).

Gardon water from Mialet: 22 ppm of calcium and 2.4 ppm of magnesium

Sea water (ppm): iron: 0.002 to 0.02; calcium: 400; magnesium: 1272; phosphorus: 0.001 to 0.01; sulphur: 900; bicarbonate < 150.

human urine

It contains: Nitrogen = 7 to 12 g/l; phosphorus = 0.5 to 0.7 g/l; potassium = 2 to 3 g/l; sulfur = 0.8 to 1.2 g/l; salt (sodium chloride) = 12 g/l; calcium = 0.13 g/l; magnesium = 0.1 g/l; iron = 0.3 mg/l; sugars = 0.15 g/l. Its "production" is about one liter per day per person.

Chilean nitrate (Salitre potásico)

This natural product corresponds to 2 NaNO3.KNO3; it contains 15% nitrogen, 18.4% sodium, 11.6% potassium, 1% sulfur (in the form of sulphates), as well as: 0.12% calcium, 0.14% magnesium and many trace elements (all the micronutrients needed for spirulina). It is colored pink. Not to be confused with pure KNO3 (white), extracted from salitre, therefore also “natural”.

Blood: Nitrogen: 350 mg/l; phosphorus: 30 to 70 mg/l; iron: 9 g/l

A15) Useful laboratory equipment: see Appendix 29

A16.1) Chemicals

Useful chemicals for spirulina

(The % indicated are the % by weight of pure product unless otherwise indicated; pm = molar weight)

- Hydrochloric acid HCl, pm = 36.5 -
Citric acid COOH-CH2-C(OH)(COOH)-CH2-COOH, pm = 192 -
Orthoboric acid H3BO3, pm = 61.8 (17.14% boron)
- Phosphoric acid H3PO4, pm = 98 (31.6% phosphorus)
- Sulfuric acid H2SO4, pm = 98 (32.7% sulfur)
- Crystallized chromium alum, CrK(SO4)2, 12 H2O, pm = 499.4
(10.3% chromium)
- Ammonia NH3, pm = 17 (82% nitrogen)
- Ammonium bicarbonate NH4HCO3, ww = 79 (17.7% nitrogen)
- Sodium bicarbonate NaHCO3, pm = 84 -
Potassium bicarbonate KHCO3, pm = 100 -
Butane C4H10, pm = 58 (82.8% carbon)
- Potassium carbonate K2CO3, pm = 138 -
Sodium carbonate Na2CO3, pm = 106 -
Sodium carbonate decahydrate Na2CO3, 10 H2O (= “soda crystals”), pm = 286 - Lime Ca(OH)2, pm = 74 (54% Calcium)
- Calcium chloride CaCl2, pm = 111 (36% calcium)
- Manganese chloride crystallized at 4 H2O, MnCl2.4H2O, pm = 198 (27% manganese)
- Potassium chloride KCl, mw = 74.5 (52% potassium)
- Sodium chloride (cooking salt) NaCl, mw = 58.5 (60.7% chlorine)
- Zinc chloride ZnCl2, mw = 136.3 (46.5% zinc) [hygroscopic!]
- EDTA (ethylene-diamino-tetraacetic acid), ww = 292 -
EDTA, disodium salt crystallized at 2 H2O, ww = 372 (78% EDTA)
- Carbon dioxide CO2, pm = 44 (27.3% carbon)
- Sodium molybdate MoNa2O4.2H2O, ww = 242 (39.7% molybdenum)
- Ammonium nitrate or ammonitrate (dry explosive) NH4NO3, pm = 80 (35% nitrogen, half of which is ammoniacal)
- Calcium nitrate Ca(NO3)2, pm = 164 (24% calcium and 17% nitrogen)
- Sodium nitrate NaNO3, pm = 85 (16.5% nitrogen; 72.9% NO3; 27% sodium)
- Potassium nitrate KNO3, pm = 101 (13.9% nitrogen, i.e. 61.4% N03; 38.6% potassium; technical grade at 91% purity)
- Molybdenum oxide, MoO3, mw = 143.9 (66% molybdenum)
- Selenium oxide, SeO2, pm = 111 (70.4% selenium)

- Zinc oxide, ZnO, pm = 81.4 (80.3% zinc)
- Phosphorus, pm =
31 - Monoammonium phosphate NH4H2PO4, pm = 115 (27% phosphorus and 12% nitrogen or 15% NH4)
- Diammonium phosphate (NH4)2HPO4, pm 132 = (23.4% phosphorus and 21% nitrogen)
- Dipotassium phosphate K2HPO4, mw=174 (17.8% phosphorus and 44.8% potassium, purity 97%) [hygroscopic!]
- Disodium phosphate, Na2HPO4, 12H2O, ww = 358 (8.7% phosphorus)
- Monopotassium phosphate KH2PO4, mw = 136 (22.7% phosphorus, 28.7% potassium)
- Disodium phosphate Na2HPO4, 12 H2O, ww = 358 (8.7% phosphorus)
- Tricalcium phosphate Ca3(PO4)2, mw = 310 (20% phosphorus, 39% calcium), insoluble - Trisodium phosphate, Na3PO4.12H2O, mw = 380 (8.1% phosphorus)

- Potash KOH, pm = 56 (70% potassium)
- Propane C3H8, pm=44 (81.8% carbon)
- Potassium salitre: 15% nitrogen (i.e. 66% NO3), 18.4% sodium, 11.6% potassium, 1.2 g calcium/kg, 1.4 g magnesium/kg, 10 g of sulfur (i.e. 30 g of SO4)/kg - Sodium selenite (Na2SeO3), pm = 173 (45.3% of selenium) [toxic]
- Caustic soda (or "soda" or sodium hydroxide), NaOH, pm = 40 - Sugar (= sucrose = sucrose = C12H22O11), pm = 342 (42% carbon)
- Calcium sulphate CaSO4, pm = 136 (29% calcium, 23.5% sulphur), very sparingly soluble - Cobalt sulphate at 7 H2O, CoSO4.7H2O, pm = 281.1 (20.3% cobalt)
- Crystallized copper sulphate at 5 H2O, SO4Cu.5H2O,pm = 249.7 (24.9% copper)
- Magnesium sulfate crystallized at 7 H2O (Epsom salt) MgSO4.7H2O, mw = 246.5 (9.6% magnesium and 12.7% sulfur, purity 98%)
- Dipotassium sulfate K2SO4, pm = 174 (44.8% potassium and 18.4% sulfur)
- Crystallized iron sulphate with 7 H2O, FeSO4.7 H2O, pm=278 (20% iron)
- Zinc sulphate crystallized at 7 H2O, ZnSO4.7H2O, pm = 287.4 (22.7% zinc)
- Urea CO(NH2)2, pm = 60 (46% nitrogen, agricultural fertilizer quality)

OXIDES (in fertilizers) (pm = molar weight)

- Phosphoric anhydride P2O5: pm = 142 (43.7% phosphorus)
- Sulfuric anhydride SO3: pm = 80 (40% sulfur)
- Potassium oxide K2O: pm = 94 (83% potassium)
- Magnesium oxide MgO: pm = 40 (60% magnesium)

Main useful IONS for spirulina (= weight of an ion-g)

- Ammonium NH_4^+ = 18
- Calcium Ca++ = 40
- Chloride Cl- = 35.5
- Bicarbonate HCO3 - = 61
- Carbonate CO3 -- = 60
- Ferrous iron Fe^{++} , ferric Fe+++ = 56
- Hydrogen (proton) H+ = 1
- Phosphate PO4---= 95 (32.6% of P)
- Potassium K+ = 39
- Magnesium Mg++ =24
- Nitrate NO3 - = 62 (22.6% N)

- Sodium Na+ = 23
- Sulfate SO4 -- = 96 (33.3% S)
- Zinc Zn++ = 65

Main sparingly soluble crystals that can precipitate in spirulina sludge

- Calcium carbonate CaCO3
- Magnesium hydroxide Mg(OH)2 Zinc
- hydroxide Zn (OH)2 Calcium
- phosphate Ca3(PO4)2 Iron phosphate
- FePO4 Magnesium ammonium
- phosphate MgNH4PO4.6H2O

A16.3) Atomic masses of the elements of interest to spirulina List of names, symbols and atomic masses (rounded) of the elements:

Nitrogen = N = 14
Boron = B = 11
Calcium = Ca = 40
Carbon = C = 12
Chlorine = Cl = 35.5
Chromium=Cr=52
Cobalt = Co = 59
Copper = Cu = 63.5
Iron = Fe = 56
Hydrogen = H = 1
Magnesium = Mg = 24
Manganese = Mn = 55
Molybdenum = Mo = 96
Oxygen = O = 16
Phosphorus = P = 31
Potassium = K = 39
Selenium = Se = 28
Sodium = Na = 23
Sulfur = S = 32
Zinc = Zn = 65.4

A16.4) Molecular masses of the main oxides and ions of interest to spir

CO3 = 60 (73.3% CO2)
HCO3 = 61 (72.1% CO2)
K2O = 94 (83% of K)
NH4 = 18 (77.8% N)
NO3 = 62 (22.6% of N)
MgO = 40 (60% Mg)
P2O5 = 142 (43.7% of P)
PO4 = 95 (32.6% of P)
SO3 = 80 (40% of S)
SO4 = 96 (33.3% of S)

A17) Standards for spirulina in France

(According to Order of 21/12/1979 + updates)

Relative to dry weight, in ppm (mg/kg):

Arsenic <= 3
Lead <= 5
Tin <= 5
Cadmium <= 0.5
Mercury <= 0.1
Iodine <= 5000

Both for fresh and dry product:

Aerobic germs (30°C) <= 100,000 / gram Faecal coliforms (44.5°C) < 10/ gram Sulphite-reducing anaerobes (48°C) < 100/ gram Clostridium perfringens <= 5/ gram Staphylococcus aureus <= 100 / gram Salmonella: absence in 25 g.

In addition, spirulina must contain less than 1 µg of mycrocystin per gram.

NB Examples of upper pH limits for the growth of microorganisms in non-dehydrated foods (in the presence of live spirulina different values could be obtained; on the other hand there could be addictions to pH > over time):

Staphylococcus = 9.8
Streptococcus = 9.3
Bacillus = 9.3 B. subtilis
= 10 Clostridium
botulinum = 8.5 Clostridium
perfringens = 8.5 Clostridium
sporogenes = 9 Lactobacillus =
8 E. coli = 10 Salmonella
(including salmonella typhi) = 9
Vibrio parahaemolyticus (cause of gastroenteritis)
= 11 Vibrio cholerae = 9.6 Pseudomonas = 8 Candida = 9.8
Saccharomyces = 8.6 Penicillium = **11** Aspergillus = 9.3 Listeria
monocytogenes = 9.6

A18) Concentration limits in the culture medium

All figures express mg/litre (or ppm). Those given in parentheses are those of Zarrouk's basic culture medium in his thesis (Zarrouk, page 4). Maximums generally include a safety margin:

Nitrate* = 440 to 6600 (1800)
Ammonium* = 0.3 to 30
Urea* < 50 Phosphate**
= 0.1 to 300 (270)
Potassium > 10 (665) and K/Na weight ratio < 5
Magnesium*** = 1 to 30 (19)
Sulfate** > 30 (675)
Iron > 0.4 (2)
Calcium**** > 0.6 (14)
Boron = (0.5)
Manganese = (0.5)
Zinc < 1 (0.05)
Copper < 0.001? (0.02)
Molybdenum = (0.01)
Chromium = (0.01)Nickel = (0.01)
Cobalt = (0.01)

Notes:

* Measurement of the "ammonium" concentration by colorimetry with Nessler's reagent actually gives the sum of ammonium ion NH4 + free ammonia NH3. It is agreed that ammonium here expresses the sum of the two.

The minimum doses only apply if there is no other source of nitrogen. The maxima for ammonium and urea are not independent since urea hydrolyses to ammonium; it is the potential total ammonium that counts, or more precisely the free ammonia. There is a balance between ammonia (NH4OH) and ammonia (NH3) in the water, the ammonia dissociating itself into ammonium (NH4) and hydroxyl (OH) ions: this balance depends on the pH and the temperature. The smell of ammonia is perceptible from 20 ppm of NH4 + NH3 at pH 10 and 20°C. The higher the pH, the more free ammonia there is at equilibrium according to the following table (at 25°C) which gives the % by weight: pH 6 = 0% NH3 (100% NH4) pH 8 = 4% pH 9 = 25% pH 10 = 78% pH 10.2 = 92% It is the free ammonia NH3 which is toxic rather than the ammonium ion NH4, which would explain that doses of ammonium + ammonia much higher than 30 ppm may not be toxic at low pH. The wavy strain (Paracas) is resistant to 75 ppm NH3 at pH 10.5 at 20°C, at least for one or two days.

The rate of urea hydrolysis itself depends on pH and temperature. It has happened to us, in the middle of the production season, to mistakenly add 350 ppm of urea without the culture dying (slow hydrolysis?, low pH?, rapid evaporation of NH3?, very resistant strain?).

According to the Melissa 2004 report (page 195), an ammonium concentration greater than 80 ppm under lighting > 3 Klux causes a high production of EPS.

There is possible reduction of nitrate to ammonia depending on the overall reaction: NO3K + 2CH2O (carbohydrate) = NH3 + 2CO2 + KOH

Note in passing that the reduction of nitrate gives an increase in alkalinity, regardless of the reducing agent. This equation means that one kilo of sugar is likely to be equivalent to 500 g of urea as potential ammonia production. **It is therefore the sum of urea plus sugar that must be considered to calculate the toxicity limit, i.e. the practical rule: "daily dose of urea + (daily dose of sugar) / 2 < 50 – 1.7 x (concentration culture medium in ammonium), where doses and concentration are expressed in mg/l** (in the absence of sugar or nitrates, there is no need to take sugar into account

in this formula).

Cases of sudden reduction of nitrates have been observed in the absence of sucrose: the reducing agent in this case would be the exopolysaccharide. This leads to be wary of nitrate levels above 200 ppm, which are nevertheless very frequent.

** According to JFCornet 's thesis : 0.7 ppm of phosphorus and 3 ppm of sulfur are sufficient. It is probable that 0.05 ppm of phosphorus is still sufficient (case of sea water). But it is not recommended to work at less than 5 mg of PO4/litre, and, to allow good productivity, it is necessary to ensure more than 20 mg/litre.

*** Mixed magnesium ammonium phosphate and magnesium phosphate, which are very poorly soluble, easily form crystals in the culture medium if their solubility product is exceeded. There is a relationship between phosphate, magnesium and ammonium,

**** At high pH (> 10.5) the solubility of calcium decreases by precipitation of limestone.

The boundaries are often either unknown or ill-defined. For example copper at the dose used by Zarrouk should be toxic. Limits may depend on growing conditions.

A.19) Elemental composition of spirulina:

Carbon = 468 g/kg
Oxygen = 279 g/kg
Nitrogen = **124** g/kg
Hydrogen = 95 g/kg
Potassium = 6.4 **–16** g/kg
Phosphorus = 6.7 – **10*** g/kg
Sulfur = 6 – **11** g/kg Chlorine = **4.2** g/kg Magnesium = 2 – **3.5** g/kg Sodium = 2 – **6** g/kg
Calcium = 1 – 7** g/kg Iron = 600 – 1800 mg/kg (= ppm)

Boron = 80 mg/kg (= ppm)
Manganese = 25 – 37 mg/kg (= ppm)
Zinc = 40 *** mg/kg (= ppm)
Copper = 8 -10 mg/kg (= ppm)
Molybdenum = 7 mg/kg (= ppm)
Nickel = 3 mg/kg (= ppm)
Chromium = 2.8 mg/kg (= ppm)
Vanadium = 2 mg/kg (= ppm)
Cobalt = 1.5 mg/kg (= ppm)
Selenium = 0.3 mg/kg (= ppm)

(values in bold have been retained to establish MEDFEED food calculations)

* or 12 when spirulina is produced under conditions where little EPS is formed (according to JFCornet 's thesis , page 166).

** highly variable: a recent work gives a calcium content of 7 g/kg (Vonshak (1997), page 149) and it is possible to reach 14 g/kg.

*** can be increased up to 1g/kg if desired.

The composition of nutritional products is given in Appendix 20. Some important differences with the table above will be noted, in particular regarding calcium, sodium and iron; the composition of spirulina is subject to variations depending on the culture conditions. Thus Cornet, (page 125) indicates for spirulina produced at low luminous flux (5 to 20 W/m²), in g/kg:

Carbon = 505 g/kg
Oxygen = 310 g/kg
Nitrogen = 100 g/kg
Hydrogen = 67 g/kg

A20) APPROXIMATE COMPOSITION OF SPIRULINA IN ELEMENTS NUTRITIONAL

Protein = 65% by weight (standard: >50)
Carbohydrates = 15% by weight
Minerals = 7% by weight (total ash: <10)
Lipids = 6% by weight
Fiber = 2% by weight
Water = 5% by weight (standard: <10)

Energy content = 3800 gtcalories or 16 kJ/gram dry.

According to Flamant Vert notices:

VITAMINS

Beta-carotene = 1400 mg/kg = 2330 International Units (IU)
E (Tocopherol) = 100 mg/kg B1
(Thiamine) = 35 mg/kg B2
(Riboflavin) = 40 mg/kg B3 or
PP (Niacin) = 140 mg/kg B5
(Pantothenic acid) = 1 mg/kg B8 or H
(Biotin) = 0.05 mg/kg B12 (Cobalamin) =
3.2 mg/kg (this B12 would not be completely assimilated by the body: see note below B12)

Inositol = 640 mg/kg K
(Phylloquinone) = 20 mg/kg

AMINO ACIDS

Alanine = 47g/kg
Arginine = 43g/kg
Aspartic acid = 61 g/kg
Cystine = 6 g/kg
Glutamic acid = 91 g/kg
Glycine = 32g/kg
Histidine = 10 g/kg
Isoleucine = 35 g/kg
Leucine = 54 g/kg
Lysine = 29g/kg

Methionine = 14 g/kg
Phenylalanine = 28 g/kg
Proline = 27 g/kg
Serine = 32 g/kg
Threonine = 32 g/kg
Tryptophan = 9 g/kg
Tyrosine = 30g/kg
Valine = 40g/kg

PIGMENTS

C-Phycocyanin = 150 g/kg
Chlorophyll a = 11 g/kg
Carotenoids = 3.7 g/kg
(including beta-carotene = 1.4 g/kg)

ESSENTIAL FATTY ACIDS

Linoleic acid = 8 g/kg Gamma-
linolenic acid (GLA or GLA) = 10 g/kg There must be no alpha-
linolenic acid (ALA): if there is, the spirulina is contaminated with another cyanobacterium.

ENZYME

Superoxide-dismutase = 1.5 million units/kg

MINERALS

Chromium = 3 mg/kg
Calcium = 10000mg/kg
Copper = 12 mg/kg
Iron = 1500 mg/kg
Magnesium = 3000 mg/kg
Manganese = 30 mg/kg
Phosphorus = 8000 mg/kg
Potassium = 14000 mg/kg
Sodium = 4000mg/kg
Zinc = 30mg/kg

NB concerning vitamin B12

It circulated in 2006 in certain vegetarian/vegan circles a virulent aversion against spirulina, accused of preventing the assimilation of real vitamin B12. For example, here is a text found on the Internet on this subject:
*“Fermented soy products, such as miso, tempeh, shiitake (dried mushrooms), algae such as **spirulina,** nori contain practically no vitamin B12. Although these foods are often sold in health stores as "excellent sources of vitamin B12" **and** are consumed by the macrobiotic community, they actually contain very little, in case they have any, of active B (cobalamin). Instead, they contain a B12-like element that is not active **and** actually block the absorption of true vitamin B12. »*

Jacques Falquet sums up very well the current state of knowledge on this important subject as follows:

> A variable (but high) proportion of the vitamin B12 present in spirulina is in fact one (or several) analog devoid of B12 activity in humans. This proportion varies according to the spirulina analyzed; that of Hawaii would contain 36%
>
> of active B12 B12 analogues exist in many food products and are naturally detectable in human plasma Vitamin B12 present in multi-vitamin tablets can
>
> spontaneously convert into non-assimilable analogues The real danger of different analogues of B12 is currently unknown (no serious clinical studies)

> The scientific literature does not report any cases of disorders related to B12 analogues of spirulina (more than 30 years of spirulina consumption in industrialized countries)
> The population of Kanem (where spirulina is traditionally consumed) does not seem to be affected by any particular disorders (but pernicious anemia is fatal and its symptoms are "spectacular").

A21) COST PRICE ELEMENTS

(Price in France VAT 20.6% included and at retail unless otherwise indicated)
(These prices are expressed in US$ on the basis of 1 €/US$ in 1999, but they remain roughly valid in 2014 in €)
(The indications of suppliers have no character of exclusivity or advertising)

SUMMARY

Pure **clay** (density 2.2 kg/l) = $0.5/kg

Movies

- Black polyethylene, thickness 0.15 mm, width 3 m = 0.35 $/m² (Arequipa)
- Black polyethylene, 0.15 mm thickness, 8 m width = $0.3/m² per 300 m² lot or $1.17/m² retail - Black polyethylene, 0.3 mm thickness, 6.5 m width = $0.98/m² per 400 m² lot - Fish farming black EVA, 0.5 mm thickness, 4, 6 or 10 m wide, guaranteed for 15 years = $5.08/m² retail - Food green PVC, 0 thickness, 5 mm, width 4 or 6 m, guaranteed 10 years = $6.77/m² retail - Black PVC, thickness 0.5 mm, non-food, width 2.05 m = $1.8/m² per large lot - Black PVC, 1.2 mm thick, food-grade and easily weldable = $6.67/m² in a large batch - gray PVC, thick. 1.2 mm, 1150 g/m², laid by company = $4.5/m² (Spain)

- Geomembrane in flexible PP (drinking water quality), thick. 1.5 mm, installed by company = $20/m² - EPDM black, thick. 1.14mm, 1161g/m², in roll 6.1-12.2m wide = $6/m² -- Greenhouse Polyethylene (at Cd), thickness 0.2mm, width 6.5m = 2 $/m² retail or $0.8/m² per roll of 390 m² (78 kg)

- Greenhouse polyethylene (at Cd), thickness 0.25 mm, width 4 m = retail $0.6/m² (Peru)

- Greenhouse polyethylene (colorless), 200 µ, width 8 m, in rolls of 3500 m² (713 kg) or in rolls of 400 m², width 6.5 m =) = $0.75/m² - Oilcloth (quality thick) = $8/m² retail *Film and tarpaulin suppliers: Celloplast, Route du Préaux, F53340-Ballée, Tel 43984602 or resellers (Mr Bricolage, Agricultural cooperatives)*

Supplier of EPDM sheets: Giscosa, Diagonal, 611-10A planta, 08028 Barcelona (in France Véronique Bellity, 06.68.54.15.55)

Geotextiles

- Bidim, 200gsm, 4m wide = retail $1.68/m²
Suppliers: Building materials

Pool cover - Fiberglass-

polyester, corrugated, 0.9 m wide, 2 m long = $15.7/m² or $11.3/m² (Arequipa) - 3 mm window glass = $20/m² - Galvanized corrugated sheet, width 0.9 m, length 2.5 m = $9.3/m² - Traditional African thatched roof on stakes and treated wood frame = $8/m² covered (Koudougou, Burkina Faso)

- Side-by-side “chapel” greenhouses, covered with anti-UV polyethylene film (all installed, computer and shade included) = $16 to $23/covered useful m² *Suppliers (greenhouses): Richel-Serres de France, Quartier de la Gare, F13810 -Eygalières,* www.richel.fr

sheets

- Flat translucent fiberglass-polyester, width 1 m = 12.3 $/m²
- Flat galvanized sheet, thickness 0.5 mm, 1x2 m = 3.3 $/m²

Wood (raw untreated spruce)

- Raw wooden planks, thickness 27 mm, length 2.5 m = 5.8 $/m² - Planed planks, thick. 14 mm, width 80 mm, length 2 m (in ayou wood) = 50 $/m² (Mr Bricolage)

- Raw wooden battens, 27 x 27 mm, long. 2 m = 0.3 $/m - Raw wooden battens, 3 x 4 cm, long. 2 m = 0.5 $/m - Raw wooden battens, 8mm x 27 mm, long 2.5 m = 0.27 $/m (Mr Bricolage)
- Raw wood rafters, 6 x 8 cm, length 5 m = $1.4/m

- Planed plaice, 14 mm x 14 mm, 2 m long = $0.83/m (Mr Bricolage)
- Edible paints: see on the Internet

Stakes -
steel (tee) painted, long. 1m. = $2.5/piece

Tubes
- 50mm galvanized steel, in 6m lengths = $3.5/m

Galvanized **screws**
- 4x40 mm = $10/200 pieces - 4 x
30 mm (cogscrew) = $0.05 each - 5 x 30 mm
= $5/100 pieces - 8x60 mm (cogscrew) =
$0.17 each - 8x100 mm (cogscrew) = $0.23
each - 8x120 mm (cogscrew) = $0.30 each -
8x140mm (cogscrew) = $0.55 each

Concrete blocks (“cement blocks” in Belgium) of 50 x 20 x 20 cm (delivered on site) = $1/piece

Sand (delivered to site) = $43/cubic meter

Shade nets
- Canisse, width 2 m = $3.5/m²; $1/m² (Bangui, CAR); $1.2/m² (Cotonou)
- Shade (“Malla Rashel” = woven plastic), black, 80%, width 4 m = 1.1 $/m² (Chile)
- Shade (woven plastic), black, 66%, 50 mx 2.8 m = $1.45/m²

Suppliers: Celloplast, Route du Préaux, F53340-Ballée, Tel 43984602 or resellers
(Mr Bricolage, agricultural cooperatives)

Horticultural lamps (complete system with ballast and reflector, Philips Son-T Agro bulb guaranteed for 10,000 hours, 13 W/klux/m²)
400 Watts = $300

Insulation
- flexible multilayer thickness 20 mm (equivalent to 200 mm of rock wool), in rolls of 1.58 mx 10 m = $15/m² - rigid extruded polystyrene in sheet 4 cm thick = $9/m²

Filter cloth supports
- “Grid” Polyethylene mesh 5 mm NORTENE, width 1 m = 4.7 $/m² retail
- Fiberglass mosquito net, width 0.6 or 1 m = $6/m² retail
- Nylon mosquito net, width 1 m = $1.35/m² (Arequipa, Peru)
- 10 mm mesh nylon net = $3/m²

Filters
- Polyester monofilament filtration cloth, 30 microns, width 1.42 m. = $51.3/m² - Polyester monofilament sieving cloth, 315 microns, width 1.58 m = $14.3/m² - Polyester (Tergal) filtration cloth, ordinary fabric for lining = 1.7 to 3.3 $/m² - Screen printing frame, 25 micron monofilament polyester fabric = $165/m² *Supplier of filtration fabrics (30 µ):*

Name of supplier: SEFAR FYLTIS
Address: BP 3175 Lyon Cedex 03, France

tel 33 4 72 13 14

15 fax 33 4 04 72 13
14 00 Postal check account: N° 7878 45 Y,
Center Lille Reference of the 30 µ canvas: Article
reference: 72556AC Designation: Width 1420
mm, length 4 meters, 07- 30 / 21 / PETEX Price (21/01/2000): 362 FF
per meter, plus 20.6% VAT (except for export) + around 4% for (insurance + transport + packaging).

vacuum cleaners

- Professional vacuum cleaner,300 m. cube/h, 20 kPa, 1200 W = $1000 -
Household vacuum cleaner = $300

Pumps

- Aquarium pump, 1000 l/h, 14 W, 220 V = $31
(Price may drop to $24 for large quantities)
(Valuable pumps can be found in Turkey at a much lower price)

- Aquarium pump, 1200 l/h, 32 W, 220 V = $37
- Vacuum-cellar pump, vortex, 16000 l/h, 1 kW, 220 V = $182
- Cellar vacuum pump, vortex, 5000 to 12000 l/h, 300 to 400 W, 220 V = $100
- Ordinary cellar pump, 5000 l/h, 200 to 300 W, 220 V = $60
-Security transformer for aquarium pumps (with insulation screen connected to earth), 500W=$100

Suppliers (Maxi-Jet aquarium pumps): Aquarium Systems 43 rue Gambetta, F57400-Sarrebourg, Tel 0387031098 or aquarium shops

Presses -

Stainless steel with upper screw, for fruit juice, 4 liters =
$190 *Supplier: Etablissements J. Perraud, 7 route Nationale, F42470- Saint-Symphorien-de Lay, Tel 0477647879*

Taps - all

plastic, 25mm diameter = $30

Water meters - all
plastic, diameter 38mm = $350

Air compressors
- Aquarium type: 300 l/h, 6 Watt = $27
- Aquarium type: 150 l/h = $12
- Without oil: 8 bars, 12000 l/h, 1100 Watt, 6 liter tank = $215
- 8 bar compressed air hose on reel, 20 m = $48
- Hose for compressed air 8 bars in spring, 5 m = 20 $
- 4 mm PVC pipe for aquarium = 0.53 $/m
- 3 Faucet Dispenser for Aquarium = $4.7

Programmers
- In 220 V alternating = 20 to 28 $ (France and Chile)
- In 12 V DC = $120

Photovoltaic
- Monocrystalline Si panel, 12 V, 22 W = $270
(+ Regulator/battery charger = $100)

- 12 V, 15 AH, waterproof = $50
- Electric current converters from 12 V DC to 220 V power 40 W = $120 power
100 W = $230

Gearmotors

- 180 W, 220 V = $251 - 30
rpm, 100 W, 220 V = $240 - 20 rpm, 80
W, 220 V = $208 - 20.8 rpm, 10 W
restored, 220 V, asynchronous motor (Crouzet ref 80667-009-INV) = $230

Extruders (Extruder guns for silicone in ladles) - manual, capacity 300ml,
SIKA model = $37 ($47 in Chile) - manual, capacity 300ml, imported from
China *(good quality)* = **$3** - manual, capacity 600 ml, SIKA MK5C model = $49 -
compressed air, 600 ml, SIKA DKR600 model = $267 - pusher (for making sausages),
stainless steel, 10 litres, manual = $500 - 60µ food-grade PE sheath, diameter 50 mm
= $24/km

Suppliers (Sika spray guns): Sika, 101 rue de Tolbiac, F75654-Paris cedex13, Tel 0153797900 or resellers (building products)

Dryers

- Electric dryer, power 600 Watt, Stöckli model with 3 trays = $67 (Switzerland); the additional tray = $1.7 *Suppliers: A. & J. Stoeckli, CH-8754- Netstal GL or resellers (in Switzerland)*

Grinders

- manual grinder (Corona) = $20 (Chile)

Packaging

- Heat-sealable metallized plastic bags, vertical or not, capacity 800 g of ground spirulina = $0.41 each per 5,000 units or $0.34 each per 10,000 units; capacity 100g = $0.078 piece per 10,000 units (unprinted) or $0.113 printed.
- electric bag sealer for aluminized plastic bags = $333 *Supplier: Bernhardt, BP 69, F62201-Boulogne/Mer, Tel 0321315091*

Chemical products

- Hydrochloric acid 33% = $1.17/litre - Citric acid
in 25 kg bags = $1.9/kg (Costa Rica)
- Phosphoric acid 78% in jerrycan (24% P) = 0.6 $/kg (Spain)
- Phosphoric acid 85% in 25 kg drum (27% P) = 1 $/kg (Costa Rica)
- Zootechnical sodium bicarbonate in 25 kg bag = $0.35/kg - USA natural sodium
bicarbonate at 99.8% purity,
in 25 kg bags = $0.4/kg (Costa Rica)
- Dietary sodium bicarbonate per 500 g = $2.7/kg
- Liquid butane = $1.3/kg in returnable 13 kg bottles; $0.69/kg (Chile); $0.713/kg
(Cotonou) + deposit
- Light technical sodium carbonate = $1/kg
- Ground raw sodium chloride in a 50 kg bag = $0.22/kg; $0.083/kg
(Arequipa), 0.117 (Spain)

- Edible sodium chloride (fine salt) in a 50 kg bag = $0.27/kg
- Food grade sodium chloride (fine salt) in 10 kg bag = $0.38/kg
- EDTA disodium salt, 2H2O, per 1 kg = $50/kg
- Ferfol (Iron chelated with EDTA at 13% iron), per 1 kg = 25 $/kg
- Liquid carbon dioxide in a 30 kg bottle = $0.863/kg
  (Iquique, Chile) bottle included, or $0.63/kg (Arequipa,
  Peru) + bottle ($2/month + $233 deposit)
- Liquid carbon dioxide in 22 kg bottle =
  $3/kg (Alès, France) + bottle ($8.8/month + $200 deposit)
- Liquid carbon dioxide in 25 kg bottle (Chile) =
  $1.25/kg + bottle ($5.8/month) [Regulator = $12]
- Bulk liquid carbon dioxide, storage rental included, excluding vaporizer (cost $4,500), for 6 tons/year
  = $0.5/kg
- Crystallized potash nitrate, fertilizer, in 50 kg bags = $0.68/kg
- Chilean soda nitrate, 16% nitrogen fertilizer, in 50 kg bags = $0.53/kg

- Trace elements in concentrated solution (J. Falquet formula) = $0.033/kg of spirulina - Bulk liquid propane = $0.5/kg - Crystallized monoammonium phosphate, fertilizer, in 25 kg bags = $1.05/kg - Phosphate technical dipotassium in 25 kg bag = $3.58/kg - Sequestrene 100 SG (Iron chelated with EDDHA at 6% iron), per 1 kg = $42.5/kg

- Anhydrous soda in 1.3 kg box = $3.33/kg, in 25 kg bag = $1.63/kg
- White sugar in a 1 kg bag = $1/kg (1.17 in Bangui)
- Crystallized brown sugar in a 50 kg bag = $0.35/kg (Arequipa)
- Crystallized dipotassium sulphate in a 25 kg bag = $0.48/kg or in a 5 kg
  bag = $2.3/kg
- Crystallized magnesium sulfate, fertilizer, in 25 kg bags = $0.32/kg
- Iron sulfate for analysis (FeSO4, 7H2O), 1 kg bottle = $35/kg
- Zinc sulphate (ZnSO4, 7H2O) for analysis, 1kg bottle = $25/kg
- Urea = urea in pearls, agricultural, in 50 kg bags = $0.25/kg; $0.28/kg
  (Spain), $0.27/kg (Arequipa)

Laboratory equipment

- Anemometer (wind speed measurement) from 0.2 to 30 m/s (at Conrad in January 2006) = 30 euros

- White food PE basin, 35 liters = $28
- Electronic scale 5 kg = 50 $
- Electronic scale 100g (at 0.1g) = $167
  - Electronic scale 250 g (at 0.05 g) Voltcraft (at Conrad January 2006) = 60 euros
  - Calibration weight 100 g = 13 euros
- CO detector (at Conrad in January 2006) = 40 euros
- Monocular microscope = $142 to $333
- Portable microscope (x 100) = $50
- Hydrometer = $17 to $29
- Alcohol thermometer = $3 to $17
- Infra-Red thermometer (contactless measurement) = $50
- Electronic thermometer-humidimeter = $25 to $98 - Professional pH meter = $400 to $580 (including electrode $60 to $100) - pH-meter-thermometer = $277 - "Piccolo" pH-meter = $154 - Simplified pH meter (type “pen”) = $58

- pH 4 -7-10 standards (60 vials) = $100 - pH 4 –
7 – 10 standards (15 capsules or “pillows”) = $22 - Aquamerck
ammonium 0.5 – 10 ppm (150 dosages) = $64 - Merckoquant
nitrate strips (100 doses) = $50 (€80 in 2018)
- Merckoquant sulfate strips (100 assays) = $37 - Merckoquant
calcium + magnesium strips (100 assays) = $37 - Merckoquant calcium
strips 10 – 100 ppm (60 assays) = $69 - CO2 analyzer in the air, IR = $400
- Digital luxmeter 50 Klux = $50 - Digital oximeter (Conrad 2007) = $216

Analytics, $/unit

- % protein = 15 - %
moisture = 7.8 - %
raw ash = 6.7
- %GLA = 97
- Total phosphorus = 18.3
- Nitrates = 24.7
- Iron = 26.2
- Other metals = 20 (average)
- Beta-carotene = 100
- Microbiology = 64
- Fatty acid profile = 150
- Cyanotoxins = 250 (for a dozen simultaneous samples)

Sets, Greenhouses

- Culture pond under tunnel greenhouse with paddle wheel (1000 m²) = 25 $/m²
- Greenhouse in PE film on steel frame (1000 m²)
tunnel type = $7/m² ventilated and
shaded multispan type = $19/m² (in 2000)
- Modern greenhouse for hydroponic cultivation (tomatoes for example) fully equipped with computer, auxiliary lighting, modulation of shade and ventilation: $125 excl. VAT/m² for ten hectares (in 2012, in Europe)
- Complete spirulina farm with greenhouse basins, rack dryers, land, etc…but excluding purification = $250/m² (in 2002)

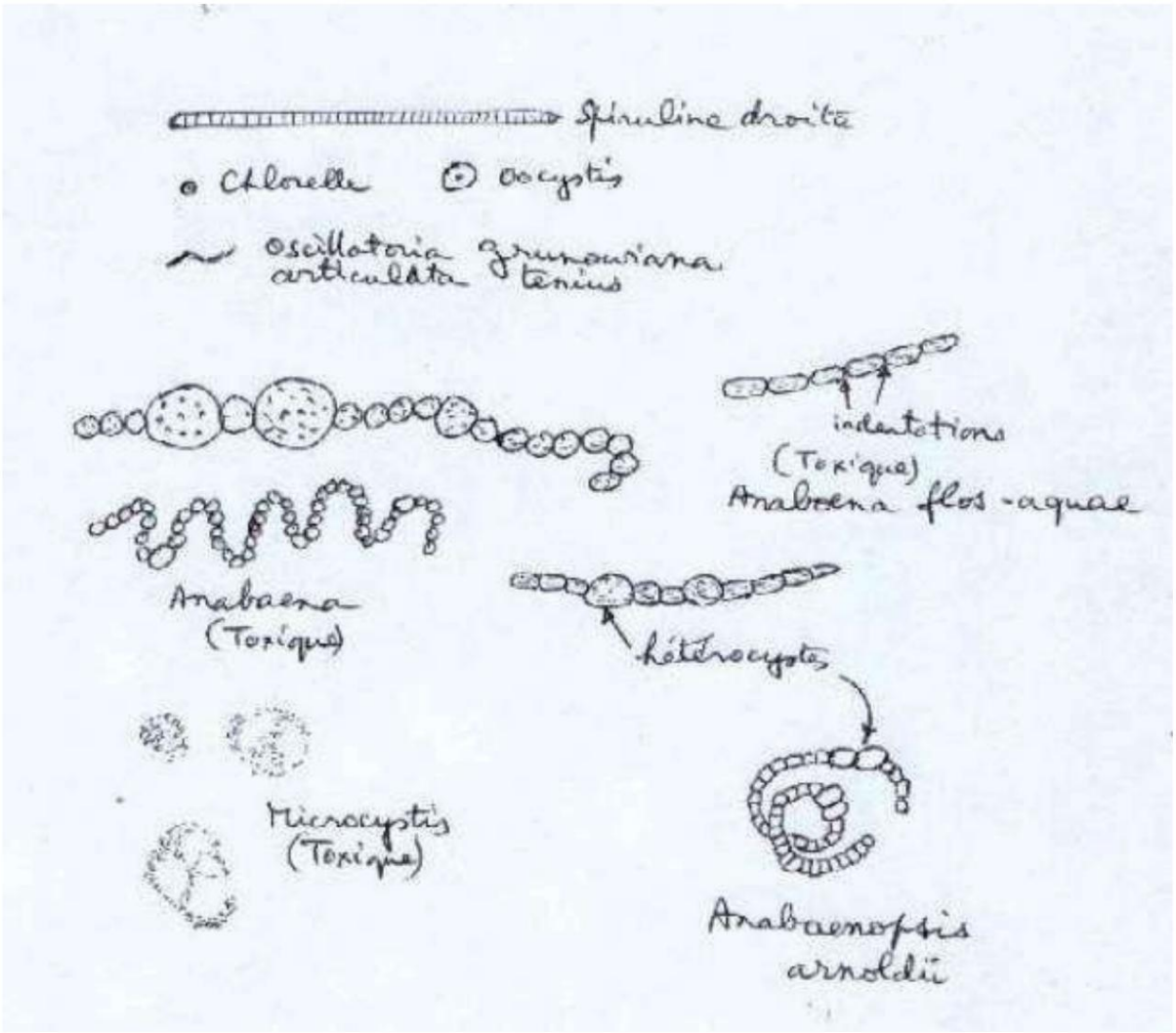
Dry Spirulina (Sale price excluding tax)

The selling price of dry spirulina is extremely variable depending on the location, the quantities, the quality, the packaging, the situation, etc. In 2012 the international price per tonne from China or India fell to around $5/kg. At the retail level, spirulina powder can be found at around $150/kg in 2014 in France, while in capsules it is sold in pharmacies at around $300/kg.

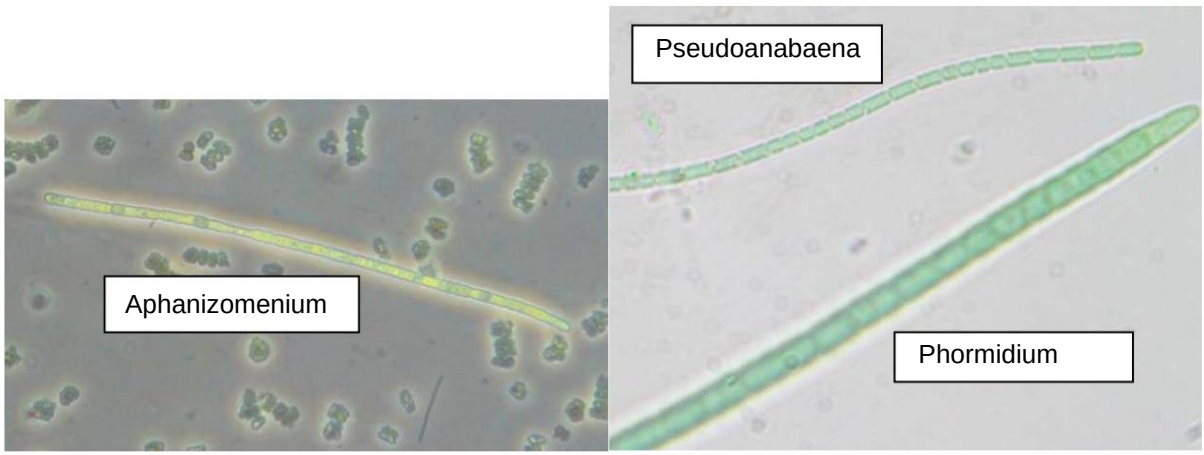
Freight

By air, from Madagascar to France = $3.33/kg

A22) BOARD TO COMPARE SPIRULINA TO OTHERS CYANOBACTERIA:



Photos of cyanobacteria seen in spirulina cultures (Sarl Limnologie, Rennes):



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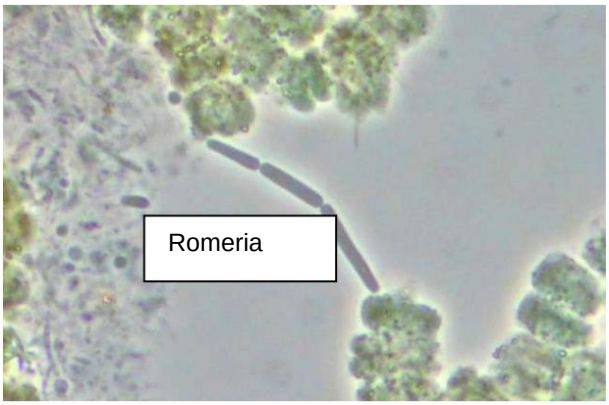
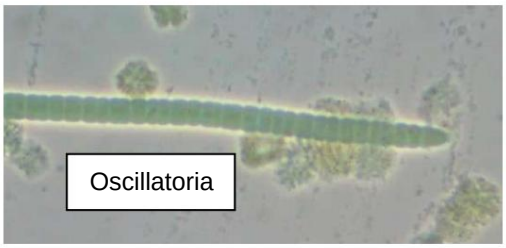
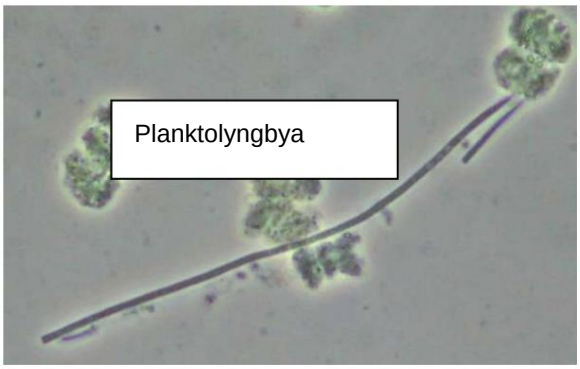
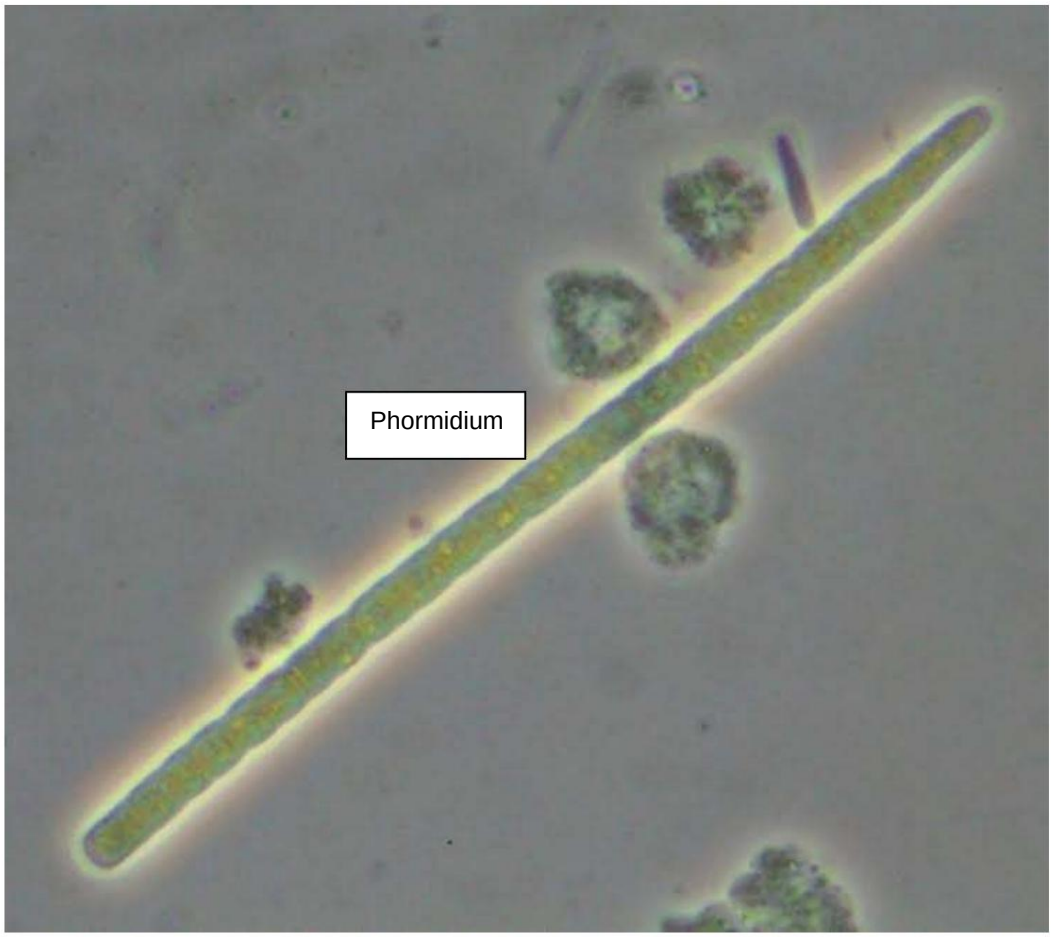
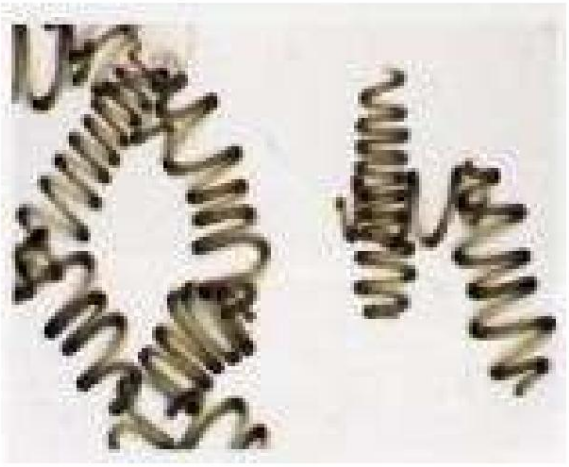








A23) SPIRULINA SEEN UNDER THE MICROSCOPE

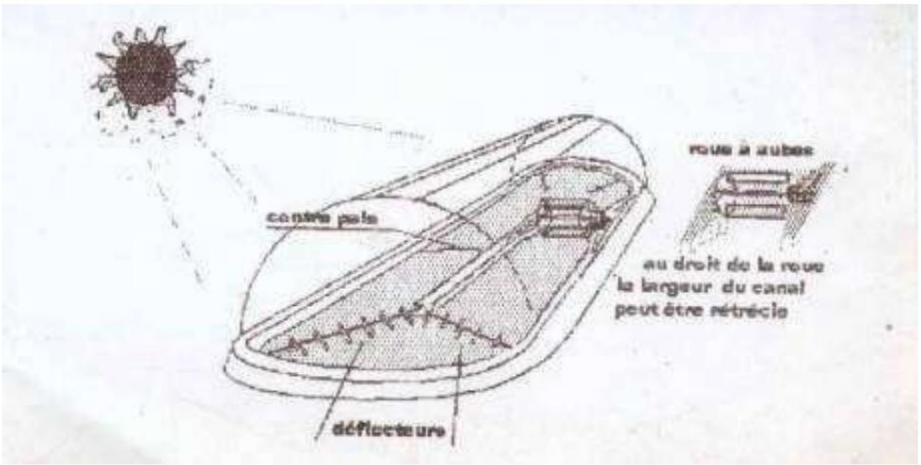


The dry weight of an average filament of spirulina is about 3µg.

The winding direction of the turns of spiral spirulina is most often counter-clockwise if you look over the spiral while descending, but not always. It depends on the strains but not on the northern or southern hemisphere. And within the same strain (Lonar for example) we can find spiral trichomes in both directions rubbing shoulders.

A24) FOR THOSE WHO HAVE ELECTRICITY:

A24.1) AGITATION BY PAD WHEEL



Paddle wheel agitated tanks are longer than wide, with rounded ends and a central partition and preferably deflectors at corner direction changes. The paddle wheel is installed on one of the sides or at one end, between the edge and the central partition. The axis of rotation rests on two ball bearings fixed on solid supports, generally concrete. At the right of the wheel the width of the channel can be narrowed without inconvenience; on the contrary, it makes it possible to reinforce the supports and to shorten the wheel, thus making it more solid.

The wheel comprises, for example, 4 or 6 blades or vanes firmly held on disks fixed to the axle and with a diameter close to 80 cm. The height of the blades is

the order of 20 cm. To minimize the damage caused to the spirulina, it is good to round the leading edge of the blades and if this edge is curved it must attack "with the back of the spoon" and not the opposite as we do intuitively. The construction of the paddle wheel should preferably be made of plastic (rigid PVC 4 mm thick or more) or 304 stainless steel or galvanized steel. An electric gear motor drives the shaft at a speed of approximately 20 revolutions per minute. Its useful power must be of the order of 1 Watt/m² of pool or more; in a greenhouse, provide an outside air inlet on the motor fan. A variable speed drive is convenient but expensive. Belt or chain transmission is recommended. For small pools, the paddle wheel can be mounted directly on the axis of the gear motor. It may have only two blades, which has the effect of causing an artificial swell propagating to the end of the basin and contributing to the agitation. It is useful to protect the bottom of the pool, if it is made of plastic film, in line with the blades: for example by stainless steel or cement plates (cement can be poured on site). The distance between the bottom of the blades and the bottom of the basin or these plates must be small, but sufficient so as not to risk touching the bottom or damaging the spirulina (5 cm seems correct).

It is accepted that the circulation speed of the culture must be 20 to 30 cm/second to obtain good agitation. To reduce flow irregularities and the accumulation of sludge in certain places, one can install deflectors or counter-blades creating whirlpool.

There is a debate concerning the best direction of rotation of the liquid in the basin: for some the best would be the anti-clockwise direction. For others the clockwise direction would be taboo! As far as we are concerned, we have no special recommendations.

A24.2) VACUUM FILTRATION

The use of a moderate vacuum (a vacuum cleaner giving a vacuum of 15 kPa – that is to say 1.5 m of water column – is sufficient) makes it possible to accelerate the speed of filtration. For this, we use a canvas resting on a rigid support (solid grid), placed on a sealed tank resistant to vacuum. This tank is connected to the vacuum cleaner. The culture to be filtered is pumped into the basin through a strainer serving as a sieve or sent to the filter cloth through a sieve. A "vortex" type vacuum pump is recommended so as not to break too many spirulina. A cellar vacuum type pump, automatically controlled by a float and equipped on its discharge with a tightly sealed non-return valve, ensures the automatic maintenance of the level of filtrate in the vacuum tank.

Instead of the vacuum cleaner+vacuum pump pair, a liquid ring vacuum pump (eg Sihi pump) capable of sucking in both air and water can be used.

During filtration, if necessary unclog the cloth with a rubber squeegee. The inflow of liquid is stopped and the biomass is waited for to be sufficiently low in water, then the biomass is recovered with a squeegee.

The filtration rate depends of course on the quality of the culture and the frequency of unclogging, but it can be around 8 kg of dry spirulina/hour/m² of filter.

A24.3) PRESSURE FILTRATION

The culture pumped through a sieve can be sent in a sleeve-shaped bag closed by a clip, floating in the basin. If the bag is vertical and outside the culture, of small diameter (< 6 cm) and of great length (> one meter), filtration can be done by gravity with good efficiency. But the bags are difficult to empty.

A24.4) CONTINUOUS FILTRATION

Various devices exist (vibrating sieves, rotating drums), but are more suited to industrial than artisanal conditions. But models adapted to small units appear and seem promising.

A24.5) VACUUM SPIN (to replace pressing)

This is a variant of § A24.2. If the biomass is left on the vacuum filter long enough (for example 10 minutes for a thickness of 5 mm), the interstitial water is eliminated as in the case of pressing. Compared to pressing, this system allows the possible washing of the biomass (operation that we generally consider useless, even harmful depending on the case, see § 8.2), but sometimes essential when the culture medium is too dirty.

It is also possible to use the vacuum filter only for dewatering; in this case the volume of liquid is low enough to dispense with the emptying pump in the tank.

A good spin can require a stronger vacuum than simple filtration.

A24.6) WRINGER SPIN (to replace pressing)

The spinning of the biomass leaving the filter can also be done in a basket spinner equipped with a filter cloth and rotating at a sufficiently moderate speed not to break the spirulina. This system also allows the washing of the biomass. We do not consider it within the reach of a craftsman, except to use a wringer washing machine.

A25.7) EXTRACTION BY COMPRESSED GAS (to replace pressing)
It is a variant of § A25.5 where the vacuum is replaced by a gas pressure of up to 5 bars without the risk of breaking the spirulina if the biomass is of the correct quality.

A25) WINTERING

In areas with cold winters, harvesting can continue as long as the maximum temperature does not fall below 15°C. Then, when the temperature of the pools is below 10°C, it happens that the spirulina settles at the bottom and turns yellow. It is necessary to avoid approaching the winter at pH < 10 and to agitate too much with the pump during the winter to avoid the risk of "bleaching" of the medium and the death of the spirulina.

If the winter is mild enough (> - 8°C) and if the environment is not deficient, spirulina can survive very well in the greenhouse and restart on sunny days, but it is prudent to shade as long as the temperature of the pool remains below 20°C. During the winter it is a good idea to agitate from time to time with a broom to resuspend and aerate the sludge at the bottom. At the end of winter, if all goes well, the culture medium is renovated (very low turbidity, little or no sludge, pH = 10, excellent harvestability). However, there is the theoretical danger that during the winter contamination may occur (possibly toxic foreign cyanobacteria): carrying out a toxicity test before starting the harvest would be good.

In areas with a strong rainy season, the basins must be covered. If this is not possible, we can continue harvesting by purging the culture medium, and adding the corresponding salts, but this is expensive in salts while the harvest may not be able to

dry off. We can therefore prefer to stop production, then empty and thoroughly clean the basins and restart cultivation when the weather returns.

It is always necessary to keep one or more reserves of good quality semen, but a fortiori in the event of an annual stoppage. The reserve should be kept in a place sheltered from bad weather, in the shade (not in the dark during the day), at a moderate temperature (20 to 30°C) and shaken from time to time. It should neither be too concentrated nor too diluted in spirulina (Secchi = 2 to 4 is fine). It is necessary to "transplant" the reserve crop, that is to say start another reserve, sown from the first every two to three months to maintain its quality. Note: a culture, even a reserve one, should never be closed tightly: it needs air, and a good way to provide it is to stir by bubbling air.

In the event of a prolonged stoppage of harvesting on a pond in production, it must be permanently shaded and agitated at least from time to time.

A26) TRACE ELEMENT FORMULAS

A26-1) Formula by Jacques Falquet, 1997 (Antenna Technology, Geneva):

(Concentrated solution to facilitate transport)
(Dissolve the products in order, one after the other, waiting for the previous one to dissolve)

Citric acid = 100 g / liter
*Borax = Na2B4O7.10H2O =
75 g/liter MnNO3.4 H2O = 45.6
g / liter ZnSO4.7H2O = 35 g /
liter CuNO3.3H2O = 9.2 g / liter
*KCr(SO4)2.12 H2O (chromium
alum) = 5.4 g / liter *MoNa2O4.2H2O (Sodium Molybdate)
= 3.5 g / liter *Co(NO3)2.6H2O = 0.2 g / liter *
Ni(NO3)2.6H2O = 2.9 g / liter *NH4VO3 (ammonium
monovanadate) = 0.94 g / liter *Na2Se2O3,H2O (sodium
selenite) = 0.2 g / liter Demineralised water = qsp 1 liter

[Note that as it ages this solution often releases a foul smell of sulphurous gas (composed of volatile and toxic selenium)].

*These elements can be omitted if necessary.

Dose to use: 5 ml contain the trace elements of one kg of spirulina harvested.

A26-2) Simplified formula from JP Jourdan (without selenium or cobalt, but with reinforced zinc)

ZnSO4.7H2O = 20 g / liter
Disodium salt of EDTA,2H2O = 7 g / liter (can be replaced by 10 g citric acid/l)
MnCl2.4H2O = 2 g / liter
CuSO4.5H2O = 0.5g/litre
MoNa2O4.2H2O (Sodium Molybdate) = 0.35 g / liter
Demineralised water = qsp 1 liter

Average dose to use = 25 to 100 ml/dry kg harvested, depending on the trace elements provided by the water in the culture medium and the inputs; if you do not know these other contributions, try 50

ml/kg and find the best dose by trial and error. The dose of 100 ml/kg provides 500 mg of zinc/ kg, which is considered nutritionally desirable by many nutritionists, but it is possible that it may interfere with spirulina.

At a dose of 50 ml/kg the cost of this formula is negligible: < 0.04 $/kg of spirulina.

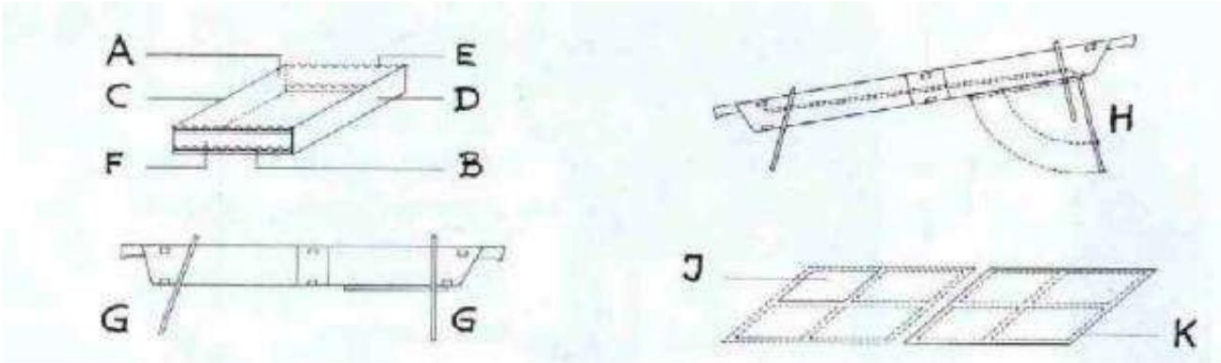
Remarks

The composition of spirulina can be modified in large proportions concerning iron and trace elements according to what nutritionists recommend. Some say, for example, that there is too much vitamin B12 in spirulina: the supply of cobalt has therefore been removed from the Jourdan formula.

A27) DRYER PLANS

A27.1) "Bangui" model solar dryer (SS4-I.1996 version) by Michel-André THELER, CH-1958 Uvrier/Sion (Switzerland), Tel. (41) 27 203 28 43

Simplified plan (Mr. Theler has complete plans):



Brief description of the element and principle of operation:

Crate (dimensions 200 x 90 x 25 cm) consisting of:

A corrugated sheet A in translucent polyester (top)
A corrugated aluminum sheet B (bottom)
Two sides C and D (plywood)
A front loading gate E (mosquito net)
A window F (mosquito net) at the opposite end This

box rests on 4 fixed feet G (in the resting state and when loading) or it is tilted in order to optimize exposure to the sun and the thermosiphon effect (raising of the rear by a double retractable foot H).

Drying by circulating hot air through 8 plastic mosquito net J frames (total useful surface area = 1.2 m²) on which the extruded biomass to be dried is placed.

Loading using 2 K frames (each supporting 4 frames) introduced when the gate is open and sliding inside the box resting on two inclined side rails.

Productivity in good sunshine: about 300 g of dry spirulina/day.

A27.2) Solar gas dryer (“Davougon” model, 1996 version) by Pierre ANCEL, F-95120 Ermont, Tel. 01 30 72 03 57

This device is built from a clean 200 liter sheet metal drum (diameter about 50 cm, height about 80 cm) to which three support legs have been welded or bolted. At 10 cm above the bottom, pluggable openings, protected by pieces of glued mosquito net, are arranged to allow the entry of fresh air and the regulation of temperature.

At 20 cm above the bottom of the metal angles are welded or screwed to serve support to the drying trays. A removable wooden or metal cover protects against rain and insects while allowing humid air to escape.

The trays are wooden frames fitted with a nylon mosquito net. They are stackable (maximum number = 5)

A butane gas stove (or a burner recovered from a gas stove, mounted on a welded metal support) is used to heat the bottom of the dryer.

Drying can also be done directly by the combustion gases, suitably diluted to adjust their temperature (by adjusting the height of the trays in relation to the burner), but on two conditions:

- good quality burner (not charring and giving a blue flame) - good quality gas (butane gas common in France is suitable)

A27.3) Solar dryer with indirect heating, designed by Claude VILLARD

The dryer consists of a matte black sheet metal box carrying 5 removable trays (wooden frame + nylon mosquito net), fitted on one side with doors allowing the trays to be loaded. The box is raised (feet or uneven from the ground) so as to be able to be supplied with hot air by thermosiphon from a solar collector with air absorber in baked bricks, inclined and oriented suitably according to the latitude of the place. The air inlet to the sensor is the low point of the system and is protected by a mosquito net; this entrance must be placed in a place as far as possible sheltered from dust and other pollutants, and of course out of water.

The box is surmounted by a large chimney, also in matte black sheet metal, surmounted by a protective hat against the rain and carrying a mosquito net to protect against insects and dead leaves. This chimney ensures sufficient draft: for this its height must be close to that of the casing.

A28) SEMI-CRAFT PROJECT OF 5 KG/DAY

It seems interesting to us to summarize here a project of 5 kg of spirulina/day that we had the opportunity to prepare at the request of an interested company in Côte d'Ivoire; it is aimed at groups with electricity, running water and CO2, and willing to invest enough to sell their production on the international market. In a hot climate, the workshop can operate all year round and produce 1.5 tonnes/year; in a temperate climate, half. It is still a process that is not very mechanized, using a lot of manpower. It was made in Adzopé (Ivory Coast), at the La Mé farm (see the video).

A28.1) Basins

4 basins of 3 mx 50 m = 150 m², under 2 greenhouses 8 m wide, with 2 basins per greenhouse, with an alley in the center of the greenhouse between the two basins. Agitation by paddle wheel with 4 or 6 wooden blades driven by a 250 W gear motor (one per basin). Drain sump at one end, drain by gravity or by vortex vacuum-cellar pump. Ventilated and partially shadeable greenhouses, equipped with mosquito nets at both ends.

As a variant, the greenhouse can be replaced by a covering of film stretched over each basin, resting on a galvanized tube resting on the central low wall. The edges of the film are buried. In this variant, access to the pool is limited.

A28.2) Building

All manipulations of spirulina are done in a 70 m² building (which can be used as staff accommodation) whose basement is converted into a harvest room. On the ground floor is the drying-grinding-conditioning of the dry product, as well as a small laboratory and the raw materials store.

The building is air-conditioned, with filtered air ventilation. This facilitates the wearing of protective clothing in force in food industries.

Half of the roof is built to be able to serve as a solar collector without glazing (sheet metal painted tile color) to supply the possible solar dryer.

A canopy houses fans, dryer, vacuum cleaner, compressor, carbonation tank and purification tank.

A28.3) Harvest

The collection device consists of a cement filtration tank, 60 cm deep, 80 cm wide and 8 m long, with horizontal edges lined with a rubber seal, on which rest 4 mobile filtration frames. . These frames have 10 cm high edges and a stretched net on the bottom. The filter cloths are simply placed on these frames.
The culture to be filtered comes from the basins by gravity through a sieve. Each pool has its own supply pipe, equipped with a water meter to know exactly the

volume withdrawn per basin. Filtration can be accelerated by connecting a vacuum cleaner to the tank.

The filtrate is pumped by an empty-cellar pump controlled by a float, located in a manhole at the low point of the tank. The discharge piping, including a non-return valve, passes through the side of the tank so as not to interfere with the vacuum seal. The filtrate is sent to the carbonation tank.

The drained biomass is dewatered in a press located near the filtration. The pressing is done on trays with 2 cm edges, with a pierced bottom (forming a grating). These trays are mobile. The biomass is wrapped in a strong cotton canvas lined inside with a fine nylon canvas, forming a flat "package" of 5 cm maximum thickness placed on one of the trays, while waiting to be put in the press. Several trays can be stacked for simultaneous pressing. The press can be screw or weight with lever arm.

The pressed biomass is loaded into a sausage-making machine (a stainless steel, hand-cranked or motorized “pusher”) and placed in a 50 mm diameter plastic food casing. String knots delimit the length of the sausages which corresponds to that of the extruder gun (about 35 cm). The sausage strings are put in the fridge as they are made. Part of the production may be in the form of shorter sausages for fresh sale. As a variant, the pusher, fixed vertically, serves as a large extruder, with the drying trays scrolling underneath.

The equipment and the ground are washed with water after use, the water being collected in a sump at the low point of the basement and sent to the sewer by a vacuum-cellar controlled by a float.

A28.5) Spirulina food

At the end of the harvest, the filtration tank is used to transfer the salts (weighed in the store located just above and transferred to the tank through a PVC chute) into the carbonation tank, using a jet of water and the pump.

This cement tank, 4 m² in section and 3 m deep, raised by 1 m. above the ground, is connected to a translucent tube allowing to know the level of liquid. It is also equipped with bubblers allowing the injection of CO2 at the bottom. The injection of CO2 (7 kg/day) is done in such a way that no bubbles come out on the surface (a scale makes it possible to monitor this surface). The injection time can be several hours. The fact that the CO2 is dissolved in the absence of light promotes the absorption efficiency, close to 100%, due to the absence of oxygen release. The bubbling also makes it possible to complete the dissolution of the salts and to homogenize the solution.

Carbonation is stopped when the desired pH is reached (generally 9.5), and the solution is then distributed in the basins in proportion to the medium withdrawn for filtration. The transfer is done by gravity.

A second tank of 12 m3, identical, is used as tank for purification of the filtrate by decantation (see Purification). It can be operated discontinuously or in daily batches.

A28.6) Drying

For the extrusion, a glue gun in Sika type pockets (“sausage” in Sika Canada language) of 600 ml capacity, operated by compressed air, is used. The loading of the gun is instantaneous thanks to the packaging of the biomass in sausages identical to the pockets of glue. Alternatively, as said in A28.3, the pusher can serve as an extruder of

big capacity.

The simplest method, and probably the cheapest in investment, is to use Stoeckli electric dryers; a dozen are needed to dry the 5 kg/day, with a night batch. Drying in an electric oven requires a little less work because the trays are larger. The oven can be coupled to a solar collector (on the roof) or to a dehumidifier to save electricity. In the latter case, which is particularly suitable for hot and humid climates, the equipment must not be thermally insulated and the circulating air must be cooled below 35°C.

The dry spaghetti is poured into an intermediate 100 liter container through a funnel of a size adapted to that of the trays. They are crushed with a pestle then ground and bagged. The packages are vacuum sealed by a machine of the type used to package cheese in Switzerland.

A28.7) Staff

This type of semi-artisanal production is particularly suitable for a couple residing on site; there is then normally no need for outside labor if it is considered acceptable to reduce production in the event of sickness or holidays.

With salaried external staff, and to ensure nominal production every day, a minimum of 3 people are needed and preferably 4.

A28.8) Cost price

The calculation program (see Annex A31) does not apply to this type of semi-artisanal project.

However, it can be used as a first approach, provided that the investment is added around $8,000, which would bring the cost price under “African” conditions to around $15/kg.

A28.9) Human conditions for the success of the project

What human conditions must be met for a small spirulina project to succeed?

A solvent demand for spirulina must be expressed before the initiation of the project, and the project must have prospects for further development, following published and recognized nutritional tests, and possibly an advertising campaign.

The local partner must have a strong desire for the project and behave like a true "boss", having the necessary powers and means as well as the material time to take care of the project. It would be good if he visited a neighboring spirulina project so that he could see what it was all about. It is very desirable that he expresses in writing his objectives both vis-à-vis his collaborators and the NGO supporting the project.

This “boss” must not be transferred elsewhere during the project.

The technical manager to be trained must be able to understand the interest of the project and be strongly involved in it. For this he must be salaried and insured

correctly (not "under the table") and work full-time on the project. He shouldn't be lazy. He has to get his hands dirty, make his harvesting tools, train his own team and ensure that there is a good team spirit. He must be convinced of the long-term interest of his new job as a seaweed farmer. He must like to eat spirulina himself and agree to taste his production to check its organoleptic quality. He must be convinced of the need to work hygienically. He must know the prices.

It is important that the leader makes some discoveries himself, or has the impression of making some. It is therefore necessary to quickly give him a certain autonomy and means (small lab), while preventing him from going outside the limits planned for the project (remain realistic).

You need good means of communication with the NGO supporting the project (at least fax), and the will to use them, and this in both directions (local NGO team and NGO-local team).

The project must be reasonably protected from theft and insurrection.

It is necessary to prohibit the access of the project to any unauthorized person, because the experiment shows that the basins are often confused with dustbins (examples of Nanoro in Burkina and Dapaong in Togo).

The staff must agree to - come
very early in the morning to harvest, - be on duty
at noon if the agitation is not automatic.
It is desirable that a member of the team lives on site.

Important visitors must come to see the project, but not too often.

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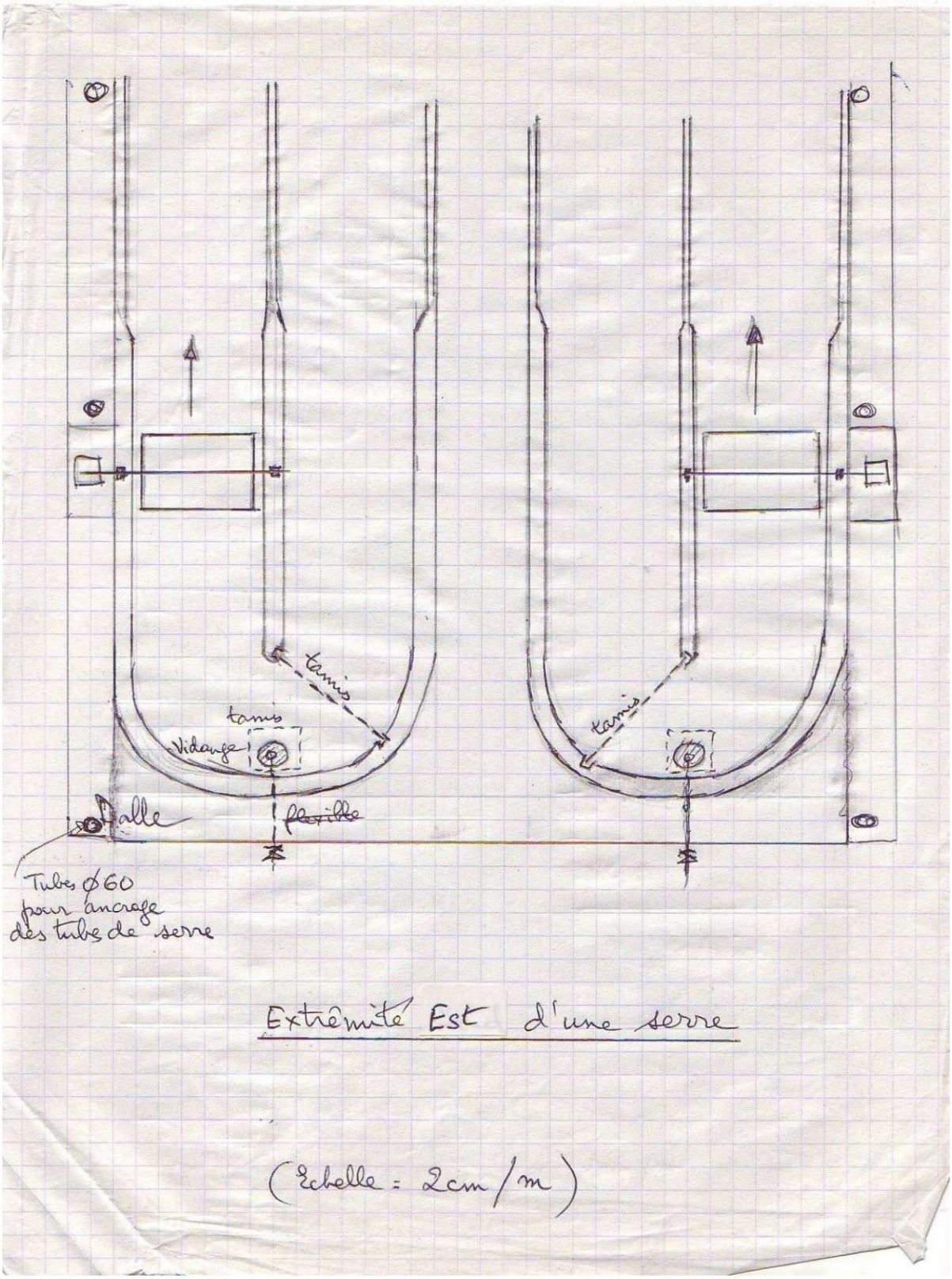
A28.10)

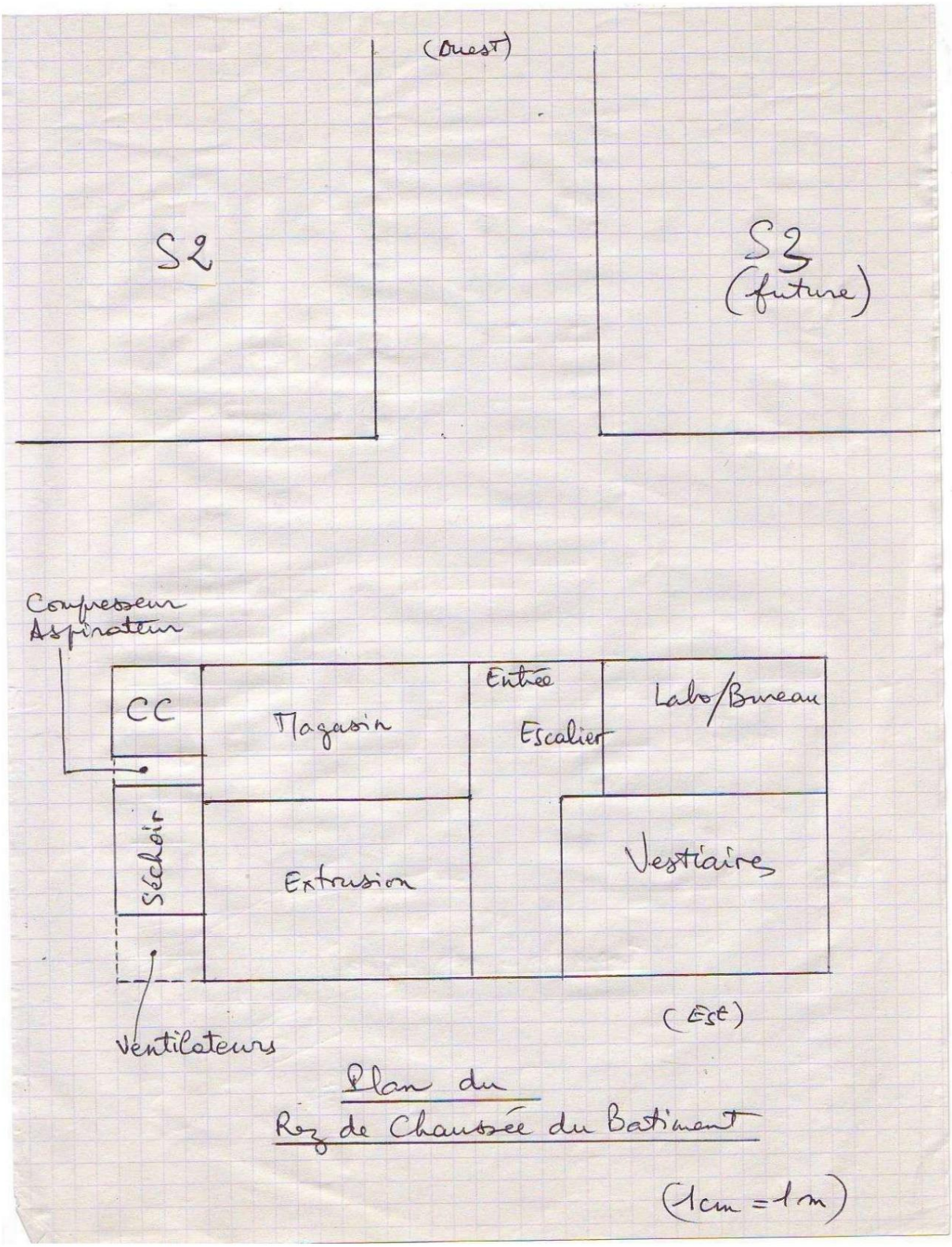
The following diagrams illustrate the above descriptions.

They served as a model for the construction of the spirulina plant at the **SAP La Mé** farm near Adzopé, with a production capacity of 5 kg/day initially and then supplemented to 10 kg/day afterwards (1200 m²). But the CO2 was not used, which changed a little from what had been planned.



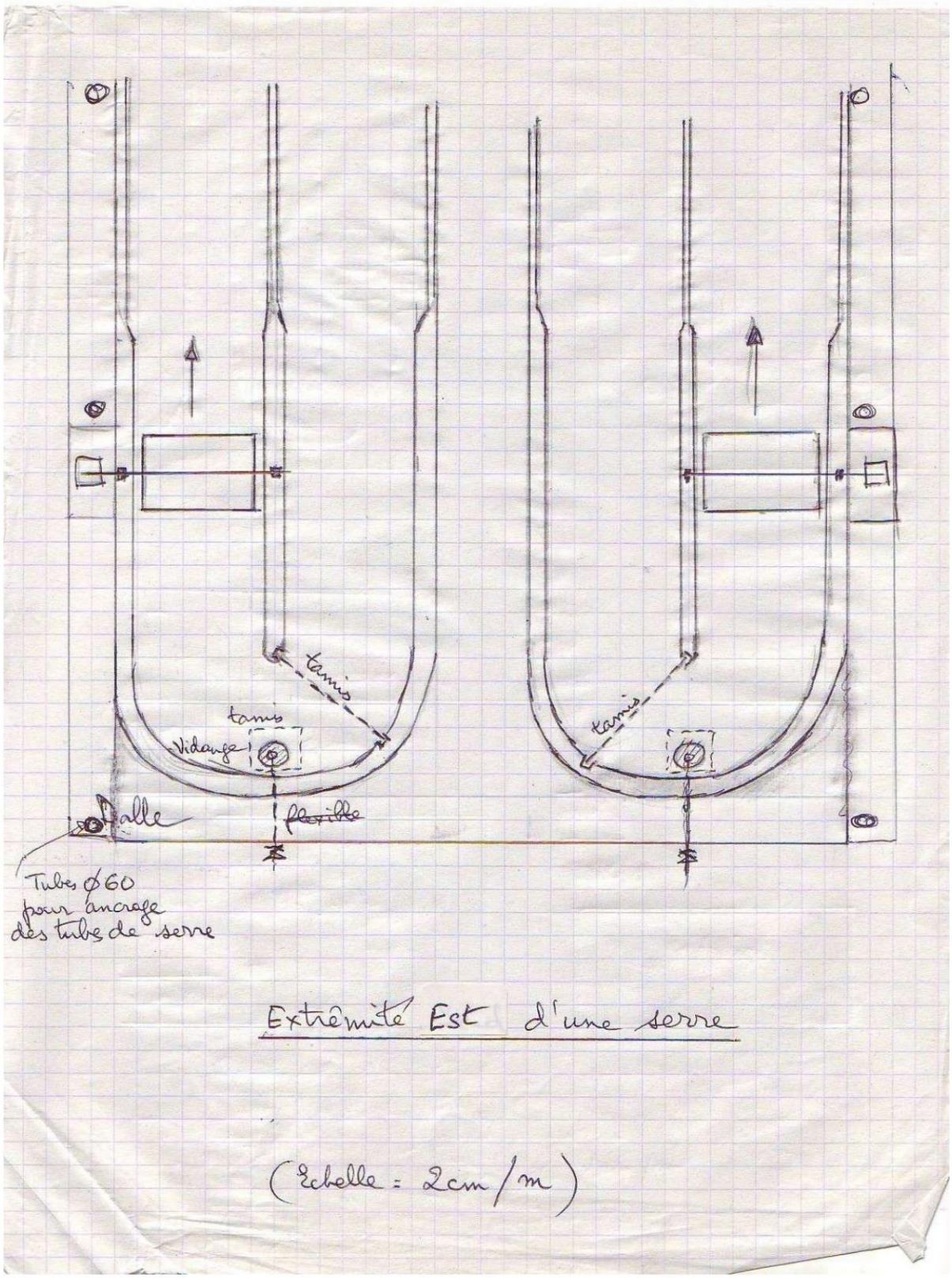
Extrémité Est d'une serre

(Echelle : 2cm/m)



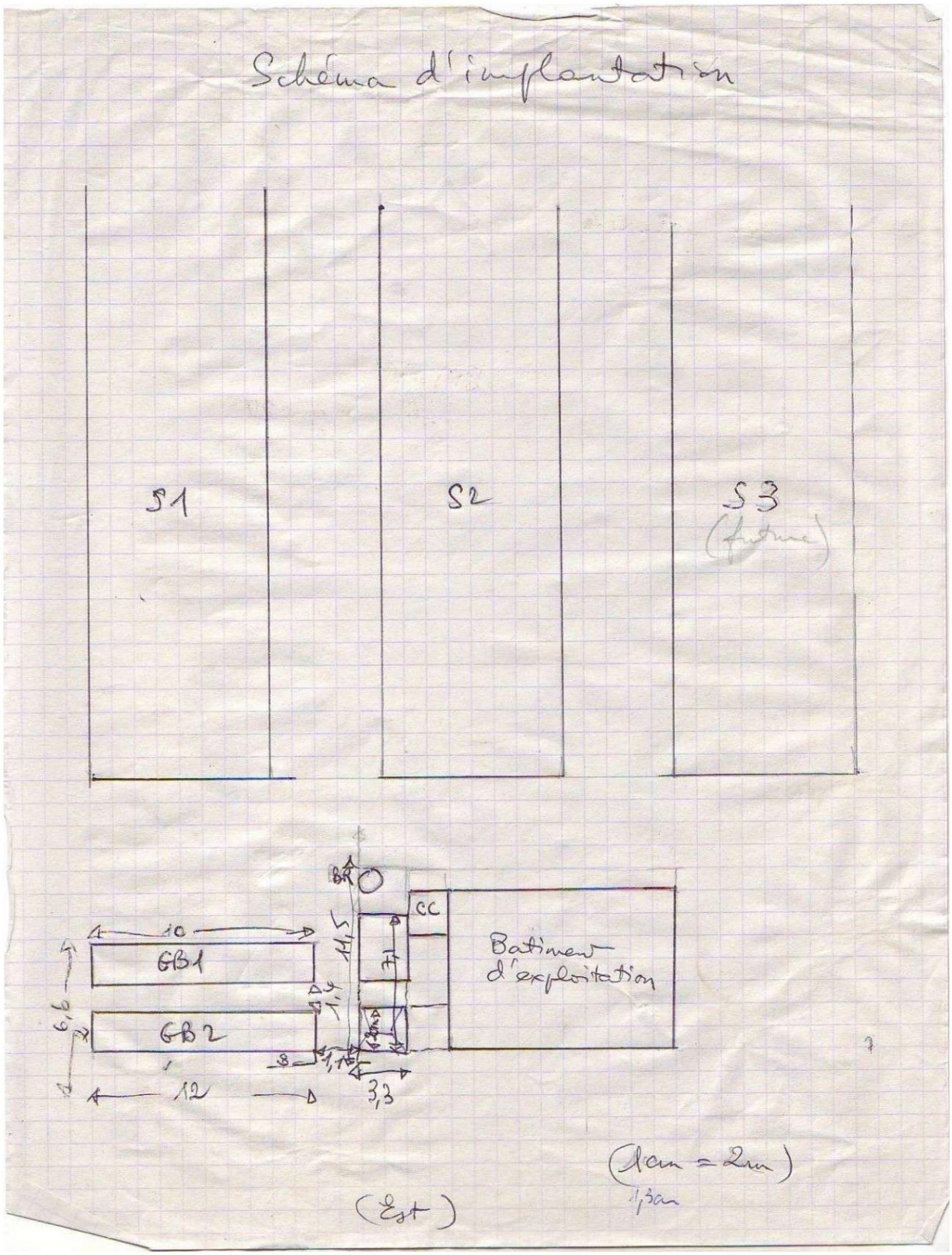
Plan du
Rez de Chaussée du Batiment

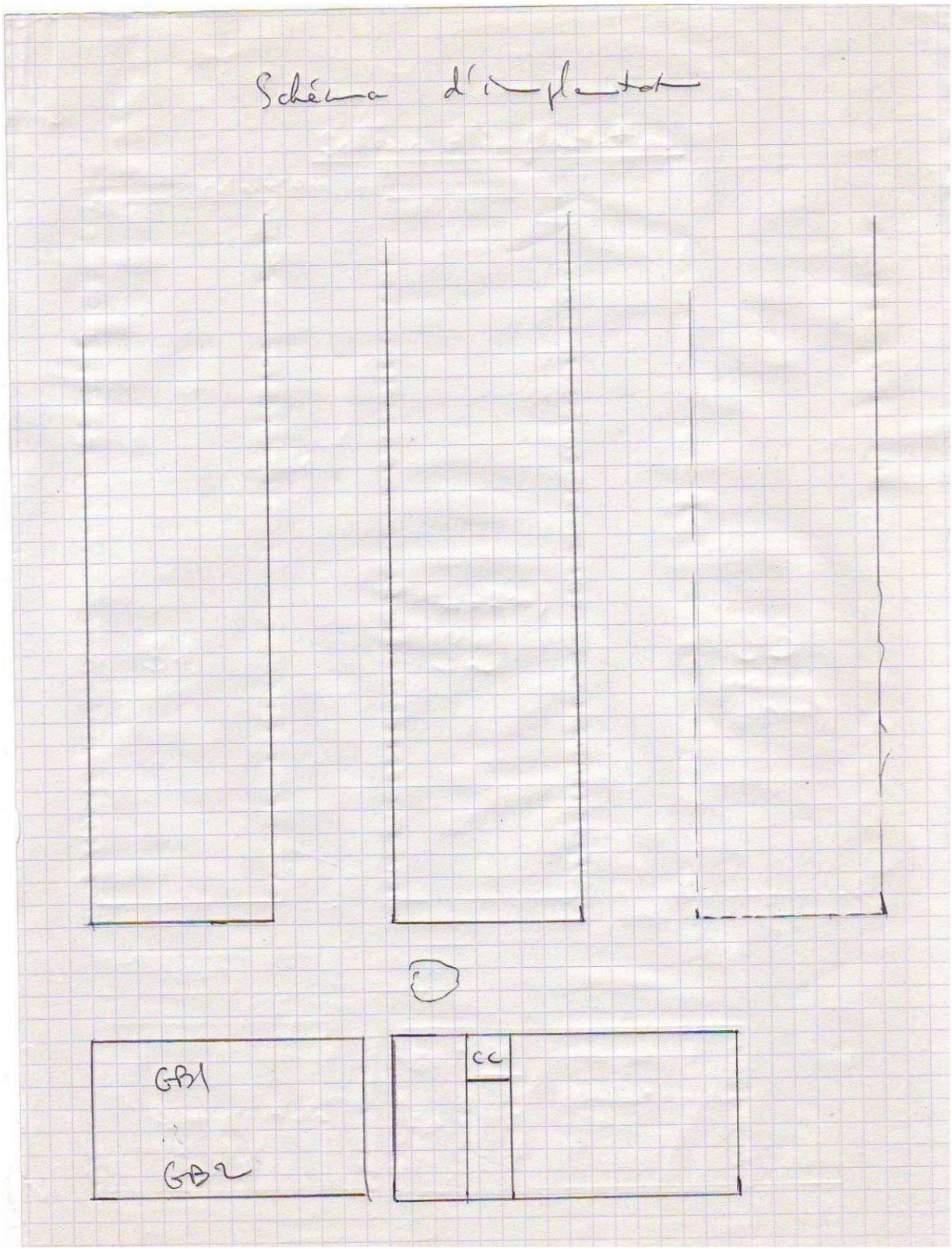
(1cm = 1m)

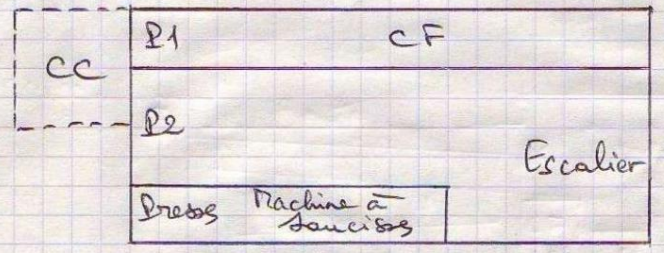


Extrémité Est d'une serre

(Echelle : 2cm / m)

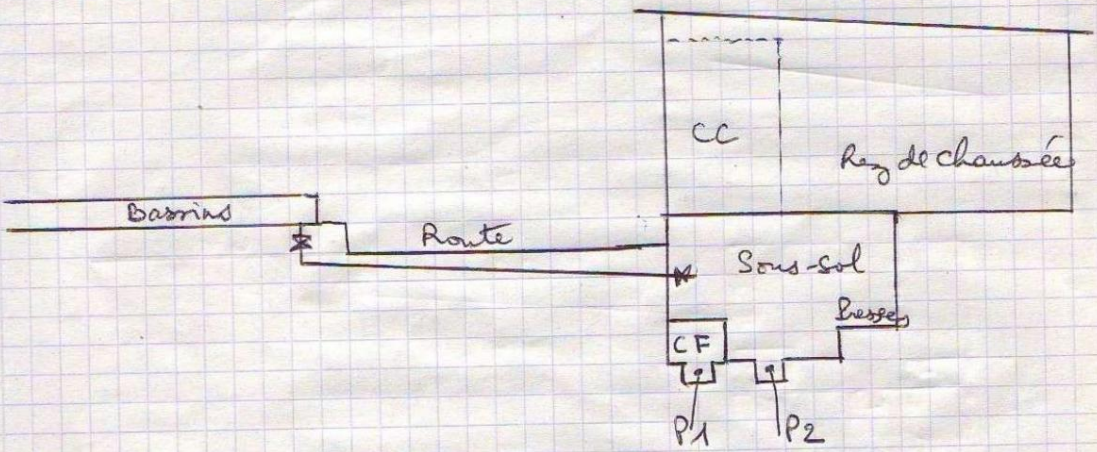






Plan du Sous-sol

(1cm = 1m)



(1 cm = 1 m)

Coupe du Batiment

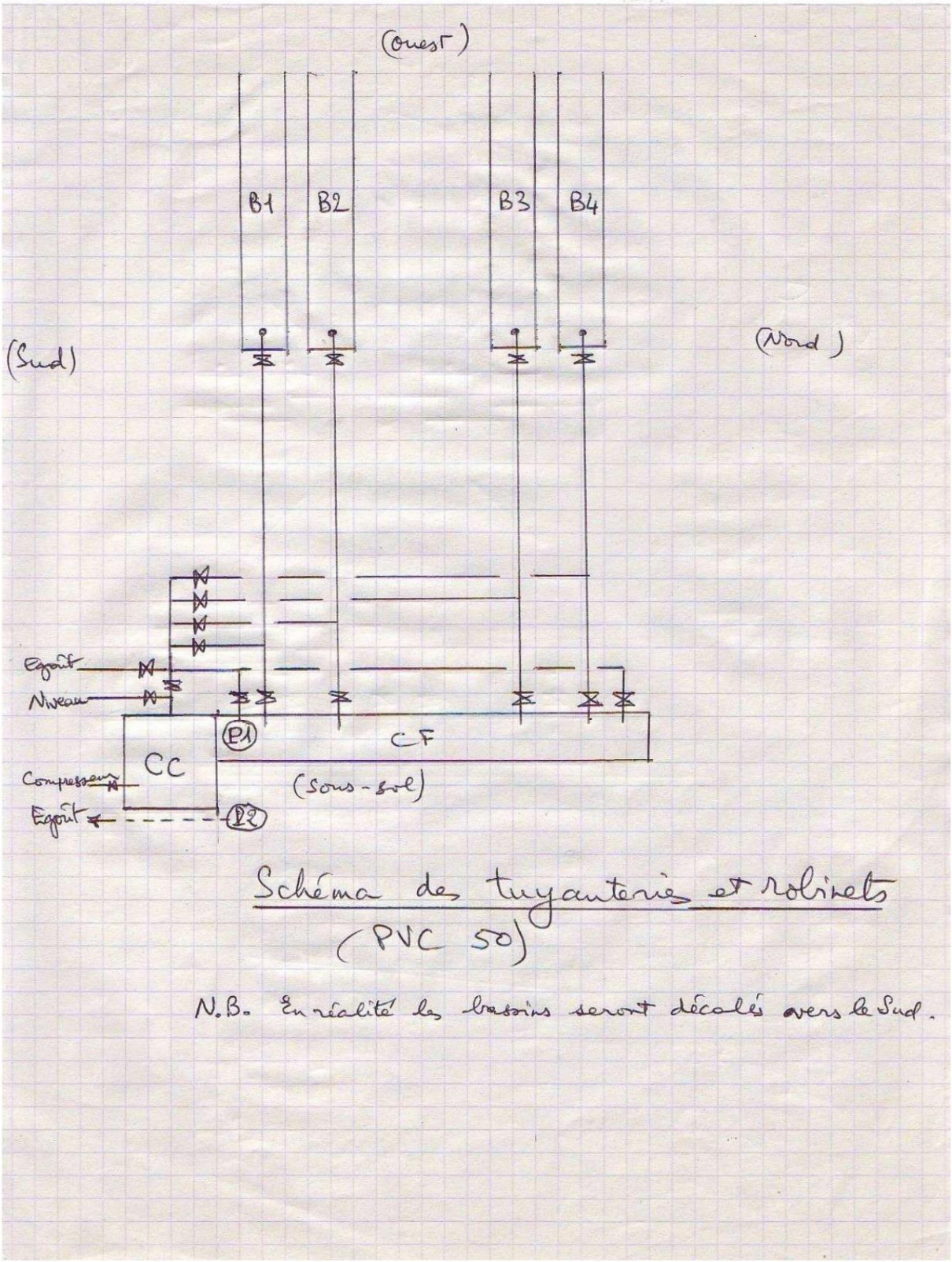


Schéma des tuyauteries et robinets
(PVC 50)

N.B. En réalité les bassins seront décalés vers le Sud.

A28.11) Description of the SAP La Mé farm (message from Lionel Raobelina at the 2008 Tulear Colloquium) :

IN IVORY COAST , AN EXAMPLE OF AN AFRICAN SPIRULINA FARM LITTLE AVANT-GARDE...THE SAP LA Mé

IN IVORY COAST, AN EXAMPLE OF PROGRESSIVE AFRICAN SPIRULINA FARM

Summary Description of a spirulina farm with many interesting points which are detailed in a message sent by its director, Lionel Raobelina, who was unable to attend the Colloquium. The farm has a capacity of 3 tons/year (1200 m² under glass, with centralized harvesting, air-conditioned clean room and thermodynamic drying).

Abstract

Description of a spirulina farm with several interesting features transmitted to the Symposium by its manager, Lionel Raobelina (unable to come to the meeting): 3 ton/year capacity, 1200 m² under greenhouse, centralized harvesting in an air-conditioned room, thermodynamic drying).

Contact details of the Agro-Piscicultural Company of the Mé:

Note :

This report was prepared for the Spirulina Symposium in Tuléar (Madagascar) in 2008.

You can also watch a 3-minute video showing the main details of this installation: click on **http://spirulinefrance.free.fr/Videos/lame2008*.m4v** (rather long loading time).

This video is from 2008, but the installation continues to produce well in 2014.

The farm is located near Adzopé, within a model farm comprising various agro-fish productions. It has 8 cement basins of 150 m² agitated by paddle wheel and **under greenhouse.** Harvesting is centralized in the basement of the building housing the office, store and laboratory. This arrangement allows the filters to be fed by gravity. The harvesting, pressing and extrusion room is treated as a “clean room” and air-conditioned so that staff have no problem wearing the recommended hygienic protective clothing.

Another special feature: the bottoms and edges of the pools are brushed daily, which considerably reduces sludge. There are no larvae.
The spiral strain used has remained the same since the origin 10 years ago, the percentage of straight lines remaining below 1%.

Among the other notable particularities, it is worth noting the method of thermodynamic drying of the biomass extruded into spaghetti: the drying is done in a current of air at 55°C, circulating in a closed circuit, in a 12 m3 cell containing 16 m² of perforated stainless steel trays (holes 1 cm in diameter); a dehumidifier extracts water from the air: it is a 3 kW refrigeration unit. The drying capacity is 10 kg/day of spirulina at 6% residual humidity. The air could be replaced by an inert gas but this has not yet happened.

The farm uses bicarbonate as a source of carbon (in addition to atmospheric air) and the purges of culture medium are sent to a reforestation area of eucalyptus, acacia mangium, koto and teak then to a water reservoir supplying a fish farming

tilapia.

The farm has a strong social role in the region (forest) by creating jobs and supplying spirulina at a humanitarian price, in particular to the anti-leprosy clinic in Adzopé, which was the first to be created by Raoul Follereau. But it makes a big marketing effort by producing spirulina in tablets and capsules in addition to granules. For this, she has taken on a pharmacist in her team.

The installed production capacity is 3 tons per year (7 g/d/m² proven) but produces only 2 tons according to the current demand of the national market (2008).

Lionel Raobelina, who has been managing the farm for 10 years, was unable to attend the Colloquium in Tuléar but he is providing the information above, as well as a film for the participants.

BRIEF HISTORY of the beginnings of spirulina in Ivory Coast

At the end of 1993 Etienne Boileau launched the production of spirulina in the Camiliens dispensary in Davougon, Benin, with the help of TECHNAP, then in August 1994 CODEPHI helped Etienne to install two 8 m² cement basins there. In 1995 Jacques Servant, CEO of Improbois (Côte d'Ivoire) visited the facility and enthusiastically signed a check for the construction of a third 8 m² cement basin.

From then on, Mr Servant decided to also set up a small experimental production of spirulina in his model farm called Société Agro-Piscicole de la Mé, near his plywood factory in Adzopé in Côte d'Ivoire. On October 4, 1998 he came to see me in Mialet (Gard) to ask for my help in order to better run his small spirulina installation and to draw up plans for a large farm on the same land. I give my agreement and propose that Lionel Raobelina, a young chemical engineer, take over from me on the spot. Lionel signs a 3 month contract.

In November 1998 I went to Adzopé accompanied by Etienne Boileau in order to better run the temporary spirulina installation and to draw up plans for a farm to produce 5 kg per day, at the request of the management of Improbois. Lionel joins us and the project, co-signed by Boileau, myself and Lionel, is handed over to the Management at the end of our mission, while Lionel takes up his post.

In February 1999 Lionel decides to extend his contract, and he will finally stay 10 years in La Mé and create a very beautiful spirulina farm there, with a capacity quickly increased to 10 kg/day.
In October 1999 the first phase (600 m²) is ready to start. In December I return to see the inventory accompanied by Boileau.

From the summer of 2000 the capacity was increased to 10 kg/day with 1200 m² of ponds in operational greenhouses. Olivier Barbaroux, from IFREMER-Nantes, comes to report on SAP La Mé and its spirulina farm.

It was a great technical and commercial success. Lionel knew how to surround himself with a team of locally recruited operators and train them thoroughly. At the same time, he collaborated with the health authorities and in particular the neighboring Raoul Follereau Center.

However, production quickly posed a sales problem and Lionel formed a well-structured marketing team, including a graduate in pharmacy, to canvass a large number of pharmacies, particularly in Abidjan. The commercial success was total in a few years so that now the demand far exceeds the capacity of the farm: we could build 2 more, but the management of Improbois has decided not to invest more in this sector.

The civil war did not stop the progress of the farm.

Lionel decided to leave Côte d'Ivoire in 2010, after ensuring that his technical and commercial teams could stand on their own two feet.

A29) CHECK-LIST FOR STARTING UP SPIRULINA ON A NEW SITE

(NB The maximum must be found on site; the rest must be brought)

Greenhouse PE film thickness 0.2 mm (for extendable basin)
“Tupperware” type containers (for humidity testing and storage of fresh biomass)

Basins (preferably white) including one with straight sides
Plastic bucket (preferably white and graduated)
Plastic broom
1 liter plastic graduated jar
Sticky notes
Filter paper type Mellita N°4 coffee filter
Plastic funnel
Straight edge plastic shovel
secchi
Sachets of salts for 8 liters of initial culture medium
Merck water analysis kit (nitrate, sulphate, ammonium, calcium, hardness)
Electronic scales 100 g (at 0.1 g) and 3 kg
Small plastic containers for weighing
Syringes, droppers
Plastic sink bottoms (for press)
Sopalin-type absorbent paper
Thermometer (0 – 100°C), hydrometer (1000 – 1050 g/l)
pH meter with spare electrode
pH 7 and 10 standards in capsules
digital hygrometer
Spare batteries
wash bottle
Aquarium compressor and pumps
Cellar pump
Plastic containers for lab cultures, bedside lamp 40 Watt
Flexible tube diameter 4 mm for air + tee with taps
Flexible tube diameter 10 mm for pump
Garden hose with adjustable jet nozzle
Programmer and multiple sockets
Pocket rule
Magnifier (x25) or microscope (x100)
Artemia cysts (eggs) and miniaquarium for toxicity testing
30 µ polyester filtration fabrics
315 µ polyester fabrics
Plastic grid for filter frames

Extruder

Electric dryer or something to build a solar dryer (mosquito net, plastic film black, fan)

Heat sealable sachets for spirulina packaging

Plastic sheeting repair kit

Stapler and staples

Basic tools (saw, screwdriver, hammer, scissors) + nails, screws

Flash light

100% spiral or wavy spirulina strain

Manual of artisanal culture (book and diskette)

Sodium bicarbonate

cooking salt

Urea

Soluble nitrate

Soluble phosphate

Magnesium sulfate

Potassium sulphate

Soluble calcium salt or lime

Trace elements

Ferfol or Fetrilon (chelated iron) or citric acid or lemon juice

Concentrated hydrochloric acid

Soda or caustic potash or soda ash (or otherwise ash)

Drinking or filtered water

A30) Humanitarian Spirulina in developing countries (Text by P. Ancel dated May 20

NB The text below reflects the opinion of its author who has a long experience of spirulina cultivation in Africa. The author of this Manual largely agrees with this text but would like to emphasize that it is always possible for you to "grow your spirulina" yourself without the socio-economic constraints that P. Ancel rightly points out.
When one cultivates for oneself or for one's own family or even one's neighbours, it is not obligatory to be "profitable" as in a company which must pay staff and present a positive financial balance sheet for lack of disappearance. The advantage of being able to consume your own **fresh** spirulina is such that it is well worth not being "profitable". All things considered, this is similar to growing tasty varieties of tomatoes in your own garden, the cost price of which you will never know, but which you will long remember the pleasure you had in eating them!

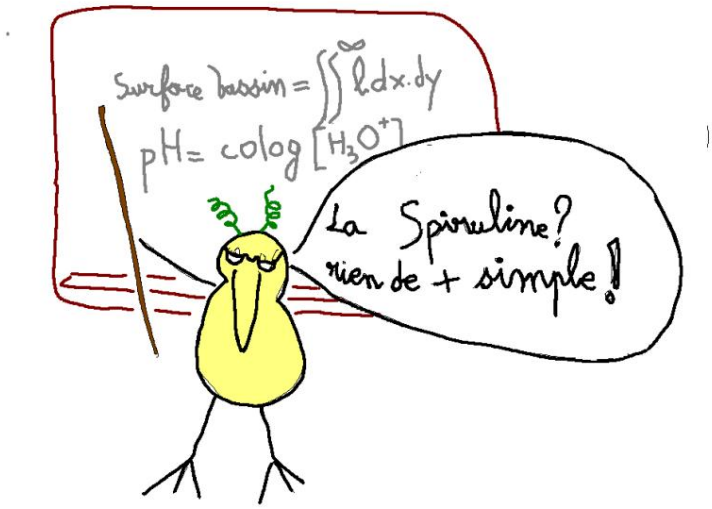
Humanitarian spirulina in developing countries: thinking about tomorrow

Introduction

When you are an NGO, wanting to set up spirulina cultivation facilities in Developing Countries is a laudable objective: local fight against

malnutrition, improvement of immune defenses for children and adults of underprivileged populations, spirulina, while waiting to have conquered the main international health organizations and the often skeptical scientific world, nevertheless has very many followers, including local health organizations, religious congregations, nutritional rehabilitation centers, doctors and nurses who were able to see the evidence of the "plus" provided by spirulina.
Many NGOs, small or medium, are therefore discovering the astonishing merits of arthrospira platensis, vulgarly spirulina, and then wanting to establish local cultures. On the principle of providing fishing rods rather than fish.

However, when you have found a good local partner, built a few ponds with him and started a culture, most of the work remains to be done by the NGO, which it is most often unaware of... -he ?



An obstacle course...

To achieve success, the NGO will encounter 3 major obstacles, which it will have to take into account if possible **before** the start of the project. Unfortunately, his efforts are generally concentrated upstream of these obstacles: choice of partner, setting up the project, agreements, fundraising, construction, starting crops absorb most of his energy... When real difficulties appear on the ground , the NGO is often not prepared.

Obstacle 1: mastering the culture

The techniques for growing spirulina are now well known… to specialists in the field! When the NGO begins in spirulina projects, it is rare that it has one of these specialists at its disposal. He is forced to begin thanks to the advice, written or oral, of the latter, or thanks to some knowledge acquired on previous missions. Let us cite the existence of the artisanal spirulina cultivation manual by Jean Paul Jourdan, that of Idées Bleues by Giles Planchon, as well as the work by Ripley Fox: “Spirulina: technique, practice and promises”. Finally, it has been possible since April 2004 to come and train thanks to a cycle of 400 hours at the Agricultural Training Center in Hyères, which should enable beginners to reach a certain maturity.

The misleading effect comes from the fact that the start of a spirulina culture does not generally pose a problem : the strain and the culture medium are new, the development conditions are optimal. This favorable period, from a few weeks to a few months, generally corresponds to the period of presence of the NGO representatives on site. They then leave with the feeling of “mission accomplished”. The first

Difficulties generally appear only after the departure of the NGO and are increased by three factors:

> the inability of the local actor, who is still inexperienced, not only to find the cause, but also to **describe** the cultural problem encountered, the difficulties and the communication delays: language, distance, telephone connection... the very rapid evolution spirulina, a cyanobacterium, just as capable of a duplication every 7 hours as of sudden death. We have thus been able to observe the simultaneous death in a few hours of "Paracas" strains in Burkina Faso, developed on three different sites 10 to 30 km apart... without any rational explanation to date...

It is clear that the cultivation of spirulina is a relatively complex art for ordinary mortals, further increased by distance. The difficulty observed, if it could finally be correctly expressed, may have several intersecting factors. Among the most frequently encountered problems, let us mention the appearance of straight spirulina, fragmented spirulina, the more or less rapid yellowing of the cultures, the difficulties of filtration, pressing, the appearance of an unpleasant taste or odor, possible contamination by other algae, etc.

To this, we should add that the equipment provided by the NGO is sometimes ill-suited or quickly taken out of service in the field: faulty or misused pH meters, outdated standard solutions and analysis kits, etc., which will make it more difficult to highlighting the causes, especially as they may be numerous, linked to factors such as temperature, sunshine, food, agitation, pH, etc.
Thus, contrary to popular belief, maintaining a cultivation unit in operation is not easy: **it is necessary to experiment for several years to be able to quickly identify, on arriving at a site, thanks to intuition and observation , the problem of culture.** Otherwise, it will be necessary to grope, restart the cultures several times before identifying the origin of the pitfalls encountered.

Barrier 2: Train local staff

1 – Acquire the know-how…

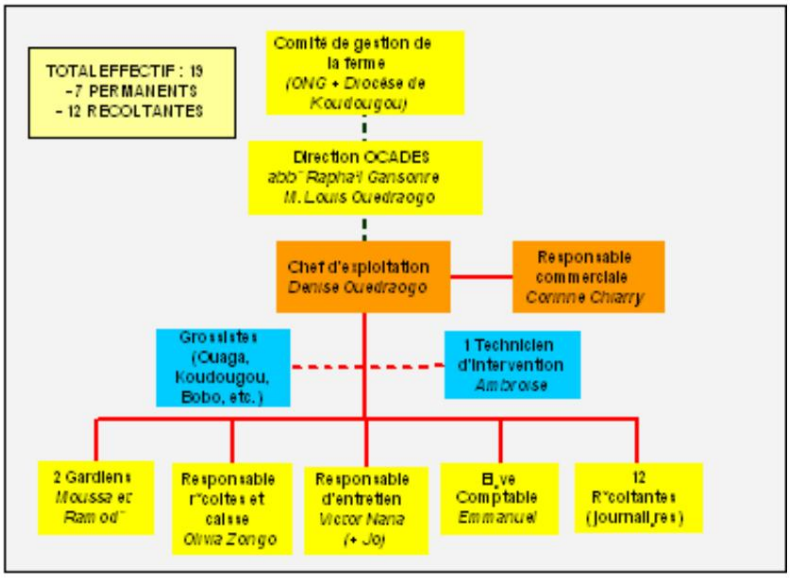
When the NGO has acquired mastery of the culture, it is then a question of transmitting this knowledge to a small local operating team. Remember that it is almost essential to have a serious and organized partner on site, facilities such as water, electricity and telephone. For the above reasons and for having experienced it ourselves, “bush” spirulina, within a village community, as desired by certain NGOs, if it sees the light of day here and there, often suffers failures. We are forced to note that the vast majority of successful establishments in Africa (life span > 5 years) are for the moment the result of stable and organized local religious congregations.

Transfer know-how? At most we can transfer *knowledge,* and wait, with the same patience we have shown for ourselves, that the local operator reaches in a few years or even exceeds our art of culture.

The transfer of *knowledge* is relatively fast: from a few hours to a few days depending on the teaching methods and the understanding of the students.
Choosing the right future operations manager is essential: the qualities required seem to us to be the following:

- know how to read, write and communicate rationally (minimum level: BEPC, preferably the baccalaureate, or bac +2) know how to count and easily practice the rule of 3 and mental calculation know how to
- observe and feel plants: in other words, have the Green hand ". This quality is often the prerogative of the
- less qualified… if the exploitation is important, knowing how to command and lead a team finally and perhaps above all, being fully involved in the exploitation and its humanitarian objectives
-
-

It should be noted that the operations manager cannot be the manager of the local organization with which the NGO has concluded a partnership agreement, the latter, given his position, being most often called upon to perform other tasks. . The farm manager will have to devote the majority of his time to spirulina (or even *all* of it for farms with more than 3 people): the rapid development of spirulina does not allow absenteeism.



For operator training, reference will be made to the existing works already mentioned. However, they will not be usable as they are, as they are often too rich. It is necessary to write a “manual of use of the farm” adapted to the particular conditions of the site. For example, the Koudougou site has an 8-page “manual”, sufficient to describe all the cultivation procedures (sowing, food, measurements and controls, problems encountered, etc.).

All that remains then is to wait over the years for the phone calls with more or less clear explanations from the operator, numerous during the first month, then which will gradually become less frequent over the years. We can estimate that **the transfer of technology is assured when there will be no more than one annual call for help...**

2 – Learn to manage your business…

As part of the training, mastering the management of a spirulina production unit, whether it has 50 or 5000 m2, is an equally long stage for the operator to acquire. From experience, it seems to us that in Africa, this aspect is even more delicate to approach, so absent from local concerns are the notions of organization, discipline, anticipation, procedures, contract, accounting, which are nevertheless pillars of any business.

How many times do we run out of sodium bicarbonate, without having thought of renewing the stock...?
How many times is the site abandoned for important funerals in the nearby village...? However, if the millet can well wait a week or two before being sown, the spirulina requires daily care if one does not want to find yellowish basins after a day of absence.
Here again, a few years of patience and advice will be needed so that, little by little, everyone at the operational level feels responsible, is efficient, and that everyone is present at 7 a.m. in the morning...
Finally, the survival of a farm, large or small, depends on the progressive professionalization of the team in place. Even if the NGO starts on a laudable alter-globalist basis, it will be forced to recognize that it will not escape the universal principles of the company.
These concepts do not in any way prevent respect for humanitarian principles and work in a good mood, on the contrary. Some useful tips:

- **Draw up an organization chart of the operation, specify the tasks of each** person using "function sheets". This will lay the foundation for efficient operation and avoid much confusion. By way of example, we give below the organization chart of the Koudougou farm (Burkina Faso).
- **Concentrate the responsibility for the operation, both technical and financial, on one and the same person :** decisions must be taken taking into account the rapidity of development of the cyanobacterium: orders for inputs, sachets, repairs, and …remittance of salaries, do not await the decision of a remote supervisor. They must be taken at all times by the operations manager.

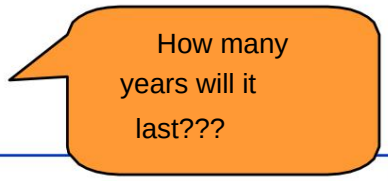
- **Establish farm accounts as soon as possible.** Whatever the operating financing solution, knowledge of operating costs is necessary. We must oppose this natural tendency in the developing countries to work from day to day, and to look for new recipes when the coffers are empty. Such truths, however banal, are not necessarily clear in everyone's mind, both at the level of the local partner and of the NGO. In principle, no project should see the light of day without the establishment of a *provisional monthly operating account, prepared in parallel with the investment budget.* This operating account will then be adjusted by detailing the key items such as wages, inputs, repairs, consumables, water, electricity, not to mention, too often victims of

African amnesia, supplies for replacements!

- **Know how to involve the staff in the operation of the farm.** The concept of salary is often quite abstract for a new recruit, most often without experience of a first job. The salary may appear as a due, regardless of the work performed. However, it is essential that each employee understands that the farm works only thanks to the will and the labor of each one, that he "is" the farm: the salary received must reflect the results of production. Productivity bonuses will be excellent means of awareness and motivation. However, money is not everything, and it will be the role of the operations manager to instil a good state of mind within his team.

Communication within his team is essential, with direct and precise contacts. Africa, but also other developing countries where the unsaid is predominant, do not always hear it that way and the human problems, in the first years, will be added to the technical problems. However, whatever the difficulties of implementing the entrepreneurial spirit, *we believe that working every day for a humanitarian cause is an essential driver of success and rapid progress within a team.*

Obstacle 3: making the operation sustainable



The third obstacle can be summarized as follows:

1 It costs a lot to **build** a crop farm. **2** It costs much more to **operate** it in the long run.
3 The NGO and the local partner tend to forget point 2.

Solution 1: Micro-installations (a few tens of m2) – operating costs borne by the local partner or by the NGO

Most often, the NGO then concentrates on the construction and start-up of the farm. **The operational aspects, in particular financial, are entrusted to the local partner:** religious congregation, associations.
They are sometimes supported in the first years by the NGO itself. This is the most common operation of small spirulina units that have been established in Africa: Davougon (Benin), Nanoro (Burkina Faso), Dapaong (Togo), Puits Bermeau (Niger), Agharous (Niger), Morandave 1ère tranche (Madagascar), Gabon,
etc

Disadvantage: a slow financial trap. At the start, neither the NGO nor the local partner has any real knowledge of operating costs. For a small installation of a few tens of m2, producing a few kilograms of spirulina per month, the monthly costs, taking into account salaries (don't forget the guards!), inputs, bagging, repairs and replacements, water, telephone, electricity, will be around 80 to 150 euros (order of magnitude for Africa), i.e. between 50,000 and 100,000 FVOIRA. These costs, even moderate, are an additional burden for the financier, of which he is often not aware at the outset. Thus, the operating costs of production units exceed the production cost in a few years, all the more quickly the smaller the unit. Finally, producing spirulina on small installations is always more expensive than importing industrial spirulina (about 15 euros per kilo). The solution of producing spirulina in a village environment or through a local humanitarian association to reduce the cost is often a decoy: no one works for free over long periods, and wages, minimal at the start, will quickly catch up. regional levels, even in the bush. On the other hand, the NGO will also have to bear the lack of local logistical and technical resources.

In conclusion, the assumption of operating costs by the local partner or the NGOs can only concern small crops. Production will remain limited to a few kilograms/month, therefore with a limited humanitarian impact in relation to the efforts made.

Advantages: making spirulina known and being able to consume it fresh. Be that as it may, it is often interesting to start with these small installations, to "get used to it", and because the investment costs are low: around 10,000 euros, if we take into account the mission expenses, ponds, a small building, the purchase of equipment and inputs for one or two years, etc. It will of course be necessary to clarify the problem of the financing of the operating costs before starting.

These facilities have the advantage of making known the technique of growing spirulina, and allowing the distribution of fresh spirulina, which is more effective and more easily tolerated. This creates "nuclei of interest" for spirulina in the developing countries, conducive to the start-up, in a second phase, of larger projects, with the objective of self-financing.

Solution 2: Self-finance operating costs

This principle concerns larger artisanal installations, currently

a few hundred square meters.

Self-financing of operating costs is obtained by marketing part of

production (%c). The other part is intended for social distribution.

Important remarks: the social share **(%s)** that a farm can provide is not an objective that can be set “a priori” but a consequence of : •the production cost price Pr •the selling price Pv , by the relation:

Ps.%s + Pv.%c = Pr (100 + operating margin)

$$\%s = \frac{Pv\ 100 - Pr\ (100 + \text{operating margin})}{HP - PS}$$

Example :
Pr (cost price) = 15 Euros/kg
Gross margin = 20%
Pv (wholesale selling price) = 23 Euros/kg
Ps (social selling price) = 6 Euros/kg

→ **%s = 29%**

How to increase **%s social percentage ?**

The degrees of freedom are finally limited:

The commercial sale price "Pv" cannot increase beyond a certain threshold : **it must take into account national and international competition, which, if it does not exist at the start of the project in the country in question, will not to settle when its success attracts attention.**

The operating margin cannot be reduced thoughtlessly at the risk of endangering the financial health of the farm. The social price depends on the purchasing power of the poorest. In some cases, it can be zero! No need to increase it...

>>> There remains the possibility of action on the cost price Pr

ÿ **How to reduce the cost price Pr ?**
We will of course seek to rationalize the exploitation, in particular by improving the techniques of

harvesting, bagging, finding cheaper inputs, reducing consumption

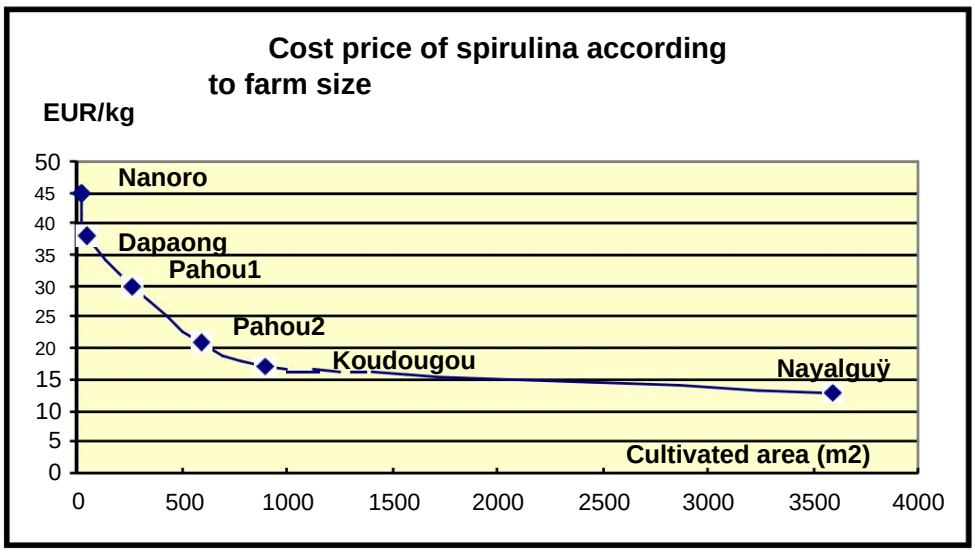
energy (water, electricity, gas), etc.

However, by far the most effective way is to **increase the surface of**

operation, in order to benefit from the scale effect.

Thus, we can:

reduce the relative proportion of non-productive personnel (fixed costs) improve the productivity of harvesters thanks to centralized workshops and the use harvest pumps
reduce specific maintenance costs (larger dryers and basins, lab equipment, IT, telephone costs) reduce the cost of inputs (bulk purchases) reduce the relative cost of bagging (bulk orders), advertising limit energy costs (water: use of boreholes – electricity: solar system connected to the grid)



By way of example, the following graph gives the order of magnitude of cost prices on different African farms.

Kind	Operating area (m2)	Productivityÿ (g/d /m2)	Annual production (kg)	Personal full-time	kg/person/year	Cost price (Euro per kg)
Nanoro, Dav ougon	20	3	22	1	22	45
Dapaong	50	4	73	2	37	38
Pahou1	260		475	5	95	30
Pahou2	600	5	1,205	9	134	21
Koudougou	900	5.5	1,807	13	139	17
Nayalguÿ	3600	5.5 6	7,884	40	197	13

[Editor's note: in this document dating from 2004 "Koudougou" designates the spirulina farm known as "du Petit Séminaire" in Koudougou and "Nayalgué" another spirulina farm also located in Koudougou whose size was to eventually reach 3600 m²: the figures corresponding to Nayalgué are an extrapolated estimate. On the other hand, the figures indicated are for operation at full capacity, whereas in practice it happens that we work under capacity for lack of outlet]

Disadvantages of self-financed operation: **this solution is only possible for medium-sized installations :** the breakeven point for profitability in Africa (but certainly also in other developing countries) is around 400 m2. Making it smaller leads to a cost price of spirulina that is most often incompatible with the international market. The investment cost on this continent being around 15,000 Euros per 100m2
(buildings included), we see that it is difficult to claim self-sufficiency in operations if one does not have a minimum of 60,000 to 100,000 Euros, which is not within reach. of all NGOs! It is then necessary to seek external financing from institutional donors, which is long and requires a certain experience.

In addition, "average" projects require a solid knowledge of the culture of spirulina, a certain rigor in the organization and management, etc. We imagine that "failing" a large-scale project will have a considerably wider impact than if we had failed in the culture of small basins...

Benefits: tackling malnutrition on a large scale. Create real local wealth
The self-financed culture farm, once its equilibrium has been reached, has many advantages:

It is obvious that setting up facilities that produce several tons of spirulina per year makes it possible to attack the problem of malnutrition on a national scale, and to treat tens of thousands of malnourished children. It is no longer a local curiosity, spirulina can be known and consumed throughout a country.

The principle of the company empowers the local partner, which the infusion by sending regular funds (solution 1) cannot do. Poorly controlled management leads the project to failure very quickly: there is therefore an obligation of result, which gradually creates at the level of the local partner the attention (and tension) conducive to success. The company thus creates real **local wealth,** which, in addition to the fight against malnutrition, employs an entire operating team, local workshops for maintenance, sales agents, etc.

Conclusion: That
the advice above, which sometimes reveals a painful reality, should not discourage beginner NGOs. There is, with spirulina, an enormous demand in developing countries, wherever malnutrition is rampant. Its success is due to its remarkable nutritional qualities, which makes it the unequaled complement to a poor diet. You have to have been in a situation of malnutrition yourself in these countries to realize the importance that a bottle of spirulina can take in your luggage, which you will nevertheless forget in a cupboard once you get home. This is the reason why, whatever the difficulties, spirulina should gradually establish itself in the years to come in most African countries.

When the water contains too much Ca or Mg compared to the needs of the spirulina, you can either add more phosphate to the pool (which will precipitate with the Ca or the Mg), or pass the water through a classic resin softener ion exchanger (but we will reject water containing chlorides, therefore pollution), or treat the water in a tank with reagents which will precipitate insoluble carbonates (and not polluting the environment).

For the latter case, we have the choice of reactants:

with soda:

$Ca(HCO_3)_2 + NaOH = CaCO_3 + NaHCO_3$

$Ca(HCO_3)_2 + Na_2CO_3 = CaCO_3 + 2\ NaHCO_3$

with lime:

$Ca(HCO_3)_2 + Ca(OH)_2 = 2\ CaCO_3 + 2\ H_2O$

A31) Softening too hard water

The choice is yours, knowing that if the Ca is in chloride form, only the carbonate will be active.

NB 1: The Mg follows the same fate.

NB 2: It is strongly advised not to store purified water in the light because there is a risk of the development of toxic microorganisms.

CALCULATIONS

The software presented here was written in VisualBasic for Windows. They replace earlier versions in QBASIC which generally can no longer be used with current Windows operating systems. They do not work on Apple and Android devices. They are provided free of charge for non-commercial use, and without warranty. For them to work it is necessary to have on your hard disk the free module

Microsoft .NET Framework Version 1.1 Redistributable

If you do not have the latter on your hard disk, *download it from the Internet (23 Mo); choose the French version (otherwise when using the software the decimal commas will sometimes be transformed into points). It is then imperative to use the comma to mark the decimals.*

The software is hosted on the Petites Nouvelles site, from where it must be downloaded **to your hard drive using the links below.**

These software cannot be sent by e-mail, because the exe are stopped by the anti-viruses.

Distribution of such software is subject to the terms of Microsoft Corporation's Visual Basic.Net End User License Agreement ("EULA").

NB These software being "exe" trigger a precautionary notice but they do not contain viruses; they can be saved and opened without fear.

Simulation of a spirulina culture by SPIRPAC-F

View and use the **Owner's Manual**

Length of day : Durday

(useful for creating new sites in SPIRPAC-F)

Raising or lowering the pH of a medium : DeltapH

Culture medium and food formulas

(see **MEDFEED NOTICE** below page 174)

1. “Classic” case: **MEDFEED1** or “Classic”
2. The same but in a “salination-free” version: **MEDFEED2** (see November 2009 PN)
3. Identical to the classic case except that seawater provides the necessary Ca and Mg: **MEDFEED3** = “Classic” but Ca and Mg provided by seawater (EDM)
4. “Classic” case where ash water (EDC) is used to provide alkalinity and to top up K, sea water (EDM) to top up Mg, Ca and Sulfur and Phosphorus being provided by phosphoric acid: **MEDFEED4** = “Classic” with K provided by EDC
5. Base case with NPK, the necessary Mg and Ca being provided by magnesium sulphate and calcium chloride (or lime) : **MEDFEED5**
6. Case at NPK where seawater brings necessary Mg and Ca: **MEDFEED6**
7. Case at NPK where seawater only provides the necessary Mg: **MEDFEED7**
8. Case in NPK where ash water (EDC) brings the alkalinity and sea water (EDM) the necessary Mg: **MEDFEED8**
9. “Classic” case but with potassium and without salinization: **MEDFEED9**

pH from C or C from pH: pHexC

(C = CO2/NaOH molar ratio in the culture medium)

CO2 consumption by photosynthesis and CO2 absorption from of the atmosphere (in fixed conditions and neglecting respiration; useful for training): **ZABSCO2**

Evolution of the pH and concentration of a culture without harvesting: SPITFIX.exe

(in fixed conditions; very useful for training)

Inclined plane: PLANINCLINE.exe

(taken from http://www.ponce.tv/onlinechannel15.php)

(For the Manning number see for example: http://www.engineeringtoolbox.com/mannings-roughness-d_799.html)

Software user manual

SPIRPAC-F.exe

(edition of October 21, 2016)

Warning

The display can be modified to adapt to the screens of mini

laptops, for example having a resolution as low as 1024 x 768 pixels. (This possibility can also be used as a virtual magnifying glass to see details of the graphics).

Before using the software, **create a C:/PERSO folder to save and print the simulation results and keep the stock of weather data from the sites studied other than those included in the software**

Summary :

In the daily results **table ,** meaning of some of the column headings:

Prod = g/d/m²
Carbur = g of fuel (pH + heating) /m²/day
COP = Performance Coefficient of the heat pump, average over the day
kWhe = electricity consumption of the heat pump, in kWhr/m²/day
kWhth = heat production by the heat pump, kWh/m²/day

NB: The lower calorific value of methane is taken = 14 kWhth/kg

Results

The results can be read either on the graphs (pay attention to the scales), or on the daily table. The display has been adjusted to better fit laptop screens (eg 1366-768 screen resolution).

The **spirulina** concentrations indicated are measured **just before harvesting,** and the **pHs indicated** are measured at **7 p.m.,** by convention

How to create new cases

Rather than changing the parameter values by the method above (§ A), it is possible to write a text file (.txt, via Accessories/Notepad for example) which is a series of 188 parameter values or delimiter characters, separated by commas. Parameter decimals must be marked **with dots.** To make it easier to locate parameters, a delimiter (an uppercase letter in quotes) is placed after each series of 12 values; it better have everything on one line for it to work well. You can save as many sites as you want in the C:/Perso folder, avoiding site name conflicts. Do not forget to adjust the atmospheric cloudiness coefficients using experimental solar irradiation data when they are known (see Appendix 3).

Here is the key to understanding the values of the variables to be fixed (the same key to understanding applies to the data printed in the results, but be **careful** in the results the decimals are marked **with commas) :**

Meteorological Data

before benchmark A (variables no. 0 to 11): average daily maximum temperatures (°C) for the 12 months of the year; mark A = n°12 **between A and B** (n°13 to 24): same for minimum temperatures, °C; marker B = n° 25 **B – C** (26 – 37): dew point temperatures, °C (calculated from the temperature and relative humidity of the air, see Wikipedia in the article “Dew point ”), but we generally take = minimum temperatures; marker C = n°38 **C – D** (39– 50): % of cloudy weather = (1 – solar fraction) x 100 NB To calculate the solar fraction from the “hours of sunshine per day” you must have the duration of the theoretical day, which is easy to calculate with the small software durjour.exe ;

D = No. 51

D–E : wind speeds , m/s E = No. 64

E – F : atmospheric disturbance coefficients (between 0 and 1, calculated with 3 decimal places so that the calculated horizontal global irradiation ratio = horizontal global irradiation given by the PVGIS; F= 77

F – G : rainfall amounts, litres/m²; G=90

After the site's weather data, come the basin operating parameters, classified in alphabetical order (the same order as in the drop-down list allowing the parameters to be viewed on the screen), and also in series of 12 delimited in the same way. way by letters (the numbers placed in front of each line are useful sequence numbers for modifying the source program):

90G

91 Maximum addition of sodium bicarbonate, g/day/m²
92 Max CO2 addition, g/day/m²
93 Maximum sugar addition, g/day/m²
94 Make-up water alkalinity, moles/l (assumed to be derived from calcium and magnesium as carbonates and bicarbonates, functions of make-up water pH)
95 Maximum alkalinity allowed in the medium, moles/l
96 Alkalinity of new medium (> or = alkalinity of make-up water), moles/l
97 Altitude, m
98 Water make-up: 1= possible, 0 = not possible
99 Crop plane azimuth (Angle degrees; 0 = South; 90 = East)
100 Maximum harvesting capacity, g/day/m² (if>=20, Rampelt system harvester)
101 Fuel (none = 0; CH4 = 1; C3H6 = 2; C4H8 = 3; MeOH = 4; EtOH= 5; CH4 in biogas = 6, CO2 in compost gas = 7)
102 CO2 absorption coefficient, moles/hr/m²/atm (normal = 25)

103H

104 Photosynthesis function adjustment coefficient (normal = 1; if very strong or exceptional agitation = 2 or 3; also depends a little on the strain)
105 Respiration function adjustment coefficient (normal = 1) (see also No. 178)
106 Shading regulation modulation coefficient (between 0 and 1)
107 Aeration regulation modulation coefficient (between 0 and 10)
108 Wind speed modulation coefficient (between 0 and 10)
109 Heat transfer coefficient (0 to 10 W/m²/°C) of the insulating cover of the pool in the cases of insulation = 1, 2 or 3. For example, insulation by 12 cm of extruded polystyrene gives 0.25 W/m²/° vs. In the case of a night storage tank in the case of insulation 1 see **explanations below: tank).**

110 CO2 concentration in the external air, vpm (around 400 in 2016)
111 Minimum concentration of “fixed” salts (chlorides, sulphates, nitrates), g/l (in principle = 12 – 40 x alkalinity if the salinity of the water = 0; but the software automatically calculates this variable as well as the addition of salt to compensate for purges)
112 Spirulina concentration after harvest, g/l (> 0.15)
113 Initial spirulina concentration, g/l (> or = 0.15)
114 CO2 consumption, kg/kg of spirulina (normal = 1.8)
115 Greenhouse ventilation rate, Nm3/hr/m²; any if no greenhouse

116 I

117 Fuel flow for pH, g/hr/m² (any if no fuel)
118 Fixed costs (depreciation, labor costs, maintenance, etc.), €/ m²/year; if = 0, calculated

automatically according to the options having an impact on the investment

119 Drying costs, €/kg 120

Packaging and analysis costs, €/kg 121 Average time

of harvest (<12)

122 Inclination of the plane of the crop, in degrees of angle relative to the horizontal 123 Insulation

upwards (not isolated = 0; total at night = 1; thermal at night = 2; thermal at night and day = 3)

124 Initial day (date in the month)

125 Lamps, power in klux/m² of pool 126 Latitude of

the location, degrees of angle (between – 65 and 65 127 ° ; < 0 in the southern hemisphere)

Maximum authorized light (in klux) to avoid photolysis when the pool is at 10°C (e.g. 45 for Lonar and 30 for Paracas in greenhouses, 30 and 20 respectively outdoors, because of UV rays); if = 0, photolysis will not be taken into account)

128 Initial month (date in the year)

129 days

130 Number of consecutive days of harvest stoppage at the weekend (0 to 7): 7

= operation without harvesting and 6 = 1 harvest/week 131 Number of consecutive

simulation days (400 max)

132 Number of days off between campaigns (cleaning, maintenance, restarting); these days will be included in the “activity period” even stopped pools.

133 Number of days without adding sodium bicarbonate, sugar or CO2 at the end of the campaign (before emptying)

134 Fixed shade inside (1) or outside (0) the greenhouse 135

Option: 0 = heating by "virtual" heat pump and/or month-by-month calculation; 1 = calculation normal and/or heating by “real” heat pump; otherwise = maximum fuel flow for heating, g/hr/m² (a good value is 75)

136 Cost price option without initial culture medium (0), or with (1) 137 pH of recycled

purified culture medium; when you do not know it, it is recommended to put the same value as the variable 140 138 pH of the make-up water (any if its alkalinity is zero) 139 pH of the initial culture medium 140 pH that you are looking for to be obtained by adding carbon (regulation setpoint)

141 Percent CO2 (by volume) in biogas or compost gas; must not be zero in compost gas _____

142K

143 Percent fixed shading (active night and day)

144 Percent night shading (heat shield at night)

145 Percent of combustion heat transformed into electricity

146 Percent of rain falling on basin surface admitted to basin (open)

147 Percent maximum daily purge allowed

148 Percentage of lamp power contributing to pool heating (> 32) _____

149 Price sodium bicarbonate, €/kg

150 Price soda ash, €/kg

151 Fuel price, €/kg (any if no fuel) (if biogas = price of CH4 content; if compost gas = price of CO2 content)

152 CO2 price, €/kg

153 Price of water, €/m3

154 Price of 220 V electricity purchased or sold, €/kWh

155L

156 Monoammonium phosphate price, €/kg

157 Price of cooking salt, €/kg

158 Price of sugar, €/kg
159 Price of dipotassium sulphate, €/kg
160 Price of magnesium sulfate heptahydrate (Epsom salt), €/kg
161 Price of urea, €/kg
162 Maximum sale price (at low production), €/kg
163 Initial depth of cultivation, cm (restored by adding water after loss of 1 cm by evaporation)

164 Maximum depth of cultivation, in the event of rain on an uncovered basin, cm (> initial depth)
165 Purge, additions of carbon, water, recycling eliminated on weekend shutdown days (1); or maintained (0)
166 Harvest only if pH >= value set here (usually 9.6)
167 Recycling of purified culture medium, litres/m²/day (if = 1, recycle = prodj automatically, normal value)
168M
169 Title of the case studied
170 Harvest yield, % (= 100 – % loss in sludge, harvest, drying and packaging)

171 Total salinity of make-up water (tds), g/litre
172 Maximum allowed salinity in culture medium, g/litre
173 Greenhouse (1 if the pool is in a greenhouse, 0 otherwise)
174 Greenhouse (0 if single wall PE, 1 if double wall PE, 2 if double wall PC, 3 if triple wall PC) (PE = Polyethylene, PC = Polycarbonate)
175 Light threshold with insulation 0 (masks) and to remove or put insulation 1 or 2 on the pool, in klux at the surface of the pool
176 Maximum authorized pool temperature, °C (42 for example)
177 Heat pump reheat temperature, °C (virtual or actual; if no heat pump: 0)
178 Rate of reduction in nocturnal breathing (0.2 for the isolated case = 1, otherwise between 0.3 and 1 depending on the degree of nocturnal agitation)
179 Temperature below which there may be photoinhibition (around 23°C); if = 0, photoinhibition not taken into account
180 Equivalent water value for pool bottoms + edges, cm
181N
182 Variation in selling price (decrease) depending on annual production (= sales), €/kg per kg/m²/year 183 Average stirring speed, cm/s (30 = maximum normal speed; > 30 = productivities exceptional)

184 Fuel heating regulation temperature, °C (if no fuel heating: 0)

185 Minimum purge rate, which can be 0 in case of powerful purge, %/day 186 Graph ordinate adjustment factor (from 0.5 to 3 which is the maximum so as not to lose the abscissa axis with large screen). But nothing prevents going beyond these limits, which can be very interesting for studying the detail over a short period (= “magnifying glass” effect), over a month for example. Usual value: 1.7

187 Graph abscissa adjustment factor (from 0.5 to 1.8 which is the maximum for not not lose the y-axis with large screen). But nothing prevents going beyond these limits up to 10 or 20: it can be very interesting to study the detail over a short period (one month for example).

188 Price of heat, €/MWh, in the event of coupling with anaerobic digestion or another source of

heat

Once written this file, save it under a name of your choice. When using it, save it under C:\PERSO 1.txt (or any of the 4 other "PERSO" available, PERSONAL 2, PERSONAL 3, PERSONAL 4 or 5)

In practice, we generally start from an existing example, we modify it on the screen, and we save it under its new name.

Use of Spirpac-F

One advantage of the software is to be able to quickly optimize the progress of a spirulina culture operating on a given site, under given climatic conditions.

It can also be used as an aid to the design of a project corresponding to a given objective, or as a tutorial (“culture simulator”).

Results

They can be read either on the graphs (pay attention to the scales), or on the daily table. The spirulina concentrations are measured just before harvesting, and the pH indicated for each day is close to the maximum for the day (measured around 7 p.m.).

The model applies to the case of an **autotrophic** culture of the cyanobacterium Arthrospira in a tank in the open air or closed by a translucent cover, with controlled ventilation. A particular embodiment of the latter case is to stretch a greenhouse film over the edges of the basin [Jourdan JP (1993) "Solarium spirulina farm in the Atacama desert (North Chile)", Bulletin de l'Institut Océanographique, Monaco , Special No. 12, page 191]; another consists of the “breathing basin” with natural ventilation by a chimney [Fox RD (1996)
“Spirulina, production & potential”, Edisud, Aix-en-Provence]; a greenhouse film sheath, placed horizontally and partially filled with culture medium also constitutes a possible embodiment (“photobioreactor”). For the simulation to apply, it is necessary and sufficient that the surface of the culture in contact with the atmosphere is equal to the illuminated surface. The air introduction mode is arbitrary. There is no limitation to the inclination and orientation of the active surface of the culture (the culture medium can thus be flowing on an inclined plane as in Setlik type photobioreactors). The latitude of the installation site must be between the polar circles. Culture in seawater according to Prof. Mario Tredici can be simulated (at seawater pH). For cold climates, optional heating and/or double glazing and/or thermal screen at night have been provided; heating is by heat pump or by combustion of clean fuel and in the latter case the co-generation of electricity is provided as an option because heating alone is too expensive in the majority of cases; but we can validly use the combustion of gases to bring CO2 into the atmosphere of the greenhouse. Another optional option is crop insulation, with several modes: either complete insulation (adiabatic) with minimal aeration at night, or allowing aeration and heating at night only or day and night. An option has been added allowing electric lighting on the pools. Another option allows to recycle culture medium after purification (and change of pH). Finally, it is possible to artificially heat or cool the pool using a heat pump.

These options are detailed below.

Principles of calculation and various options

The program simulates the operation of the basin from its seeding to its shutdown after a fixed number of days (up to 400). From a culture medium at a given pH inoculated at time zero, the growth of the spirulina is calculated hour by hour; a thermal balance and a carbon balance (absorption of CO2 from the air + injection – consumption) make it possible to calculate, also hour by hour, the temperature and the pH, which themselves determine the speed of growth.

The harvest takes place once a day (at a time of your choice), except on days without harvesting, and it brings the concentration of spirulina to a value of your choice, except that the daily harvest capacity cannot exceed the fixed limit. But there is no harvest if the pH is below a limit to be set (usually 9.6 is chosen) or during weekend holidays (0 to 7 consecutive days off per week). At the end of the campaign, a total harvest is made (until the sowing concentration is regained), and a certain number of days are devoted to inter-campaign operations. The average productivity and the cost price take into account these inter-campaign days. **The cost price can only be calculated if the number of campaign days is 365 (including the inter-campaign days = days when the pools are stopped).**

The ambient air dry bulb temperature and the radiation absorbed by the crop are calculated hour by hour from the loaded weather data (monthly mean values). The air dew temperature, haze factor and wind speed are assumed to be constant during the day. Each decade (period of 10 days) of a month the monthly cloudiness percentage is concentrated on 100% cloudy days (where the light at each hour is taken equal to 20% of that of 100% sunny days), plus one day partly cloudy and the remaining days are 100% sunny (without correction for ambient air temperature based on insolation). This distribution of cloud cover is not arbitrary since it is generally observed that periods of good weather alternate with cloudy periods, lasting a few days each, but it suffers from too few partially cloudy days; it therefore tends to give a less favorable result than in reality, which constitutes a safety factor. We should add that it is always possible to carry out calculations by modifying the climatic data (temperatures and % of clouds above all) and thus obtain specific daily values.

The distribution of monthly rainfall follows the same principles as that of cloudiness. A percentage (choice) of the rain enters the basins in the open air (a filtrate purge is performed to maintain the level in the event of excessive rain). We also purge to maintain the alkalinity and, if possible, the salinity below the set maximum. The desired salts and optionally water are added to maintain the quality of the culture medium.

Water is also added to compensate for evaporation and maintain the level between the normal level (= initial) and a minimum set at 1 cm below the normal level.

You can also perform a purge to maintain the quality of the medium, even if there is no need to purge for other reasons: this is the "minimum purge" (variable 185), which can be set to 0 if there is sufficient purification (next paragraph).

Independently of the purge, it is planned to be able to send the filtrate to a **purification** system that eliminates organic matter and can modify the pH. An equal volume

is recycled simultaneously with the basin; it is accepted that this recycling changes neither the basicity nor the "fixed" salinity (non-carbonated salts) of the medium, nor the level of liquid in the basin, nor the temperature. The pH of the recyclate is freely fixed, but generally close to 10.
If a pH < 8 is set, the value used will automatically be that corresponding to the equilibrium with the outside air, except that if it is set to 0 the pH will be that of the culture.

An option (variable no. 165) is used to decide whether purges, water make-up, carbon supply (pure CO2, sugar, sodium bicarbonate) and recycling are possible or not on **weekly shutdown days ;** if they are possible on these days, the volume of medium to be purged or purified is filtered, and the recovered biomass is assumed to be returned to the basin. If it is decided that they are not possible, this makes it possible to simulate an absence for leave for example, and to set parameters (particularly shading) so that the culture survives during this time.

The culture can be shaded and/or heated and/or thermally insulated. We neglect in all the cases the thermal losses by the sides and the bottom of the basins. On the other hand, upwards night losses are governed by variable 123 (“insulated” option): at night the crop can thus be completely insulated upwards (both thermally and from the atmosphere, with heating switched off: insulated option = 1) or partially insulated (only convective and radiative exchanges upwards can be suppressed or reduced, with ventilation and heating maintained: insulated option = 2). In case of complete isolation (isolated = 1), minimal aeration is maintained to avoid culture anoxia (breathing can be reduced to 20% of normal, thanks to variable No. 178) but it is neglected from the point of view of thermal effect and evaporation. Insulation option 2 is reserved for greenhouse ponds. Insulation options 1 and 2 are only effective if the natural lighting on the pool is below a predetermined threshold (variable 175), a very sensitive variable. There is another insulation option (isol = 3), requiring lamps, which stays in place day and night. Variable 109 (referred to as the "thermal insulation coefficient") represents the total upward heat transfer coefficient through the insulating cover. In the isol 1 option, the culture can be stored in a tank overnight: if the surface of the lid of this tank is x times smaller than the surface of the basin, variable 109 must be assigned the value of the insulation coefficient heat divided by x.

The multiple glazing options of the greenhouse, thermal insulation and shading are compatible.

The single-glazed greenhouse (greenhouse option = 0) has a single polyethylene film. The greenhouse option = 1 has a roof with double polyethylene film transparent to Infra-red (but not to UV). The option 2 greenhouse has a double-walled honeycomb polycarbonate roof and option 3 has a triple-walled honeycomb polycarbonate. These polycarbonate roofs have an anti UV side placed on top.

Two types of daytime shading are provided, and are cumulative; they are expressed in %; so-called “automatic” shading, adjustable, is automatically installed if the temperature and light conditions require it; the so-called “fixed” shade is permanent night and day and can be installed outside or inside the greenhouse. The interior shade makes it possible to better heat the greenhouse. A heat shield (or “night shading”) can be installed at night to reduce nighttime cooling; it is combined with any fixed shading, but it has no effect with thermal insulation (variable 174). NB: automatic shading is installed outside the greenhouse so as not to thwart the desired cooling, and this complicates its installation.

NB: When two shades A and B are “cumulated” this means that the light penetrating the crop is multiplied by (1 – shade A/100) x (1 – shade B/100).

The aeration of the greenhouse includes a fixed element to evacuate the oxygen produced (< 35 Nm3/hr/m²) and an "automatic" element that can be modulated by means of a coefficient to be chosen and implemented automatically according to the temperature of the basin. .

The contribution of CO2 by combustion of fuel (which generally represents only a small fraction of the heating needs) is decoupled from the heating of the basins, which allows better regulation and above all better quality of the combustion gases. It requires a greenhouse. The supply of pure CO2 from a liquid stock of food quality remains however the preferred solution if possible.

The **possible heating of the basins** is done by circulation of hot water in tubes under the bottom of the basin. The heat source can be the combustion of fuel in a boiler or, preferably, via a generator (= cogeneration, recovery of heat not transformed into electricity). Combustion takes place with outside air, with 10% excess. The heating is done night and day except that it is automatically cut off at night in the isolation option = 1. A maximum fuel flow must be defined by variable 135 (known as “Option”) which must be chosen with care , neither too high nor too low because it has a significant influence on fuel consumption the value 1 is given to this variable 135 we are in “real heat pump option”]. It should be noted that electricity production is only done if heat is needed (cogeneration), but that this electricity production is assumed to be fully recovered. We can specify that the production of electricity is nil, in which case all the heat, within the efficiency, will be sent to the heating.

Heating can also be done by heat pump (PAC), this is the origin of the name of the application (the F being the French version). The coefficient of performance (COP) of the heat pump is taken as a quarter of the theoretical value, i.e. (273 + pool temperature)/(temperature difference between pool and outside air) / 4. The results table provides the average COP of each day the heat pump is in operation, as well as the daily electricity consumption and the peak power of the heat pump. To obtain heat pump heating, give variable 135 the value of 1 (for normal calculation) or 0 (for month-by-month calculation) and set a heat pump heating setpoint temperature using variable 177 other than 0.

The mixing of heating modes (fuel and heat pump) is not planned.

On the other hand, it is possible to simulate **heating by heat** coming for example from anaerobic digestion. In this case, a "virtual heat pump" is used, which is easier to calculate than heating by fuel: to do this, the value 0 must be assigned to variable 135 and the kWh consumed by the (virtual) heat pump are obviously free. With the value 1 for variable 135, the calculation of the cost price is done with HP electricity at the normal price; in this case, disregard the numbers in the box with a white background at the bottom left of the screen.

Caution: for pool temperatures > **37.4°C** the aeration flow may increase suddenly (see rules page 160) to avoid dangerous overheating. To partially counter this effect, the aeration regulation coefficient (variable 107) can be lowered.

Procedure to follow to calculate a spirulina methanization coupling month by month (from the time when the legislation provided for a bonus based on the by-product heat recovered)

Data: by-produced heat to be consumed per m² of pool / **month.**
Goal: consume as much of this heat as possible to maximize the premium (according to legislation, etc.) while usefully producing spirulina.

We play mainly on the setpoint temperature of the pools, the aeration flow and the CO2 flow while aiming to maximize production and get as close as possible to the heat to be consumed. You can also adjust other important parameters such as harvesting capacity and auxiliary lighting. Then we make a calculation for the whole year with the same configuration (in particular the
harvesting capacity) giving approximately the same production and the same heat consumption: this calculation makes it possible to obtain the fixed costs and an annual cost price corresponding to the chosen configuration, then to recalculate a "truer" annual cost price on the basis of total consumption and production month by month.

NB The results screen includes a window (bottom left, on a white background) giving the main useful values for these coupling calculations (but this window has been kept for ordinary calculations because it may prove useful).

Artificial lighting of greenhouse ponds is possible between 4 a.m. and 9 p.m. when the natural light (measured under possible shade) is lower than the light output of the lamps (these are partially or totally turned on automatically to maintain the level illuminance to the value corresponding to fully lit lamps). In the option insulation = 1, when the culture is isolated the lamps cannot be used. The average power consumption of the lamps is assumed to be 13 mW/ lumen (i.e. 13 W/m²/klux, which corresponds to an average between fluorescent tubes (17) and moderately used high-pressure sodium vapor type horticultural lamps (10); when new, these can drop to 6.5 mW/lu.

The future certainly belongs to LEDs: but in 2014 household-type LED bulbs (for 220 V alternating current) still consumed 16.4 mW/lu for a lifetime corresponding to approximately 15 years of operation in Brittany, for example, in the event of coupling with anaerobic digestion.

In the night and day thermal insulation option, the lamps are switched on from 4 a.m. to 9 p.m. The portion of the heat given off by the lamps admitted into the greenhouse to contribute to heating is adjustable from approximately 32 to 75% (in thermal insulation options 2 and 3 the lamps are located under the insulation).

In the case of pools in the open air, these options are obviously not all available; fixed shading and thermal screen at night are possible, but the pools cannot be heated by fuel combustion (they can be by heat pump).

The **rate of photosynthesis** can vary according to the strains used or the circumstances (mortality, predators); it is therefore adjustable by means of a coefficient.

The calculation program does not take into account the disappearance of spirulina by mortality or because of predators (it is accepted for these two cases that there is recycling of carbon inside the culture).

To **take into account the production of exopolysaccharides** not included in the harvest, which may vary according to strains or conditions, and to also take into account possible variations in the composition of spirulina depending on the strains, the consumption of CO2 per kg of spirulina does not is not considered fixed but as an adjustable variable.

We must also set the **harvest yield** which takes into account the loss of spirulina in the sludge, and between filtration and storage of the finished product.

The **program ignores :**

- the influence of the internal air circulation speed on evaporation, - the effect of shading due to the edges of the basin by non-vertical sun, - the variations in oxygen content in the internal atmosphere of the greenhouse, - the possible acidification or alkalinization of the environment under the effect of nutrients (nitrates and urea), - the possible hardness of the make-up water.

Salinity, alkalinity and pH of the make-up water are taken into account, which allows the use of brackish and/or alkaline water. **It is assumed that water provides the necessary calcium.**

The carbon supply, controlled by pH regulation, can be done either by direct addition to the culture medium of sodium bicarbonate or sugar or pure CO2 gas, or by CO2 enrichment of the greenhouse air. by compost gas or by the clean combustion of fuel directly in the greenhouse; a material balance on the CO2 between the air entering the greenhouse and its exit makes it possible to calculate the CO2 content of this air (assumed to be homogeneous). The calculation takes into account the CO2 provided by the urea *(at the rate of 300 g/ kg of spirulina, non-modular package, i.e. 220 g of CO2 or 12% of the carbon of the spirulina produced),* by the fresh air for ventilation and through recycling. There is nothing to prevent mixing the various carbon sources. The pH can be regulated by burning fuel in the greenhouse, even if the greenhouse is not heated with fuel.

The risk of photolysis at low temperature is indicated during the calculation when the light on the pool exceeds a threshold in klux for more than one hour, evaluated as follows: Threshold = 1.2 x (maximum light assumed authorized at 10°C = variable 127) x (pool temperature in °C/10) x (spirulina concentration/0.4) x (shaking speed/30)
The calculation of this limit is quite arbitrary and serves above all to draw attention to the risk of photolysis. This risk depends on the strain cultivated (for example for the "Paracas" strain we can take 30 for the variable 127 against 45 for the "Lonar" in the greenhouse but 20 and 30 respectively in the open air because of the higher dose of UV).

Summary of the thermal limitation rules adopted in greenhouses :

Night (unless insulation 1) and day :

(tblim = maximum temperature allowed for the pool)

Aeration

If pool < 37°C, normal flow
Between 37°C and tblim-2.5°C: normal flow + 10 times the coefficient automatic ventilation
Between tblim-2.5 and tblim-2°C: normal flow + 15 times the coefficient
Between tblim-2 and tblim-1.5: normal flow + 20 times the coefficient
Between tblim-1.5 and tblim-1: normal flow + 25 times the coefficient
Between tblim-1 and tblim-0.75: normal flow + 30 times the coefficient
Between tblim-0.75 and tblim-0.5: normal flow +35 times the coefficient

If the pool temperature approaches the authorized limit by less than 0.5°C:
normal flow rate + 40 times the coefficient

Fixed shading = invariable (active night and day)

Night (unless insulation = 1):

Nocturnal shade (heat shield)

Day :

- Automatic shading = 0 if
  pool < 30°C 50% x
  coefficient if pool between 30 and 35°C 75% x
  coefficient if pool above 35°C

Summary of the thermal limitation rules adopted without a greenhouse :

Night and day :

- Fixed shading

Night (unless insulation = 1):

- Nocturnal shade (thermal screen)

Day :

- Automatic shade = 0 if pool
  < 30°C 50% x
  coefficient if pool between 30 and 35°C 75% x
  coefficient if > 35°C

NB
For a pool without any shading device, the variables n°106,143 and 144 must be set to a zero value.

Absorption of atmospheric CO2

The rate of absorption is proportional to the absorption coefficient and to the difference in the vapor pressures of CO2 in the air and on the liquid. The vapor pressure of CO2 over a sodium carbonate/ bicarbonate solution is given in the literature. Kohl and Riesenfield (1960) give in "Gas Purification" [Kohl AL and Riesenfeld FC (1960) "Gas Purification", McGraw-Hill Book Co. temperature, alkalinity and ratio c (moles of CO2/mole of base) , in mmHg:, on page 117], a formula having as variables the

$$pCO2 = 68.5 \times b1.29x (2c - 1)^2 / [(1 - c) \times (333 - 1.8\ xt) \times (0.0487 - 0.0006\ xt)]$$

where b = alkalinity of the absorbing medium, gmoles of strong base/
litre c = CO2/base molar ratio corresponding to the pH of the medium
t = temperature of the medium,°C

The absorption of CO2, expressed in g of spirulina/day/m² (assuming 1.8 kg of CO2 per kg of spirulina) is then equal to 0.772 x ka x [0.00076 x vpm x (1 – alt/10000) – pCO2], formula where:

ka = absorption coefficient,
gmoles of CO2 absorbed/hour/m²/atmosphere
vpm = CO2 content in the air, volume ppm alt =
altitude, meters 0.772 = (44 x 24)/(1.8 x 760)

The absorption coefficient of CO2 through the surface of the basin is adjustable (variable 102), but generally it is taken equal to the experimental value of 20 to 25 gmoles/hour/m²/atmosphere.

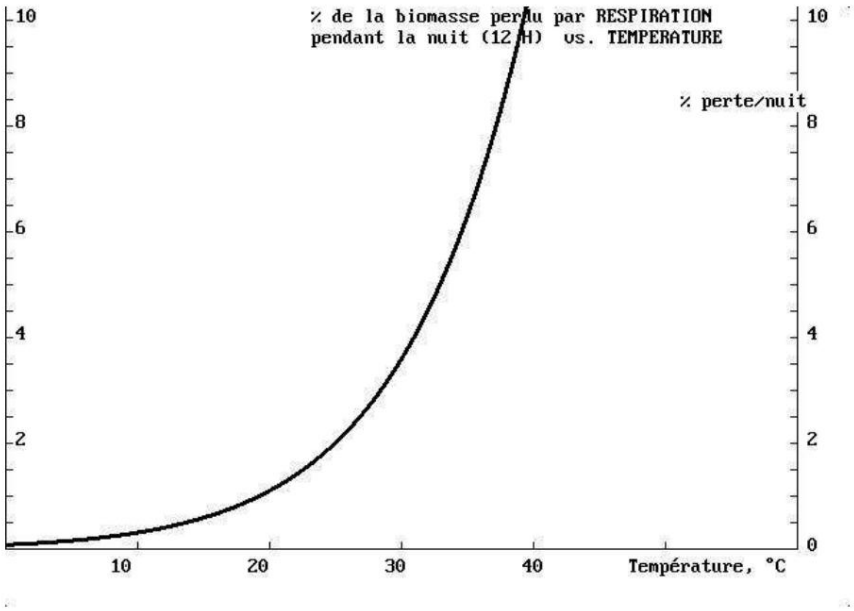
The software combines these two formulas to calculate CO2 exchanges between the atmosphere and the basin.

spirulina breath

Spirulina only breathes in the absence of light, and in the presence of oxygen. By day we admit that it sees the light only in the superficial layer of height equal to the "Secchi" (we have adopted the Secchi curve corresponding to the so-called "spiral" strain, with non-turbid culture medium) and that there is no breathing in this layer, but below there is breathing; this assumes that the medium is agitated (homogeneous). Respiration is set at 20% of its normal value in the event of complete nocturnal isolation (in accordance with several experiments showing that this was well supported by spirulina).

To quantify normal breathing, we use the results of JFCornet [Cornet JF (1992) “Kinetic and energetic study of a photobioreactor” Doctoral thesis, University of Paris-Sud Center d'Orsay, 02/27/1992, p. 115] for the variation with temperature, but for the base value at 20°C we take an average between the indications of Cornet and those of L. Tomaselli et al. [Tomaselli L., Giovanetti L., Pushparaj B. and Torzillo G. (1987) “Biotecnologie per la produzione di spirulina”, IPRA, Monografia 17 (page 21)].

This is of course an approximation as respiration also depends on the carbohydrate content in spirulina. These hypotheses serve as the basis for the simulation and they are represented by the graph below as a function of temperature when oxygen is not a limiting factor:



NB It is possible to modify this curve to adjust it, if necessary, to reality, thanks to the parameter called adjustment coefficient of the breathing function = variable No. 105 (see list of variables). It is also possible to modify the importance of respiration by applying a reduction factor to it (variable No. 178) to be chosen between 0 and 1 depending on the oxygen content of the culture.

Growth by photosynthesis

Biomass concentrations greater than 0.1 g/l (general case)

We admit that the growth of spirulina by photosynthesis is the product of an adjustment coefficient and 5 supposedly independent factors detailed in Annex 2:

- factor depending on salinity -
factor depending on temperature -
factor depending on pH - factor
depending on illumination - factor
depending on the degree of agitation of the culture medium

This postulate, which is at the heart of the program, is not scientifically substantiated. The comparison of figures 4 and 19 of Zarrouk's thesis authorizes to admit that the function of the temperature is independent of the illumination; Productivity measurements on our basins show that the influence of pH, temperature and light are in fairly good agreement with the hypotheses contained in this postulate.

Moreover, this postulate implies that the speed of photosynthesis does not depend on the height of liquid, nor on the concentration of spirulina, nor on the concentration of mineral nutrients (other than sodium bicarbonate), therefore that photosynthesis is proportional to the illuminated surface. In other words, we make the assumptions, largely verified in practice at the concentrations considered, that growth is in the linear phase, not limited by mineral nutrients, with total absorption of light entering the basin. Note that the amount of spirulina per m² (height of liquid x concentration) has an influence on productivity through respiration (see previous §).

We also admit that the growth of spirulina is uniquely autotrophic. If a

mixotrophic growth, or even possibly heterotrophic, occurs, it will be strongly competed by the heterotrophic organisms cohabiting with the spirulina in the medium (bacteria, zooplankton). The error committed on growth can in any case only be by default. We therefore admit that in the event of a supply of carbon by the sugar, this is oxidized into CO2 by any mechanism whatsoever (fermentation).

Photoinhibition is a very complex phenomenon of reduced photosynthesis under the effect of too much light, aggravated by too low a temperature. The software tries to take this phenomenon into account as follows: if the pool temperature tb is below a threshold ts (variable No. 179) and if the light exceeds 20 klux on the pool a very approximate formula calculates a coefficient of reduction of photosynthesis equal to: 0.035 x (klux-20) x (1 + tb/ts)/2).

There is another form of photoinhibition when the temperature approaches the maximum allowed: it is not taken into account in the software (it is rather recommended to maintain the temperature quite far from the maximum allowed).

For biomass concentrations below 0.1 g/l, the growth is assumed to be exponential, which the model translates by multiplying the speed calculated previously by ten times the concentration (in g/l).

Note on the spectral distribution of the light energy used and the efficiency of the lamps :

Spirulina being able to use a very wide spectrum thanks to its richness in various photosynthetic pigments, the differences in spectral distribution between solar lights at different angles, latitudes, altitudes, haze factor, through greenhouse glazing, etc. and artificial lights are neglected. The "klux" (of visible light, as measured with a luxmeter) are assumed to have the following equivalence with the total power dissipated:

10 Watt/m²/klux for the sun
13 Watt/m²/klux for modern lamps (iodide or sodium type).

The use of LEDs will be included, but this technology is still too recent, and progressing too quickly to be studied now (2017) here.

Climatic data (temperatures and solar radiation)

It is assumed that the ambient temperature varies linearly between its minimum at sunrise and its maximum at 2 p.m. (solar time)

The solar radiation absorbed by the crop is calculated as is done in the solar collector with or without glazing, from the classical astronomical and thermal equations recalled for example in Chouard, Michel and Simon [Chouard Ph., Mich and Simon MF (1977) “Thermal balance of a solar house”, EDF]. The global horizontal irradiance on the ground calculated by the program by summing uh hour over the duration of the operating campaign (here the month considered), of an estimated monthly disturbance coefficient, is indicated in the results: this allows adjust it by successive approximations until obtaining the global horizontal irradiation on the ground given by the statistics for the site considered [see **App**

This is our way of calculating this haze coefficient (defined in o Chouard, Michel and Simon, page 8 where it is called “B”). Three decimals are sufficient, but this calculation remains a major preliminary work required for the site studied.

Thermal balance

The temperature of the culture is calculated by heat balance between the heat contributions (including solar radiation and combustion heat) and the various heat losses (we neglect the losses towards the ground and the sides of the reactor, but we take into account a " "equivalent water value" of the bottom and sides by adding it to the height of liquid.

It is assumed that the culture and the internal air of the greenhouse are homogeneous in temperature and at the same temperature, and that the thermal inertia of the air is negligible, but the heat capacity of the air flow passing through it is taken into account. the greenhouse which extracts heat by sensible heat and by becoming saturated with water. Additions (water, nutrients) are assumed to be made at crop temperature. We obviously take into account the heat gains by heating and lamps.

It is assumed that shading reduces incident solar radiation and thermal losses by radiation by the same percentage, without affecting thermal exchanges by convection.

We also take into account the solar energy consumed by photosynthesis by taking as calorific value of spirulina 20.9 kJ/g [Cornet JF (1992) "Kinetic and energetic study of a photobioreactor" Doctoral thesis, University of Paris -South Center of Orsay, 02/27/1992, page 263)|. The thermal losses by convection towards the atmosphere and by radiation towards the sky are calculated as for a solar collector according to the classic equations, for example those recalled by R. Gilles (1976) [Gilles R. (1976), Promoclim A, N ° special “Outdoor swimming pools” (page 269), SEDIT, Paris] and Chouard, Michel and Simon (1977) [Chouard Ph., Michel H. and Simon MF (1977) “Thermal balance sheet of a solar house”].

The influence of any inclination is neglected as justified by PI Cooper [Cooper PI (1981) “The effect of inclination on the heat loss from flat plate solar collectors”, Solar Energy, Vol. 27, No. 5, pages 413-420].

Electricity consumption/production

For the calculation of the electricity consumption for agitation (or pumping in case of cultivation on an inclined plane), a very simple and more or less arbitrary equation has been adopted. The consumption of a possible heat pump is dealt with in § heating.

If fuel is used (except in the case of coupling with anaerobic digestion on the farm), electricity can optionally be produced by a generator; excess electricity is usually available for sale or other uses. There are miniaturized gas turbines, but we turn instead to engines for small capacities. Beware of impurities in exhaust gases. A simple burner can give purer gases than an engine. The generator could in the hopefully near future be a clean fuel cell.

The electrical power for ventilation has been neglected (natural ventilation admitted sufficient), but that consumed by any lamps, which is very high, is obviously taken into account, as well as that of agitation.

Electricity is assumed to be bought and sold at the same price, with connection to a network, the needs and the production of electricity not necessarily being in phase (especially if lamps or a heat pump are used).

Cost price calculation

The model includes an economic component, making it possible to calculate a cost price, taking into account a system of costs provided by the user.

The calculation of the cost price is based on the specific consumption corresponding to the following formulas:

- culture medium formula without nitrate containing, in addition to salt and carbonate and/or sodium bicarbonate, per liter of medium: 1 g of K_2SO_4 + 0.02 g of urea + 0.08 g of $NH_4H_2PO_4$ + 0.16 g of Mg sulphate + 0.001 g of Iron
- food formula comprising, in g/kg of spirulina: 300 g of urea + 50 $NH_4H_2PO_4$ + 40 K_2SO_4 + 30 Mg sulphate + 0.5 Iron - cost of iron (and trace elements) negligible.

The semen is counted at the fixed price of €10 per kilo counted dry.

An option makes it possible to remove the culture medium from the calculation (in the case where recycling or effective purification is available, for example). In this case choose an initial concentration of spirulina equal to the concentration after harvest.

The calculation charges the fixed costs in proportion to the days of use (number of days of operation + “inter-campaign” days). If the installation does not work all year round, this must be taken into account correctly, for example by including the annual shutdown in the "inter-campaign" days, otherwise the calculation will not be made: it is only done for a period 365 day campaign.

In the automatic evaluation of fixed costs (= labor + depreciation + maintenance = 25% / investment excluding tax) the model uses the following values in €/ m^2/year:

base case (basin and agitation): 20 with single-wall PE greenhouse, 16 without greenhouse supplement for double-wall PE: 1 supplement for double-wall PC: 2 supplement for triple-wall PC: 3 supplement for fixed shading: 2 supplement for modular shading : 2 supplement for night heat shield : 2 supplement for night insulation = 4 supplement for harvest = if harvest capacity is specified < 20 = 0.5 x (harvest capacity, g/d/m^2) / (post harvest concentration, g/litre); otherwise the use of Rampelt automatic harvesters is assumed = 0.05 x (harvest capacity, g/d/m^2)/(post harvest concentration, g/litre) and the harvest capacity is displayed as 21 automatically supplement for depuration and recycling = (harvesting capacity, g/d/m^2) supplement for heat pump = 4 + 11 x maximum heat pump power in kWe supplement for lamps = 2 per klux supplement for generator = 6

The investment for simple fuel combustion is neglected.

If we admit that the labor is concentrated in the harvesting station (manual) and represents 2/3 of it, the standard investment (basins, agitation, double-walled greenhouse, night insulation, harvesting, purification and recycling) corresponding to these hypotheses would be approximately €240/ m² for a fresh biomass productivity close to 1 to 3 kg/year/m² (expressed in dry matter), in France.

Note that **drying is not included here :** the model only applies to the production of fresh biomass (but expressed in dry matter). To estimate the cost of the dry finished product, we specify drying, packaging and analysis costs different from zero (variables N° 119 and 120), expressed in €/ kg dry.

Commercial costs and taxes are also not included in the cost price thus calculated.

The model also proposes a calculation of profit (before tax) based on a sale price of dry product assumed to vary linearly according to the level of sales reached. We can admit that it represents the remuneration of the spirulina grower plus any commercial costs.

All prices listed in this section are exclusive of tax.

> **Note that the "Cost prices" in question here are only examples of orientation for school cases, not estimates for a real project!** In practice, it is still used, but prices are generally lower than those of artisanal spirulina growers, in particular because of the failure to take into account here the "uncertainties" of cultivation, nor any marketing costs.

Presentation of the results

During the calculation a graph appears on the screen, giving the daily harvest and various other results depending on the day. This graph can be copied and printed via the screenshot and the clipboard.

The complete results are published at the end of the calculation, at the same time as the corresponding data, and can be printed (they are automatically copied and referenced in the file: C:/perso/SPIRPAC-F.txt).

It should be noted that the productivity appearing in the results **takes into account the estimated yield** (losses during harvesting and drying) and is expressed in dry matter.

Influence of temperature, light, alkalinity, salinity and pH on spirulina photosynthesis

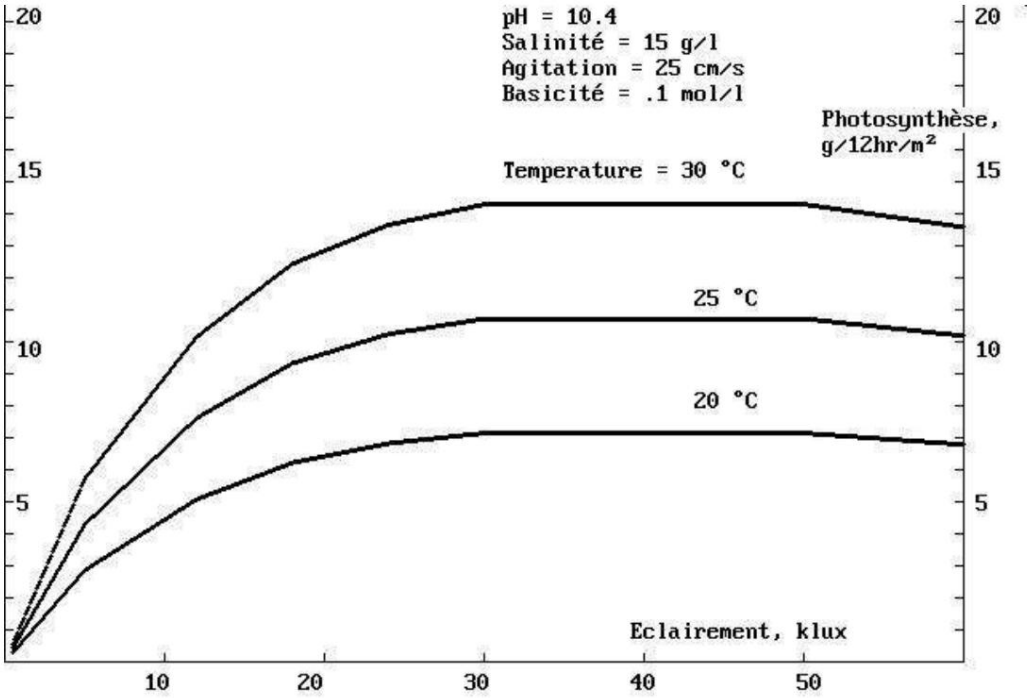
We can assume that the maximum rate of photosynthesis, in a well-agitated pond, and under the best conditions of temperature, light, alkalinity, salinity and pH, is close to 1.8 g/hour/m² of pond.

This speed can also vary depending on the strain of spirulina and the presence of catalysts.

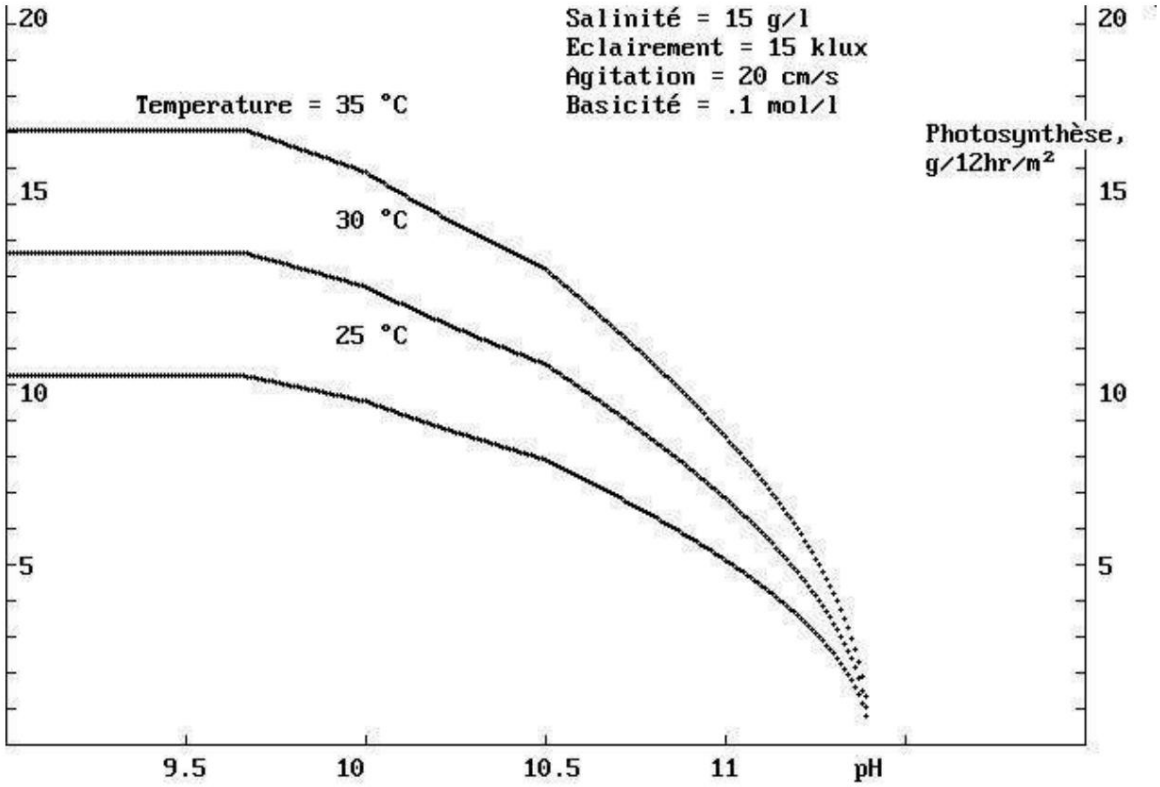
In the simulation programs it is assumed that the photosynthesis function is directly proportional to functions of temperature, light, salinity, pH and degree of agitation:

Photosynthesis rate = kxf(T) xf(klux) xf(salinity) xf(pH) xf(agitation)

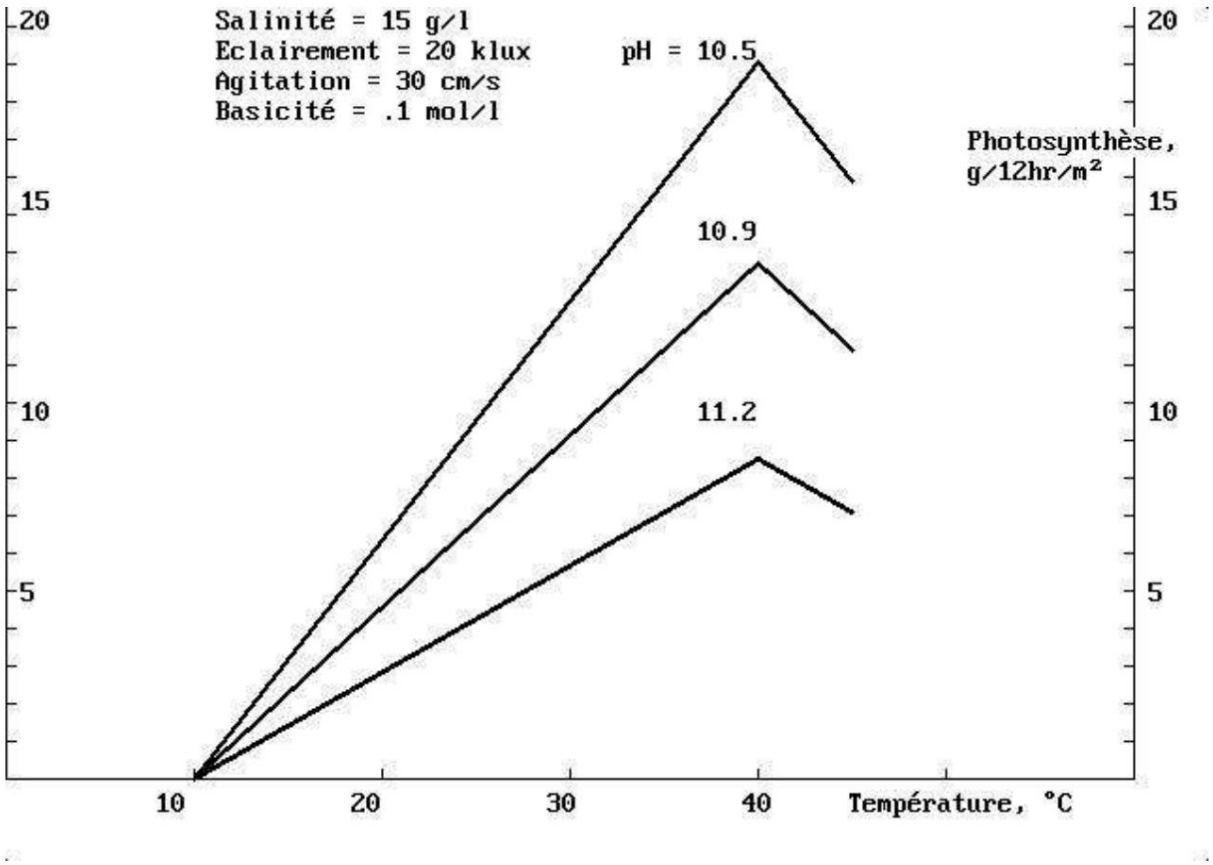
This assumption has no real scientific basis, but it makes the calculations easier and it does not give such bad results.
Here are some examples of these functions, which are largely inspired by Zarrouk's thesis (also taking into account experimental results):



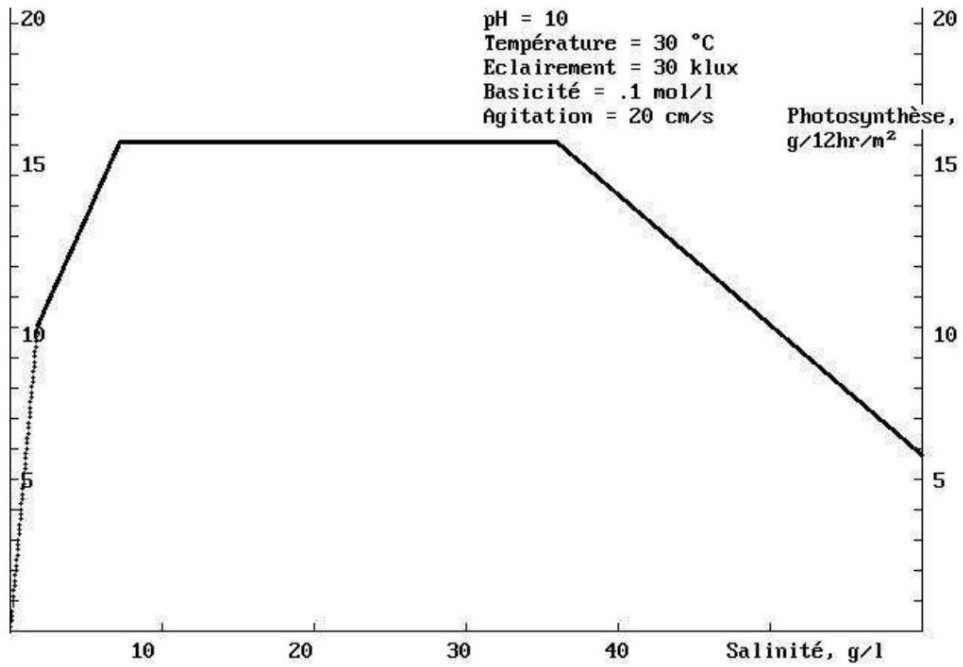
Photosynthesis rate of spirulina as a function of illumination according to Zarrouk's thesis, Fig. 3 [Zarrouk C. "Contribution to the study of a cyanophycea: influence of various physical and chemical factors on the growth and photosynthesis of Spirulina maxima (Setch and Gardner) Geitler", Doctoral thesis, Faculty of Sciences of University of Paris, 06/12/1966]



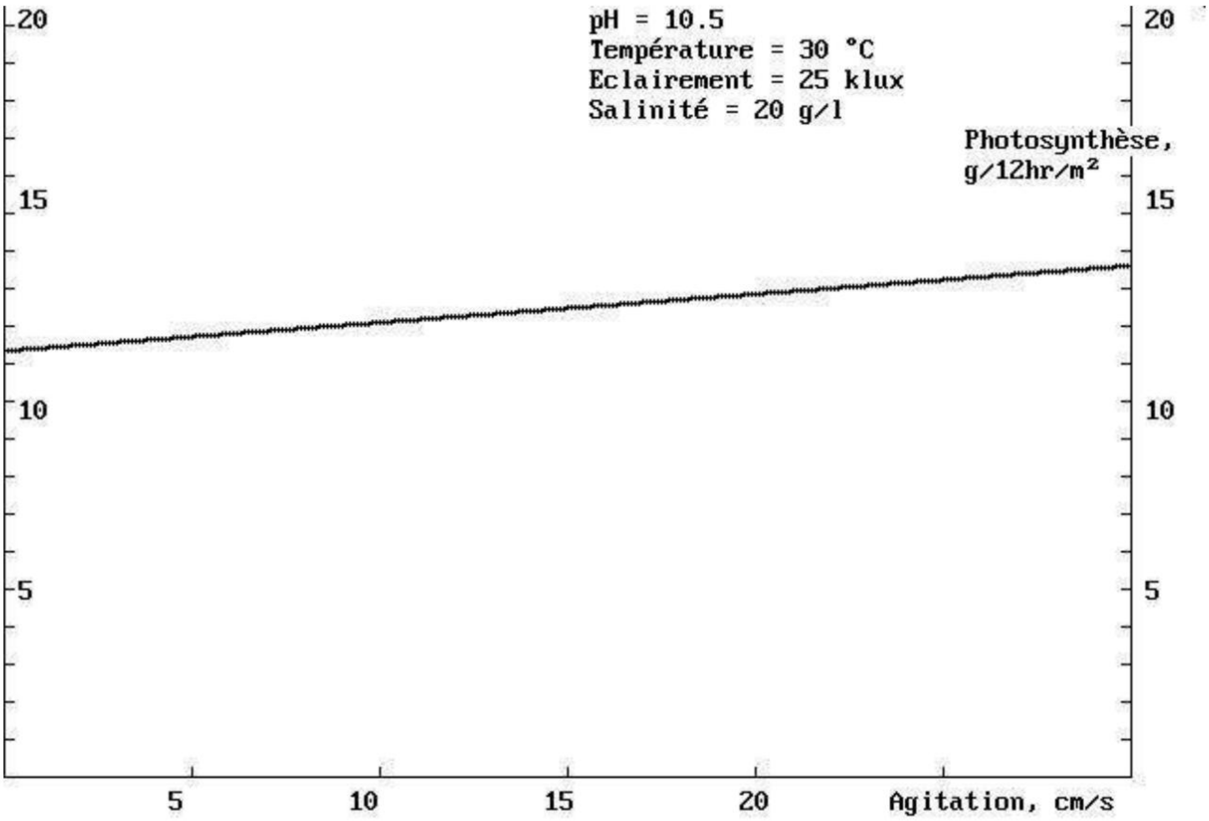
Photosynthesis rate of spirulina as a function of pH according to Zarrouk's thesis, Fig. 20



Speed of photosynthesis of spirulina as a function of the temperature of the culture according to Zarrouk's thesis, Fig 19.



Speed of photosynthesis of spirulina according to the salinity of the medium according to Zarrouk's thesis, Table IV



Speed of photosynthesis as a function of agitation (more or less imaginary function, in which the pH also intervenes, valid for usual agitation systems up to the speed it is possible that of photosynthesis, but we have not experienced it beyond 30 cm/s; clearly the speed with perfected systems it is possible to increase by 30% the function is provided to take this effect into account, but without any quantified experimental basis)

APPENDIX 3: climate data (solar)

1. Global horizontal ground irradiation data (monthly averages) can be obtained free of charge for all **of Europe** here : **http://re.jrc.ec.europa.eu/pvgis/apps4/pvest.php** generally with the altitude and longitude of the site in question.

NB for the PACA region in France the same data is available in another form (with very detailed maps) here: **http://www.atlas-solaire.fr/atlas-solaire-paca**

2. Similarly for **Africa** and Asia : **http://re.jrc.ec.europa.eu/pvgis/apps4/pvest.php?lang=en&map=africa**

NB

a) The reproduction of these data is free on condition that their origin is mentioned. b) When using these data, to obtain the figures for the site, it is in principle necessary to point the cursor in the middle of the point representing the site or site of the farm (not on its name), it is therefore necessary to expect small variations depending on pointing accuracy.

APPENDIX 4: Practical tips for optimizing with Spirpac-f

a) Set variable 131 (Number of consecutive days…) to 365

b) Set variable 132 (Number of inter-campaign days) to approximately 80, which will give the number of effective market days = 365 – variable 132

c) By trial and error find the initial month giving the result optimum

APPENDIX 5: Precautions on Vitamin K

Extract from Dr Vidalo's book (2016): on page 54 a note has been added referring to page 130 which explains that spirulina would essentially only contain vitamin K2 which does not interfere with the coagulation process. Clearly, no precaution to take… Further investigation is therefore necessary on this subject. In the meantime disregard page 173 of this Manual.

En effet, la vitamine D pénètre dans l'organisme par deux sources : l'une dite « exogène », c'est celle qui est apportée par l'alimentation, essentiellement les corps gras, et singulièrement les graisses de poisson, l'autre dite « endogène » provient de la synthèse de cette vitamine D par la peau exposée aux rayons du soleil. La vitamine D régularise le taux de calcium sanguin, améliore son absorption et minimise son élimination.

Elle favorise la synthèse de l'insuline, protège la peau et certaines parties du cerveau et elle agit sur certaines tumeurs malignes.

Outre le rachitisme, sa carence engendre des retards mentaux chez l'enfant, des déformations osseuses, des retards de croissance et des fractures spontanées.

La source végétale la plus importante de vitamine D est la spiruline.

La vitamine E ou tocophérol

est la vitamine de la longévité. En 1926, Evans et Bishop découvrent une substance extraite du germe de blé et des feuilles vertes à laquelle ils donnent le nom de vitamine E. C'est un excellent antioxydant qui encourage l'oxygénation des cellules, retarde le vieillissement, protège des maladies cardio-vasculaires et même de certains cancers. Il est important pour les femmes enceintes de n'avoir aucune carence en vitamine E.

Même si la teneur en vitamine E de la spiruline est insuffisante pour satisfaire l'apport journalier recommandé, elle en contient d'avantage que les germes de blé, souvent pris comme référence. Selon la FDA, deux cuillers à café de spiruline apportent 20 % des besoins quotidiens en vitamine E.

La vitamine K ou phylloquinone

est la vitamine antihémorragique. La vitamine K – avec un K comme *Koagulation* en danois – a été découverte par deux danois, H. Dam et Schönheyder, à l'occasion de recherches sur le métabolisme du cholestérol entreprises en 1929 sur des poulets. La vitamine K est transformée par le foie en prothrombine.

L'organisme l'utilise pour combattre les hémorragies, divers troubles hépatiques et colitiques. Un déficit en vitamine K se traduit par des hémorragies et des retards de coagulation. Son absorption se fait par l'intestin grêle et nécessite la présence de sels biliaires.

Il y a deux sources d'apport en vitamine K :

- L'une exogène, par l'alimentation. Les 4 principales sources végétales de vitamine K sont le chou frisé, les navets, les épinards et la spiruline.
- L'autre, endogène, est donc interne. En fait notre organisme ne sait pas fabriquer la vitamine K mais il sait capter celle fabriquée par les bactéries dites « saprophytes » contenues dans l'intestin, bactéries « utiles » qui participent activement à la dégradation du bol alimentaire circulant dans la lumière intestinale. Il récupère la vitamine K ainsi produite par absorption au niveau de la muqueuse de l'intestin grêle, en fonction des besoins. C'est là un remarquable exemple de symbiose biologique, ou de « xénie ».

La vitamine C

est le seul micronutriment essentiel à la vie et qui ne se trouve pas, ou alors en quantité négligeable, dans la spiruline.

Heureusement, la vitamine C est présente dans quasiment tous les fruits et légumes. Et pas seulement dans les agrumes comme il est de tradition de le proposer : le sempiternel « jus d'orange » du petit-déjeuner, à condition de consommer le fruit pressé, mais aussi dans d'autres agrumes comme les pamplemousses, pomelos, clémentines etc. Le kiwi est une véritable « bombe » de vitamine C naturelle comme le sont les baies, framboises, cassis, mûres, la petite cerise acérola bien sûr. Mais aussi, l'immense majorité des fruits et légumes frais. Même la pomme de terre en contient ! Il faut bien intégrer que notre organisme a besoin d'apports en vitamine C quotidiennement, à la différence d'autres vitamines, liposolubles notamment, car il ne sait pas la stocker. Par contre les sources alimentaires sont tellement nombreuses qu'il est très aisé d'équilibrer ses apports en ayant une alimentation simplement variée.

Quant à notre petite spiruline, elle possède, elle, les coenzymes qui permettent à la vitamine C de jouer pleinement son rôle, notamment de puissant antioxydant.

VITAMINES	Composition pour 10 g	% AJR*	Rôle
A (Béta-Carotène)	14 mg	1000 %	Vision, croissance, antioxydant
E	1 mg	10 %	Lutte contre le vieillissement
B1	0,35 mg	30 %	Participe à l'équilibre du système nerveux, des muscles et du cerveau
B2	0,4 mg	30 %	Lutte contre le vieillissement de la peau, rides, lésions oculaires
B3	1,4 mg	9 %	indispensable au bon fonctionnement des cellules du cerveau et du système nerveux
B5	0,01 mg	0,2 %	Accroît la résistance à la fatigue et au stress et combat le vieillissement cellulaire
B6	0,01 mg	4 %	Facilite la digestion et l'assimilation des aliments. Stimule le système immunitaire
B8	0,005 mg	0,5 %	Appétit, perte de cheveux
B9	0,01 mg	2,5 %	Nécessaire à la croissance et à la division des cellules
B12	0,032 mg	1000 %	Fatigue, circulation, croissance
I	6,4 mg	-	Action sur le système nerveux, anticancéreux
K	0,224 mg	300 à 500 %	Favorise la coagulation, lutte contre le vieillissement

MEDFEED SOFTWARE USER MANUAL

(Edition dated February 1, 2014)

1. Purpose

Facilitate calculations of spirulina culture media and food; by hand these calculations are simple but tedious and repetitive. Only about ten cases are presented, but they already cover a wide range.

2. System Requirements

Written in Visual Basic language, this software runs on any PC (Windows XP, Vista or 7…). To be able to print the results, **a C:/Medfeed folder must have been created.**

3. Basis of calculations

Medium, in mg/litre of medium

Ca >= 40

Mg >= 20
K >= 100

S >= 50

P = (Ca / 2 + 0.9 x Mg) / 3 + 10
N ammoniacal <= 20

K / Na < 5 by weight

Cl >= 607 (based on 1 g of salt/litre minimum)

Food, in g/kg of dry spirulina

NH4 >= 160 (in general an excess of 15% above the minimum is expected)

P >= 10 + ((Ca – 7) / 2 + 0.9 x (Mg – 3.5)) / 3
K >= 18

S >= 11

Ca >= 7

Mg >= 3.5
Na = 6.4

[For the cases without salinization we base ourselves on the values

lower except for nitrogen]

4. Principles

Account is taken of the elements (Ca, Mg, K, S) contained in the water used (including ash water, sea water and water used as culture medium and to compensate for evaporation), in salt and in N It is assumed that the Ca and Mg of the water used to make the culture medium and to compensate for evaporation are in the form of bicarbonates.
It is assumed that 1/3 of the Ca and Mg phosphates precipitate (we s that they have in practice a strong tendency to remain in solution even when their solubility products say that they must precipitate)
We give up using nitrate as a source of nitrogen, even in the new culture medium; in practice, it is often preferable to add 2 g of nitrate/litre for ease when starting new crops, except in cases “without salinization”.

We try to keep to the lower limit allowed for the element
rare
In the case of using ash water to provide alkalinity, it is assumed that the ash water contains dipotassium sulphate, and that it is carbonated at pH 10.

In the case of using sodium bicarbonate to provide alkalinity, one can choose the initial pH of the medium, and the pH adjustment agent (soda ash or sodium hydroxide).
In the case of use of NPK, it is assumed that it contains sulfur in the form of diammonium sulphate (usual case).
In the case where calcium must be provided in addition to that found in the raw materials, it is provided in the form of anhydrous CaCl2 or lime (at the rate of 2/3 by weight relative to the calcium chloride).

"Magnesium sulphate" is Epsom salt with 7 moles of water of crystallization
If the available phosphoric acid or one of the other acids used is not at 100% concentration, this must obviously be taken into account by increasing the values found.

5. Comments

Decimal numbers must be typed with a comma
Cases with **NPK,** ash water and/or sea water are more

particularly designed for developing countries.

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LATEST REVISIONS

Revision of January 15, 2018: better definitions of variables and addition Appendix on vitamin K page 173

Revision of September 29, 2017: improvements to the phycocyanin test Revision of July 15, 2017: Small improvements to the formulas of trace elements Revision of July 3, 2017: it is specified that the calculated cost price is excluding taxes Revision of June 7, 2017: it may be necessary protect your hands from the high pH of the culture medium and the risk of electrocution; if rinsing the biomass, use an isotonic solution at the same pH as the culture.

Revision of May 20, 2017: modification of the “Jourdan” oligos formula by adding Molybdenum to promote nitrogen fixation. And (minor) modification of the filtration test.

Revision of October 21, 2016: modifications to improve the use of SPIRPAC-F

Revision of September 30, 2016: Modified the Calculations chapter to take into account the fact that hypertext links to software no longer work in the new version of Word, but remain active in PDF.

Revised July 7, 2016: added FSF input recommendations (Food)

Revision of June 9, 2016-06-09: in appendix A3 deletion of the mention of the calculation program (which is no longer available)

Revision of May 30, 2016: K increased to 18 in Medfeed with potassium without salinbization Revision of April 29, 2016: variable 163 is better explained in the list of SPIRPAC-F variables Revision of April 23, 2016: It is specified that we admit to simplify that 12% of the carbon contained in the spirulina produced comes from urea (= 300 g of urea/kg of spirulina) regardless of the quantity of urea actually used (it can be zero if we use ammonia or nitrate, for example, or in the case of nitrogen fixation in the air).

Revision of January 8, 2016: detail improvements

Révision du 19 septembre 2015 : concerne des modifications dans le calcul du prix de revient dans Spirpac-f surtout en relation avec le couplage avec sources de chaleur

Révision du 9 septembre 2015 : précisions concernant les variables 174 et 123 dans la notice Spirpac-f

Révision du 23 juillet 2015 : modifié les variables 105 et 178 dans la notice Spirpac-f

Révision du 22 avril 2015 : améliorations de détail, y compris sur Spirpac-f concernant le calcul mois par mois

Révision du 6 novembre 2014 : légère amélioration du chapitre Nourriture

Révision du 29 septembre 2014 : modifications de Spirpac-f concernant surtout l'épuration (pH du recyclat)

Révision du 19 mai 2014 : explications sur le calcul en cas de couplage avec méthanisation

Révision du 22 avril 2014 : ajouté dans le tableau de résultats journaliers de Spirpac-f une dernière colonne indiquant la chaleur de la PAC en kWh/jour ; ajouté une 188ème variable Spirpac-f, inutilisée pour le moment mais disponible.

Révision du 14 avril 2014 : modifié légèrement le § "Comment créer de nouveaux cas" de la Notice Spirpac-f (page 151)

Révision du 29 mars 2014 : corrigé dans Spirpac-f la fonction fnray, ce qui a pour effet d'augmenter la chaleur de chauffage; et ceci rend efficace l'écran thermique nocturne.

Révision du 14 mars 2014 : corrigé une erreur dans SPIRPAC-F concernant la variable 136 (option prix de revient dans milieu de culture initial)

Revisions from March 4 to 6, 2014: slight modifications to the text of the Manual and SPIRPAC-F, without effect on the calculations, except for the “virtual HP” option for coupling with methanation (variable 135)

Revision of February 15, 2014: corrected daily table of results in Spirpac-f
General revision of 8/2/2014
Revision of January 8, 2014: energy content of spirulina reduced from 500 to 380

Revision of October 30, 2013: slight modification to the measurement of alkalinity Revision of June 20, 2013: modified Spirpac-f by adding the calculation of the cost price with biogas as a source of CO2 (biogas methane at €2.43/kg)
Revision of June 4, 2013: some modifications to the drying chapter.
Revision of April 9, 2013: in the Spirpac-f manual, added a paragraph about LED lighting; added to the Appendices a paragraph of good practice for the brine shrimp test.
Revision of February 6, 2013: added to SPIRPAC-F the month-by-month calculation of the heat requirement/m² in the event of heating by fuel, and the automatic adjustment of the number of days per month (provided to start with January).
Revision of November 23, 2012: modification of SPIRPAC-F which does not affect the calculation itself but provides comments.

Revision of August 4, 2012: modified SPIRPAC-F to limit the action of variable 175 (threshold) to the options isol =1 and 2 and remove the taking into account of "masks" in the manual.
Revision of July 14, 2012: enriched SPIRPAC-F with the possibility of modulating the size of its graph to be able to adapt to small screens, which are increasingly numerous Revision of June 9, 2012: slightly modified the paragraph on the calculation of photoinhibition in Spirpac-f (without changing the formula for calculating the photosynthesis reduction coefficient)

Revision of June 4, 2012: slightly modified the formula for automatic calculation of fixed costs in Spirpac-f (to facilitate manual calculation)
Revision May 29, 2012: Adjusted the display of Spirpac-f to be more suitable for laptops (e.g. 1366-768 screen resolution) and removed the exceptional influence of shaking speeds above 30.
Revision of March 14, 2012: added to Spirpac-f two sites: Barcelona and Madrid. The site of the Canaries is now called Santa Cruz de Tenerife Revision of March 10, 2012: indicated how to calculate the dew point from the temperature and the relative humidity (in Notice Spirpac-f); improved the alerts in spirpac-f Revision of March 7, 2012: improved Spirpac-f by allowing to modify the respiration rate (variable N° 178) and by expressing the average horizontal irradiation in Wh/m²/day Revision of February 10, 2012 : modified the Instructions for use of Spirpac-f (in § heating)

Revision of October 28, 2011: corrected the erroneous link for the flow formula in an inclined plane Revision of October 11, 2011: added a description on Edible Paints in

Appendix A21

Revision of September 22, 2011: compliance of the Spirpac-f title with the revision of August 10, 2011, same for the corresponding source program.
Revision of August 30, 2011: slightly modified the summary in English (Grow your…)
Revision of August 10, 2011: in Spirpac-f

adjusted haze coefficients to match monthly horizontal global irradiation data for French and African sites (provided on page 153 of the pdf Manual) between program lines “ *vasy:* ” and “ *saturday:* ” replaced 30 by 365/12 = 30.4

Revision of August 4, 2011: modified Spirpacf to increase the influence of clouds in the months of January, February and December so as to be more consistent with the horizontal global irradiation data for Paris.
Revision of August 1 , 2011: modified Spirpac-f to add the Angers case and to start adjusting the global horizontal irradiation of the French and African sites

Revision of July 22, 2011: modified Spirpac-f to include the calculation of the global horizontal irradiation over the simulation period, in order to allow the adjustment of the haze factor so that this irradiation is close to those given by the climatic statistics of the place. Added in Annex 3 of the notice of the sources of such statistics for Europe and Africa.

Revision of July 18, 2011: added to the Seeding chapter the § 5.7 concerning the strain drifts; corrected title of § “Sudden Deaths” in § 7.20; pointed out that lines can float.

Revision of July 2, 2011: incorporates several detailed modifications, in particular on the atmospheric CO2 analyzers and filtration of air entering non-thermodynamic dryers.

Revision of May 27, 2011: added to the Spirpac-f software the calculation of the heat supplied by the heat pump and made detailed improvements to the software, and to its instructions in the § heating (without changing the date of update of the software which remains on May 1st)

Revision of May 12, 2011: minor improvements Revision of May 10, 2011: additions made to the "Environment" chapter concerning purification and recycling Revision of April 25, 2011: added to the "Environment" chapter the possibility of bringing phosphorus through sodium tripolyphosphate.

Revision of April 4, 2011: Modified DeltapH software by improving it Added risk of photolysis at 39°C.

Revision of March 12, 2011: Modified appendix A26 concerning paddle wheels.

Revision March 2, 2011: Modified results output from Spirpac-f and Spitfix software to address appearance flaws with certain computer parameter settings

Revision of February 5, 2011: provided details on the method of calculating the cost price and fixed costs in the Spirpac-f program manual; slightly modified the description of the brine shrimp test

Revision of February 1 , 2011: made a small correction to the SPITFIX program

Revision of December 30, 2010: improved the formula of HCl N in chapter A5 (measurement alkalinity)

Revision of December 22, 2010: Added the dosage formula of allophycocyanin.

Revision of December 15, 2010: clarified the definition of variable 175 in the Spirpac-f leaflet and added the risk of anaerobic sulphite-reducers in the event of insufficient agitation.

Revision of October 1 , 2010: slightly revised § 4.5 of the Manual Revision of September 4, 2010: corrected a small error in Spirpac-f concerning variable 109 (“thermal insulation coefficient”) in the event of complete night insulation. This variable actually represents the heat loss and not the coefficient. Not changed the title of the software = "version of August 23, 2010")

Revision of August 31, 2010: corrected a small error in Spirpac-f concerning the complete nocturnal insulation case (previously the loss of heat through the insulation was neglected); not changed the title of the software = "version of August 23, 2010")

Revision of August 23, 2010: modified the warning of the risk of photolysis in Spirpac-f by putting the condition that this risk be over two consecutive hours (instead of one before) and by removing the condition that the temperature be lower than 25 °C.

Revision of August 19, 2010: slightly modified the explanation of the photolysis threshold formula in the Spirpac-f leaflet Revision of August 13, 2010: added a warning not to confuse caustic soda and soda crystals Revision of June 30, 2010 : specified that the Spirpac-f model only applies to autotrophic cultures

Revision of April 23, 2010: lowered from 0.05 to 0.04 the dose of urea in case of variable geometry.

Revision of April 16, 2010: corrected a corrupted link

Revision of March 5, 2010: in the Spirpac-f simulation software, increased the maximum duration of weekend leave to 7 days, modified variables n°112 (which becomes the concentration after harvest) and n°113 (which becomes the initial spirulina concentration) and made variable n°175 effective in all cases (simulates masks in cases where there is no insulation of the pool)

Revision of January 11, 2010: added variable 185 to Spirpac-f, and put the software: Absorption de CO2, Zabsco2, and Spitfix in VisualBasic Revision of July 16, 2009: modified the chapter Culture Revision of July 4, 2009: modified the chapter Presentation Revision of June 30, 2009: added to the Food chapter an example of very high nitrogen "fixation" Revision of May 14, 2009: added to the chapter Drying the finish of drying in the dryer Revision of January 14, 2009: in "Harvesting" added Nayalgué's practice of washing biomass with 5% salt water.

Revision of January 7, 2009: in Spirpac-f, if the harvest is spread over the whole day, the average harvest time variable is assigned the value 12, which automatically divides the fixed harvest costs by 4. (Modification not made in Spiru-f).

Revision of November 15, 2008:

improved Spirpac-f by adding double and triple glazing options in PC, and slightly modified the translucency of the PE double glazing. **These modifications have not been made in the Basic version (Spiru-f), for lack of space, except for the translucency modification.**

Corrected the calculation of electricity surplus in Spirpac-f (which was based on the total production excluding resetting to its initial level of the final spirulina concentration). This error is not in Spiru-f.

In the "Food" chapter added a § to note c to explain that mixed urea/nitrate food is compatible with the use of urea alone

Revision of November 13, 2008: corrected the formulas of butane and propane in "fuel"

Revision of November 12, 2008 in spirpac-f and spiru-f: following a light measurement campaign in 100% overcast weather, the light was increased by 15 to 20% (% of light in clear weather)

Revision of November 7, 2008: in spiru-f and spirpac-f:

waived the cost of fertilizers when using biogas

corrected the gas heating and the temperature regulation in the case of insulation N°3

Revised October 30, 2008:

corrected the initial calculation of the pool temperature in case of fuel heating in spiru-f

corrected heating fuel consumption in spiru-f and spirpac-f

Revision of October 22, 2008: corrected an error in an alert in Spirpac-f

Revision of September 29, 2008: in spirpac-f and spiru-f correction of an error in calculating the calorific value of fuel in the case of biogas

Revision of September 22, 2008: in spirpac-f and spiru-f the gas flows are separated to

regulation and for heating
Revision of September 10, 2008: simplification of the formula of trace elements, according to the recommendations of Cogné et al. (Clermont Ferrand)
Revision of August 19, 2008: corrected a small programming error concerning the option isolation = 3 in Spiru-f and Spirpac-f Revision of July 20, 2008: slightly modified the Culture chapter (§ Agitation), and made a minor correction in Spiru -f (print results).

Revision of July 11, 2008: in the Appendices, pH measurement: modified the temperature coefficient
Revision of June 30, 2008: in Spirpac-f and Spiru-f modified the impact of the risk of photooxidation on automatic shading, and introduced fixed costs for shading.

Revision of June 26, 2008: in Spirpac-f and Spiru-f divided by 4 the fixed harvesting costs in case there is no drying (since we can harvest all day)
Revision of June 23, 2008: modified Deltaph.exe in Visual Basic Revision of
June 5, 2008: improved functions C ex pH and pH ex C in SPIRU-F and in SPIRU-E.BAS (impossible to transcribe it in .EXE, program too long), and in CEXPH.
Revision of March 13, 2008: slightly modified SPIRPACF (renamed SPIRPAC-F) and SPIRU-F to allow them to deal with the case of sea water.

Revision of January 12, 2008: major modifications to the SPIRPACF simulation software, to integrate the modifications of SPIRU-F and SPISIMP3.

Revision of September 15, 2007: major modifications in the SPIRU-F simulation program, not made in SPIRU-E nor SPISIMP3. These changes include the introduction of variables 86 (photoinhibition option) and 92 (interior shading option) as well as the photolysis danger alert.

Revision of August 10, 2007: modified SPIRU-F, SPIRU-E and SPISIMP2 (modified in SPISIMP3) with regard to the limit of illumination at low temperature.

Revision of July 23, 2007: modified SPIRU-F, SPIRU-E and SPISIMP2 with regard to the low temperature illumination limit.

Revision of July 7, 2007: deleted variable 86 (global temperature increase) in SPIRU-F and SPIRU-E

Revision of June 16, 2007: modified SPIRU-F, SPIRU-E and SPISIMP2 (in particular increased by 5°C the lower operating temperature limits)

Revision of June 14, 2007: added a comment on the difference between Zarrouk and Vonshak in the chapters Calculation and Appendices Revision of June 9, 2007: in Appendix Cultivation/Shade, added additional

precautions to avoid photolysis.

Revision of June 4, 2007: in the Calculation chapter, deleted regulation by automatic aeration at temperature < 38 °C Revision of May 28, 2007: added to the Middle Chapter a remark about the

injection of phosphoric acid Revision of May 10, 2007: added a notice regarding fresh spirulina in the Consumption chapter

Revision of April 20, 2007: small addition to § Packaging of the Drying chapter

Revised April 12, 2007

Minor improvements in the “Sowing” and “Cultivation” chapters

Revised March 31, 2007

In the CALCULATION chapter:

added that the total night insulation option is possible even without a greenhouse

added that the CO2 absorption coefficient and the photosynthesis adjustment coefficient can be increased by up to 50% in the event of waves and foam on the surface of the bowl

- added that the relationship pH/CO2/base ratio, established experimentally between alkalinities of 0.02 and 0.30, is accepted as valid outside these limits.

Revised March 19, 2007

Modified the § dealing with organic spirulina in the Food chapter

Reduced urea in food to 300 g/kg in the Food chapter and in the Milnour software

Revised March 7, 2007

Removal of rotifers as predators of straight spirulina

Revised January 22, 2007

Modified Spiru-f, Spiru-e (and Spisimpl and Spirpacf) so that full night insulation does not cut greenhouse ventilation (but cuts fuel supply). The use of complete nocturnal insulation (for example in connection with basins on inclined planes) becomes possible while supplying the greenhouse with diluted CO2 during the day, which was excluded before. It is also possible with outdoor pools.

Revised January 6, 2007

Modified Spiru-f, Spiru-e (and Spisimpl and Spirpacf) to set up night insulation (options isol = 1 and 2) only when the illumination drops to a predetermined threshold (variable 87) and to make possible the option 1 for variable 77 (automatic calculation of recycling recycle = prodj) in all cases, independently of the automatic calculation of fixed costs.

Some other (minor) changes in Chapter Calculation

Revised December 28, 2006

Modified Spiru-f, Spiru-e and Spirpacf to allow the full nocturnal insulation option (isol = 1) without greenhouse, and, in Spiru-f and Spiru-e, to update the estimate of the fixed costs calculated when variable 71 = 0 Modified in a minor way the Chap. Culture and Power Point Presentations Corrected a small error in Case 1 of the MILNOUR utility program in the Calculation chapter

Revised December 1 , 2006

Addition to the Culture chapter/lines and chlorellae eliminated by rotifers

Addition to the Sowing chapter of the recommendations on the initial concentration

Revised November 23, 2006

(After crash of the author's main hard disk on 1/11/2006, reconstitution of the Manual in its state after October 16).

Added to the Calculation chapter the way to avoid that the download of the exe is blocked by the security parameters.

Added to the same chapter the main source programs in QBasic.

Revised October 16, 2006

Small additions to the Consumption and Harvesting chapters

Revised September 18, 2006

Addition to the chapter "Culture", § carbonaceous food, of an addition on the optimum dose of sodium bicarbonate

Addition of remarks in the "Calculation" chapter, § Cost price

Revision of September 7, 2006

Modifications of SPIRU-F and SPIRU-E: introduction of a variable (Proportional workforce), removal of cost updating, and harmonization of the two software.

Revised August 28, 2006

Made a slight correction (without impact on the results) to SPIRU-F and E

Revised August 5, 2006

Made some additions to the "Culture" Chapter and "Seedling"

Revised July 1 , 2006

Modified slightly the final recommendations

Revised June 6, 2006-06-06

Small correction concerning recycling in SPIRU-F

Revised May 1 , 2006

Some improvements on packaging (Drying chapter) and on Chlorella contamination (Culture chapter)

Revised April 15, 2006

In CALCUL, slight modification of SPIRU-F concerning recycling

Revised February 20, 2006

In the MIDDLE chapter, added the method of making magnesium sulfate from ashes

Revised February 8, 2006

In the Calculation chapter, modified the passages concerning recycling (consumption of water and electricity from recycling), and consequently SPIRU-F and SPIRU-E

Revised February 2, 2006

In the Calculation chapter, corrected the link to Notice Spirpacf Revision of January 24, 2006 Translated SPITFIX.EXE into French Revision of January 17, 2006

In Calculation, in SPIRU-F a new result has been introduced: the tank volume for water autonomy when you have a greenhouse.

Revised December 12, 2005

In Calculation, in SPIRU-F and SPIRU-E, temperature regulation by adjustable ventilation has been restored in the case of night and day insulation, and the "opmil" option has been introduced, which makes it possible to calculate the cost price without initial culture medium.
Modified WEATHER accordingly.

Revised November 29, 2005

Small improvements to the Culture chapter (straight lines, EPS and Purification paragraphs)

Revised November 22, 2005

Small modifications to the text of the Calculation chapter (combustion gas), and to the crop simulation software.

Revised November 10, 2005

Addition of the automatic calculation of fixed costs option in SPIRU-F

Revised November 8, 2005

Fixed Milnour.exe,
Improved Spiru-f.exe and Calculation

Revised October 27, 2005

Added to the Spiru-f simulation software the possibility of supplying CO2 to composting (diluted CO2)

Revised October 13, 2005

In the Appendices, in § A13, corrected the table of soda/sodium bicarbonate mixtures

In the Middle chapter added a paragraph on adding phosphate after soda

Slightly modified the paragraph on larvae in the Culture chapter

Revised September 23, 2005

Added in the Sowing chapter the address of J. Falquet Added

in the Environment and Food chapters details concerning the use of urine Added in the Culture chapter

a complement on the purification of used culture media.

Revised September 19, 2005

Small additions to the Culture Chapter (on bleach)

Revised September 15, 2005

Modification of case 1 in MILNOUR.exe

Revised September 12, 2005

Minor improvement to Culture and Calculation chapters

Revised September 8, 2005

Minor changes to Environment and Food chapters

Revised August 30, 2005

In Chapter Culture, added a precision concerning the appearance of amoebas

Revised August 28, 2005

In the Culture chapter, added that mosquitoes may not be sterilized by consumption of spirulina.

In the Index, corrected the link to the summary in Spanish; in Cultivo, added links in the summary.

Revised August 19, 2005

In the Culture chapter, § Iron, added the composition of Ferfol

Revised August 14, 2005

In the Culture chapter, § Agitation, added that it is good to agitate at night.

Revision of August 9, 2005

In the CALCUL chapter put in capital letters the names of files SPIRUL, etc.

Revision of August 1st

Slightly modified in the Technical Annex the filtration and turbidity test

Revised July 27, 2005

Added to the Environment, Culture and Appendices chapters: do not store fresh water in the presence of light.

GROW Update

Revision of July 21, 2005

Improvements made to the Culture chapter, concerning chlorella.

Revision of July 14, 2005

Modifications (minor) in Chapter CALCULATION, concerning the Visual Basic version of the main simulation software.

Corrected a spelling mistake in the Bibliography (Puyfoulhoux)

Revised July 13, 2005

Renovation of a certain number of links with a view to the installation of the Manual on the site of Petites Nouvelles (due to work making it temporarily unusable on the Antenna site)

Revision of June 30, 2005 In

Chapter Calculus added the Cexph.exe to Small Utility Programs, and a link towards these.

Revised June 28, 2005

In Harvest chapter, added that we must put the biomass in the fridge before and after pressing

Revision of June 24, 2005

Add technical appendix A31 on water softening Revision of June 16,

2005 Detailed improvements made to the Calculation chapter and to

meteo.exe, spiru-e.exe, spiru f.exe

Revised June 8, 2005

In the Culture chapter, added the possibility of flocculation in weak green lumps in EPS.

Revision of June 6, 2005

Amended § 7.8 regarding the use of glucose as a replacement for sugar as carbon input

Eliminated a small "bug" in the spiru-f and spiru-e and SPIRPACF software

Revision of 05/23/2005

Added in the summary a link to the summary in Spanish

Revision of 03/18/2005

Added to spiru-f and spiru-e a variable N°86: increase in the temperature of the Earth in relation to current weather conditions.

Adapted this software to treat environments with very low alkalinities

Corrected small errors in the Visual Basic version of these software (Spirpacf)
Revision of 02/23/2005

Reintroduction of the "minimum pH for harvest" variable (No. 85) in meteo, spiru-e and spiru-f
Revision of 02/22/2005

Minor correction made to Spiru-f Revision
of 02/14/2005 Important modification in the

Calculation chapter: removal of the Spirpac software, and integration of the heat pump in the normal Spiru-f software (or Spiru-e).

This does not change the results, but operation without a heat pump requires that variable N°79 has the value 0, and variable N°80 the value 45.
Revision of 4/02/2005

Corrected the simulation programs (spirpacf, spirpac, spiru-f, etc), which slightly improves productivity and cost price in the results
Revision of 01/25/2005

Fixed Salt in Milnour.exe results
Revision of 01/22/2005

Addition in Calculation and Notice Spirpacf of an explanation concerning the accumulation of shading on a culture of spirulina.
Revision of 6/01/2005

In the medium and food calculation formulas, reduce the P (excluding Ca and Mg) from 14 to 10 mg/l and g/kg.

GROW YOUR OWN SPIRULINA

Revised on August 30, 2011

NOTICE

This is the condensed version of a "Manual of small scale spirulina culture" written in French and distributed by Antenna Technology.

No warranty is granted on the contents.

This is not one more book on spirulina. Excellent ones are available*, dealing with such topics as: - what is spirulina? - what is its natural habitat?

- how did the Aztecs harvest it and eat it?

- how was it rediscovered 30 years ago? - what nutrients, vitamins, minerals does it contain? - what are its food-grade specifications?

- what are its numerous benefits for your health? -
how does industry manufacture and market spirulina? - why
is spirulina ecologically friendly? - why has it such a brilliant
future?

The sole purpose of this little manual is to bring my field experience on small scale spirulina production to those who would need it: the answers to the above questions are assumed to be well known.

To make the presentation shorter, easier and more accurate, I decided not to avoid using common technical terms: in case some would confuse you, look up for an explanation in a chemistry college handbook.

What is called "spirulina" here actually bears the scientific name of *"Arthrospira platensis",* a cyanobacteria. But the common name "spirulina" is universally used.

* See for instance "Earth Food Spirulina", by Robert Henrikson, published by Ronore Enterprises, USA (1994), and "Spirulina, Production & Potential", by Ripley Editions Cell-biology and Biotechnology, edited by A. Vonshak, published by Taylor & Francis (1997)

CLIMATIC FACTORS

Temperature is the most important climatic factor influencing the rate of growth of spirulina, provided there is enough light available.

Below 20°C, growth is practically nil, but spirulina does not die. The optimum temperature for growth is 35°C, but above 39°C spirulina is in danger.

Growth only takes place in light (photosynthesis), but no more than 16 hours a day. During dark periods, chemical reactions take place within spirulina, like synthesis of proteins and respiration.

Respiration decreases the mass of spirulina ("biomass"); its rate is much greater at high temperature so cool nights are better on that account, but in the morning beware that spirulina generally cannot withstand a strong light when cold (below 20°C).

Light is an important factor but full sunlight may not be the best illumination: 30% of full sun light is actually better, except that more may be required to quickly heat up the culture in the morning.

Individual spirulina filaments are destroyed by prolonged strong illumination ("photolysis"), therefore it is necessary to agitate the culture in order to minimize the time they are exposed to full sunlight.

Rain is beneficial to compensate for evaporation, but it must not be allowed to cause overflowing of the culture pond.

Wind is beneficial for agitating and aerating the culture, but it may bring dirt into it.

Artificial light and heating may be used to grow spirulina, although they might not be economical. Fluorescent tubes and halogen lamps are both convenient, but LEDs will be even better.

PONDS

Spirulina thrives in alkaline, brackish water. Any water-tight, open container can be used to grow spirulina, provided it will resist corrosion and be food grade type. Its shape is immaterial, although sharp angles should be avoided to facilitate agitation and cleaning. Its depth is usually 30 to 40 cm (twice the depth of the culture itself). It can be as small as 1 m^2 but 5, 20 or 100 m^2 are more economical. Dimensions are only limited by the necessity of accessing for agitation and cleaning. The bottom should have a slight slope and a recess to facilitate cleaning and emptying. Two ponds are better than just one, for practical reasons.

The most economical ponds are made of UV resistant, food grade plastic film of 0.5 mm thickness or more, with sides supported by bricks or a wooden structure or metal tubes. If termites are present, a layer of dry ash plus a layer of sand should be placed under the film to protect it, and of course wood should not be used.

Concrete ponds are a good, durable solution where experienced labor is

available. Before starting the culture, the cement should be well cured and whitewashed.

A greenhouse over the ponds offers many advantages, provided it can be aerated and shaded. As a matter of fact, covering the ponds is necessary in many instances.

Agitation can be manual, with a broom, once every two hours. If electricity is available, aquarium pumps are practical to agitate the culture (one watt/m² is enough). "Raceway" ponds agitated by paddlewheels are standard in the industry, but somewhat outside the scope of this manual.

MEDIUM CULTURE

Spirulina can live in a wide range of compositions of water; the following is an example of a convenient analysis:

Anions	Carbonate	2800 mg/lite4
	Bicarbonate	720
	Nitrate	614
	Phosphate	80
	Sulfate	350
	Chloride	3030
Cations	Sodium	4380
	Potassium	642
	Magnesium	10
	Calcium	10
	Iron	0.8
Urea		< 40
Total dissolved solids		12847
Density @ 20°C		1010 g/liter
Alkalinity		0.105 N (moles strong base/liter)
pH @ 20°C		10.4

In addition, the solution contains traces of micronutrients.

Such solution can be obtained by dissolving various combinations of chemicals; here is one example convenient for many typical soft waters:

Sodium carbonate (soda ash)	5g/liter 5
Sodium chloride, crude	
Potassium nitrate	2

sodium bicarbonate	1
Potassium sulphate, crystallized	1
Monoammonium Phosphate, crystallized	0.1
Magnesium sulphate, crystallized, MgSO4, 7 H2O	0.2
Lime	0.02
Ferrous sulfate, crystallized, FeSO4, 7 H2O	0.005

The water used should be clean or filtered to avoid foreign algae. Potable water is convenient. Water often contains enough calcium, but if it is too hard it will cause mud which is more a nuisance than a real problem. Brackish water may be advantageous but should be analyzed for its contents or tested. Seawater can be used under some very special conditions, outside the scope of this short manual.

The culture medium described above is used to start new cultures. Yo increase the volume of culture the make-up medium should best be as follows: carbonate is replaced by bicarbonate (8 g/l in total), urea is up to 0.06 g/l, and nitrate is omitted.

Certain ions can be present in concentrations limited only by the total dissolved solids which should not be much over 25 g/l; these are: sulfate, chloride, nitrate, and sodium. Sodium or potassium nitrate can replace urea, the advantage being a large stock of nitrogen; urea is more efficient and chaeper to supply nitrogen but it may kill spirulina at too high concentration. Spirulina can grow on either nitrate or urea alone, but spirulina prefers urea (or ammonia).

Phosphate, magnesium and calcium cannot be increased much without precipitating magnesium or calcium phosphate, possibly leading to imbalances in the solution.

Potassium concentration can be increased at will, provided it does not become more than five times the sodium concentration by weight. This makes it possible to use potash solution extracted from white wood ash to replace sodium carbonate/bicarbonate should these not be available (let the potash solution absorb CO2 from the air until its pH has come down to 10.5 before using it). If fertilizer grade chemicals are used, they should be of the "soluble" or "crystallized" type, not of the "slow release", granulated type. Iron sulphate sold for treating lawns is not suitable.

Trace micronutrients contained in the water and in the chemicals are sufficient to support the initial growth.

In case of necessity ("survival" type situations), nitrogen, phosphate, sulfate, sodium, potassium and magnesium can all be brought by urine (from persons or animals in good health, not consuming drugs) at 5 ml/liter dosis and iron by a saturated solution of iron in vinegar (use about 0.1 ml/l).

Solutions of iron should preferably be introduced very slowly and under agitation into the medium. Dripping is best.

SEEDING

Choose a spirulina strain containing a high proportion of coiled filaments (less than 25% straight filaments, and if available 0%), easy to harvest, and containing at least 1% of gamma-linolenic acid (GLA) based on dry weight.

Concentrated spirulina seed culture can be obtained either from the floating layer (if any) of an unagitated culture, or by rediluting a freshly filtered biomass (beware of lumps). A concentration of up to 3 g spirulina (dry) per liter is permissible if storage and transportation last less than a week's time, and provided the seed culture be aerated at least two times a day. If aeration can be continuous, the concentration may be up to 10 g/l (weights of spirulina always refer to contained dry matter).

It is advisable to maintain the growing culture at a fairly high concentration in spirulina after each dilution with new culture medium, about 0.3 g/l: the "Secchi disk" reading (see Annex 1) should not be above 5 cm, ie the color of the culture should be clearly green (otherwise shading is mandatory). The rate of growth is about 30 % /day when light and temperature are adequate and the make-up culture medium is based on bicarbonate (without carbonate). As the growth is proportional to the area of the culture exposed to light, it is recommended to maximize this area at all times (ie use the minimum feasible depth during the expanding area period, generally 5 to 10 cm).

When the final area and depth (10 to 20 cm) are reached in the pond, let the spirulina concentration rise to about 0.5 g/l (Secchi disk at about 2 cm) before harvesting.

HARVESTING

When the spirulina is in good condition, separating it from the water ("harvesting") is an easy operation, but when the culture gets too old and "sticky" harvesting may become a nightmare (see § "Taking care").

The best time for harvesting is early morning for various reasons: - the cool temperature makes the work easier, - more sunshine hours will be available to dry the product, - the % proteins in the spirulina is highest in the morning.

There are basically two steps in harvesting:

- filtration to obtain a "biomass" containing about 10 % dry matter (1 liter = 100 g dry) and 50 % residual culture medium, - removal of the residual culture medium to obtain the "fresh spirulina biomass", ready to be consumed or dried, containing about 20 % dry matter and practically no residual culture medium.

Filtration can be simply accomplished by passing the culture through a fine weave cloth, using gravity as the driving force. Synthetic fiber cloth (especially polyamide or polyester) with a mesh size of about 30 to 50 microns is the preferred filtering medium. Supporting the filtration cloth by a net will somewhat accelerate the filtration and protect the cloth against rupturing, but a simple bag made from the cloth works well also.

The filter may be installed above the pond to directly recycle the filtrate.

The culture to be harvested should be passed through a sieve (mesh size about 200 microns) to remove any foreign matter such as insects, larvae, leaves and lumps of polysaccharide or muds from the bottom of the tank.

When the spirulina floats, which is often case without stirring, it is efficient to scoop out the "cream", using a straight edge pail. Harvesting the floating layer (generally richer in spiralled spirulina) will tend to increase the % straight spirulina in the culture. Straight spirulina is more difficult to harvest. So actually it is not recommended to harvest the floating layer when both straight and spiralled spirulina are present.

The filtration is accelerated by gently moving or scraping the filter cloth. When most of the water has filtered through, the biomass will often agglomerate into a "ball" under the motion, leaving the cloth clean (this desirable condition happens mostly when the biomass is richer in spiralled forms and the culture medium is clean).
Otherwise it may be necessary to scrape it out from the cloth.

The final dewatering is accomplished by pressing the biomass enclosed in a piece of filtration cloth plus a strong cotton cloth, either by hand or in any kind of press. The simplest is to apply pressure (0.15 kg/cm² is enough) by putting a heavy stone on the bag containing the biomass. The "juice" that is expelled comes out first colorless, later it turns slightly green and the operation must then be discontinued otherwise too much product will be lost. For the usual thickness of cake (about one inch after pressing), the pressing time is about 15 to 20 minutes. Practically all the interstitial water (culture medium) is removed, and some rinsing may be effected by the internal juices from ruptured cells. The pH of the well pressed biomass is near 7 (neutrality).

This pressing operation effects a more efficient separation of the residual culture medium than washing the biomass with its weight of water on the filter.
Washing with fresh water may cause rupture of the cell wall of the spirulina due to osmotic shock, leading to a loss of valuable products; it may also introduce germs contained in the wash water. Washed biomass is more prone to fermentation than pressed biomass. Pressed biomass contains twice as much dry matter as unpressed biomass, which reduces the drying time.

When the biomass is too "sticky", for instance 100% straight filaments, it may not be possible to dewater it: in such case, it must be washed.

FEEDING THE CULTURE

The nutrients extracted from the culture medium by the harvested biomass should be replaced to maintain the fertility of the culture medium.

The main nutrient is carbon, which is spontaneously absorbed by the medium from the air, as carbon dioxide (CO2), whenever the pH of the medium is above 10. However the air contains so little CO2 that this absorption is a slow process, corresponding to a maximum productivity of 4 g spirulina/day/m². This maximum rate is reached at or above pH = 10.5. Extra CO2 can be introduced to increase the productivity. Pure CO2 gas (from fermentation or from a cylinder) is introduced into a pipe through which the culture is being pumped and returned to the tank.

Another popular, although usually costly, means of feeding carbon is bicarbonate. Adding bicarbonate is an easy and efficient way of reducing the pH, but it increases the salinity and alkalinity of the medium; to maintain the quality of the medium, it is mandatory to drain part of the culture medium from time to time and to replace it by new medium made from bicarbonate. Disposal of the drained medium may be an environmental problem and the cost may of the chemicals consumed may be uneconomical.

The amount of gas or bicarbonate to be fed is adjusted so as to control the pH at around 10.4. A pH lower than 10.2 may cause an overproduction of undesirable exopolysaccharides (EPS). A good practical dose of carbon feed is the equivalent of 40% of the spirulina produced (ie about 0.8 kg of CO2 per kg of dry spirulina harvested).

Apart from carbon, spirulina requires the usual major biological nutrients: N, P, K, S, Mg, Ca, Fe, plus a number of micronutrients. In many cases, the micronutrients and the calcium need not be fed to the culture, being supplied as natural impurities contained in the make-up water and as impurities in the chemicals used as food for the spirulina. In some locations, the water contains a large excess of calcium, magnesium or iron, that may become a nuisance by producing abundant mud. In such case pretreatment of the water is preferred.

The major nutrients can be supplied in various ways, preferably in a soluble form, but even insoluble materials will slowly be dissolved as the corresponding ions are consumed by the spirulina in the medium. Availability, quality and cost are the main criteria for selecting the sources of nutrients, but their content in valuable micronutrients may also affect the choice.

If fertilizer grade chemicals are used, they should be of the "soluble" or "crystallized" type, not of the "slow release", granulated type. Beware of the contents in "heavy metals" (mercury, cadmium, lead and antimony), as the spirulina readily absorbs these and strict specifications must be met.

Natural nitrate from Chile, where available, is a good source of nitrogen, not only on the basis of its low cost but also because it contains many valuable

micronutrients apart from nitrogen. But very generally the cheapest source of nitrogen is urea. Urea, made up of ammonia and CO2, is an excellent nutrient for spirulina but its concentration in the medium must be kept low (below about 50 mg/liter. Excess urea is converted either to nitrates or to ammonia in the medium. A faint smell of ammonia is a sign that there is an excess of nitrogen, not necessarily harmful; a strong odor however indicates an overdose.

Here is a feed formula convenient in most locations, per kg of harvested spirulina (dry product):

Urea	300g
Monoammonium phosphate*	50g
Potassium sulphate	30g
(or 40 g if no potassium nitrate is used in the culture)	
Magnesium sulphate**	30g
Lime	10g
Iron sulphate**	2.5g
Micronutrient solution***	5ml

* Concentrated liquid phosphoric acid may replace the phosphate.

** The usual commercial product, crystallized with 7 molecules of water (crude iron sulfate sold for treating lawns is unsuitable)

*** Optional but useful to make the biomass easier to harvest and also to reduce the need for renewing the culture medium; click on A26 to obtain the recipes, however in French.

In case of necessity ("survival" type situations), all major nutrients and micronutrients except iron can be supplied by urine (from persons or animals in good health, not consuming drugs); add daily doses equivalent to about 15 to 20 liters/ kg spirulina produced. Iron can be supplied by a saturated solution of iron in vinegar (use about 100 ml/kg) mixed with some lemon juice or citric acid.

Ferilizers other than urea can be fed every month or so, but urea (or urine) has to be fed daily, preferably based on the average production expected.

TAKING CARE OF THE CULTURE

Apart from harvesting and feeding, a spirulina culture requires some attention in order to be kept in good condition.

Agitation is a requirement. Continuous agitation however is not required.

One third of full sun will saturate the photosynthetic capacity of spirulina, but shading is not required except to reduce the consumption of water (evaporation) or the temperature (< 38°C) or the pH (< 11.3). The temperature will practically never be

too high, but the pH may soon become too high if insufficient carbon is supplied.

The depth of culture must be kept between 10 and 20 cm. Evaporation must be compensated for by adding water. Without a grrenhouse, rains must be compensated for either by evaporation or by draining part of the medium (in the latter case, add the chemicals corresponding to the volume of medium drained).

Accumulation of mud on the bottom may cause some to float due to anaerobic fermentation gases, and this will disturb the harvesting process. Therefore it is recommended to agitate the mud layer with a broom from time to time. If too much mud accumulates at the bottom of the pond, it can be removed by pumping or siphoning (preferably while the spirulina is floating, in order to reduce the loss). Add new culture medium to replace the volume removed. Of course another way to remove the mud is to provisionally transfer the culture into another pond and clean the bottom.

In large industrial spirulina farms, continuous monitoring of the elements contained in the culture medium makes the exact make-up of individual micronutrient possible. But this is too costly for small scale operators, who then have to rely on renewing the culture medium plus the addition of minor amounts of a concentrated solution of micronutrients as mentioned above.

Excessive production of exopolysaccharides (EPS) by the spirulina or its too slow biodegradation will cause "stickiness" of the biomass and/or a flocculation of spirulina into undesirable aggregates. To control this, maintain pH, nitrogen and iron contents at a higher level in the culture medium. The pH should be above 10, preferably above 10.3. Partial or total renewal of the culture medium also helps remedy the "stickiness" of the biomass.

Excessive turbidity of the filtrate may be reduced by slowing down the growth of spirulina and/or maintaining agitation during the night. This applies to the organic mud and EPS also. The culture is an ecosystem inside which various microorganisms (useful bacteria and zooplankton) live in symbiosis, resulting in a continuous, but slow, cleaning effect of the medium. If pollutants are produced more rapidly than this biological cleansing system can absorb, renewal of the medium will be necessary to keep it clean. Slowing down the growth may be obtained by shading or by reducing the rate of harvesting.

When stressed by a sudden pH or salinity variation, for instance by a heavy rain (more than 10% of the culture volume), the spirulina may sink to the bottom of the pond, where it will be in great danger of dying from suffocation. In order to facilitate the recovery, agitate the bottom often to give thespirulina filaments more chance to disentangle from the mud.

The culture may become colonized by predators living on spirulina, like larvae of mosquitoes and Ephydra flies, or amoebas. In our experience these invaders cause no other trouble than somewhat reducing the productivity. Often they can be controlled by increased salinity, pH or temperature, or they disapear by themselves

after a few weeks, or due to a change in the weather.

If the concentration of spirulina is too low, the culture may be invaded by chlorella (a unicellular, edible alga). Fortunately, chlorella passes through the filter during harvesting: so you can harvest all the spirulina, recover the wet biomass, wash it with some new culture medium and use it to restart a new tank; The contaminated medium can either be discarded or sterilized. The same procedure should be applicable to diatoms.

Toxic microalgae like anabaena, anabaenopsis arnoldii and microcystis rarely grow in a well tended spirulina culture, but for safety's sake it is recommended to have the culture checked by a microscopic (with phase contrast) examination and/or have the cyanotoxins analyzed once a year. A culture of young artemias can be used to check the absence of toxic algae: boil a little of the spirulina culture to be checked (10% of the artemias culture) during one minute, cool it and mix it with the artemias culture: observe the small animals; if they retain their vitality for at least 6 hours, there is no toxic algae. Artemia eggs are sold by aquariophilic stores. Actually theis artemia' test is not quite sure.

Usual pathogenic bacteria do not survive more than two days at the high pH (> 9.7) of a spirulina culture in production; however a microbiological assay of the product should be made also at least once a year. Contaminations generally occur during or after harvesting.

The color of the culture should be deep green. If it turns yellowish, this may be due to either a lack of nitrogen or an excess of light (photolysis) or of ammonia (excess of urea). In the latter two cases recovery is generally possible within two weeks while resting and shading the culture.

STORING THE PRODUCT

There is no question that freshly harvested, pressed biomass is superior to any other forms of spirulina. However it will not keep more than a few days in the refrigerator, and no more than a few hours at room temperature.

Adding 10% salt is a way to extend these keeping times up to several months, but the appearance and taste of the product change: the blue pigment (phycocyanin) is liberated, the product becomes fluid and the taste is somewhat like anchovy's paste.

Quick freezing is a convenient way to keep fresh spirulina for a long time.

Drying is the only commercial way to store and distribute spirulina. If suitably Packaged under vacuum in aluminized heat sealed plastic bags, dry spirulina is considered good for consumption up to five years. In that kind of storage, most microbes disappear. But drying is an expensive process and it generally conveys the product a different and possibly unpleasant taste and odour, especially if the product

is spray dried at high temperature as is the case in very large, industrial plants.

DRYING (see also Annex A6)

The industrial type of spirulina dryer is the spray dryer which flash dries fine droplets at very high temperature and yields an extremely fine powder of low apparent density. This type is outside the reach of artisan producers. So is freeze drying, the best way of drying but far too expensive and complicated.

Sun drying is the most popular among small producers, but requires a few precautions. Direct sun drying must be very quick, otherwise the chlorophyll will be destroyed and the dry product will appear bluish.

Whatever the source of heat, the biomass to be dried must be thin enough to dry before it starts fermenting. Basically two types of shapes are used: thin layers of rather fluid biomass laid on a plastic film, and rods ("spaghetti") laid on a perforated tray. In the former case the air flows horizontally over the film, while in the latter one it flows either horizontally or vertically through the tray. The rod shape is better as evaporation can take place all around; rods are obtained by extrusion to a diameter of 1 to 2 mm. But rods must be sturdy enough to maintain their shape, so this type of drying is restricted to biomasses that can be dewatered by pressing into a paste of firm consistency.

Warm, dry air passed over or through the biomass to be dried must have a high velocity at the beginning of the drying process. Later on in the process the velocity of the air is less important than its dryness (therefore it is usual to end up with air heated at 68°C). The total duration of the drying should not exceed a few hours, preferably 2 hours.

During the drying process as well as afterwards the product must be protected against contaminations from dust and insects and should not be touched by hands.

Drying temperature ideally should be limited to 42°C in order to avoid destruction of enzymes, vitamins and phycocyahin and anyway be limited to 68°C, and drying time to 7 hours.

Incipient fermentation during drying can be detected by smelling during the drying process as well as afterwards. However it is customary that a rather strong smell evolves from the biomass at the very beginning of the drying.

The dry chips or rods are usually converted to powder by grinding in order to increase their apparent density. The best storage is in heat sealed, aluminized plastic *bags.*

CONSUMPTION

Those persons who cannot stand the taste and odor of spirulina most probably were once exposed to a low quality product. Good quality fresh spirulina is so bland it can replace butter on toast and can enrich almost any dish; cold drinks can be prepared by mixing it with fruit juices. Fresh spirulina is a paste easily mixed, diluted, extruded, etc.

There are literally thousands of possible recipes making use of spirulina either fresh, frozen or dry, raw or cooked.

Above 68°C the gorgeous green color often turns brown in the presence of water. So you can choose your preferred color for soups and sauces.

APPENDIX

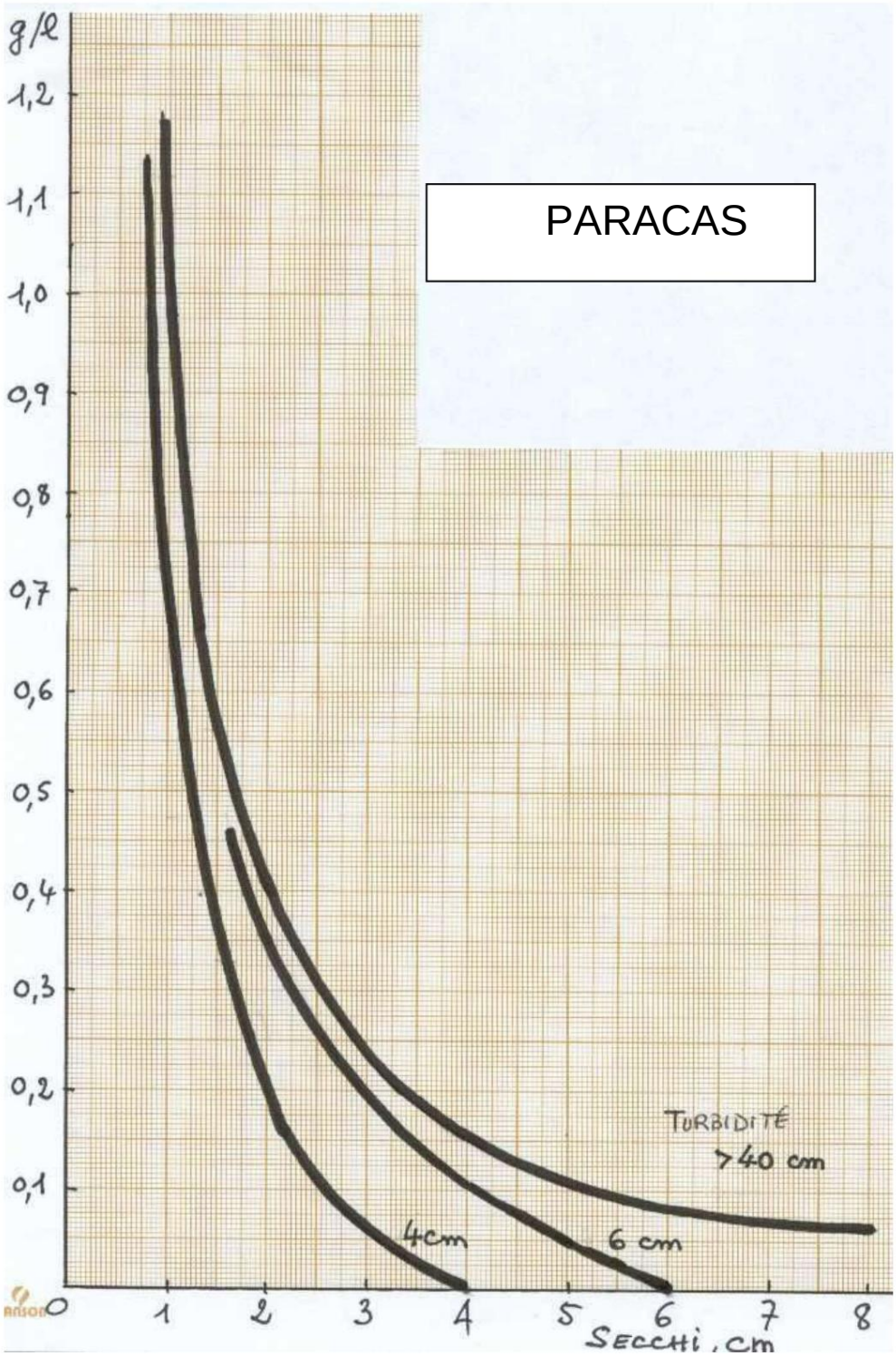
A1) MEASURING THE CONCENTRATION IN SPIRULINA WITH THE SECCHI DISK

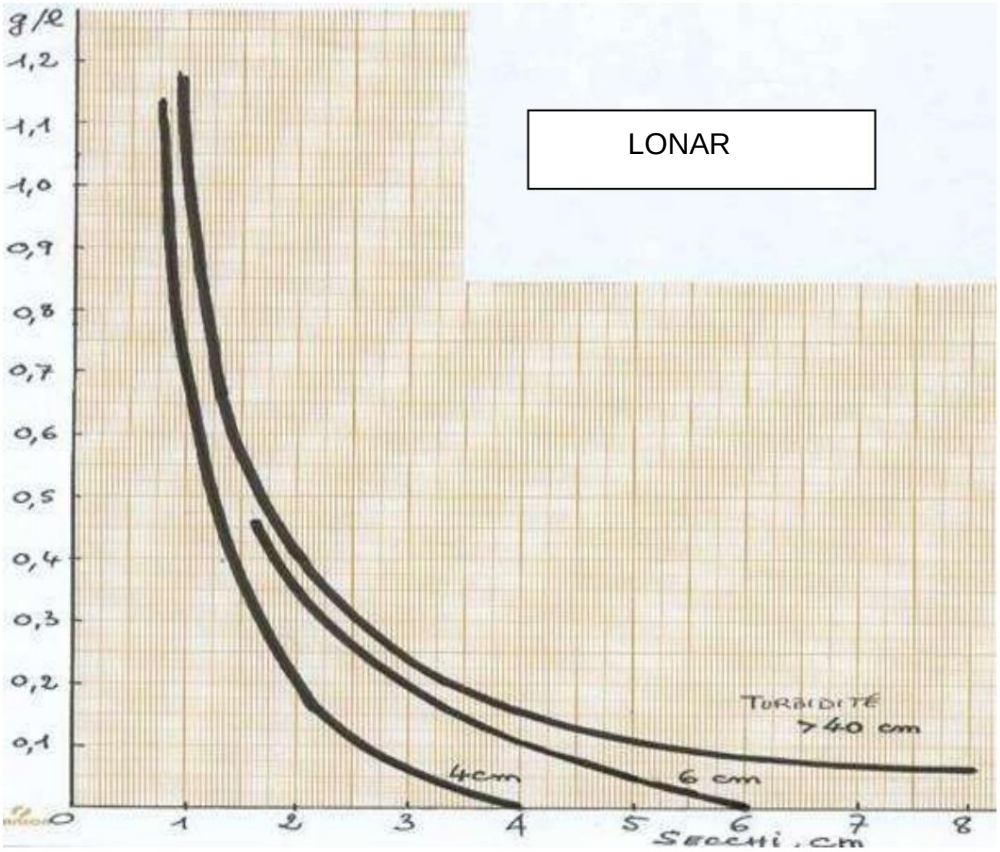
The "Secchi disk" is a self-made instrument: a piece of white rigid plastic fixed at the tip of a graduated rod. Dip it vertically into the spirulina culture until you just cannot see the white piece; the reading in centimeters gives an approximate value of the concentration. If the medium itself (the filtrate) is turbid, use the appropriate curve (the turbidity of the filtrate can be measured using a black Secchi disk, and is expressed in cm in the same way as the concentration of spirulina).

As the reading depends on the eye of the operator, every one should make his own graph, based on absolute measurements of the concentration (by filtering a given amount, drying in the oven and weighing).

The reading also depends on the shape of the filaments.

The following graphs were established by the author for the Lonar (coiled) and for the Paracas (loosely coiled, almost straight) strains. They can be used as approximations.





A2) MEASURING THE SALINITY OF THE CULTURE MEDIUM

Use a densitometer calibrated for densities above 1.

Temperature correction:

$$D = DT + 0.000325 \times (T - 20)$$

Where D = density at 20 °C, DT = density at T °C, expressed in kg/liter

Salinity SAL is calculated from D by the formulas:

If D > 1.0155, SAL = 1275 x (D - 1) - 0.75, g/liter

Otherwise, SAL = 1087 x (D-0.998)

A3) MEASURING THE ALKALINITY OF THE MEDIUM (ALCALIMETRY)

Titrate the medium using normal hydrochloric acid (concentrated acid diluted 10 times with water). Use pH 4 as the end point.

Alkalinity (moles of strong base/liter) is the ratio of the volume of acid used to the volume of the sample of medium.

A4) MEASURING THE PH

The pH meter should be calibrated from time to time. If standard calibration solutions are not available, self-made solutions can be made for calibration as follows (pH at 25°C):

- pH 11.6: 10.6 g sodium carbonate per liter water (freshly made solution or flask kept closed) - pH 9.9: 5.5 g sodium bicarbonate + 1.4 g caustic soda per liter water, gold: 4.2 g sodium bicarbonate + 5.3 g sodium carbonate per liter water; maintain in contact with the atmosphere and make up for evaporated water.
- pH 7: 5.8 g monoammonium phosphate + 11 g sodium bicarbonate per liter of water; maintain in a closed bottle. - pH 2.8: standard vinegar (6% acetic acid, density 1.01).

Temperature correction on pH:

$$pH \text{ at } 25°C = pH \text{ at } T°C + 0.00625 \times (T - 25)$$

A5) COMPARING SPIRULINA SAMPLES

Protein, iron, gamma-linolenic acid, heavy metals contents and the microbiological analysis can only be performed by a competent laboratory, but a few home-made tests can give an idea of the quality of a spirulina sample by comparing with a reference product.

Examination of color, odor and taste may reveal significant differences between samples. The green color should tend more towards the blue than the yellow color.

The "pH test" reveals the degree of removal of the culture medium from the biomass. On fresh spirulina simply measure the pH: if should be near 7. For dry spirulina powder, mix a 4% suspension in tap water and measure the pH: the initial pH should be near 7 (for many commercial products it is near 9 or even 10), and after 12 hours it usually falls down to well below 6. For biomasses that were washed with acidified water, the initial pH may be acidic (< 7).

To assay the blue pigment phycocyanin content proceed as for the pH test on dry samples, mixing several times the suspension. After 12 hours, put one drop of the decanted solution on a white filter paper (for instance the "Mellita" filter paper for coffee making) maintained horizontal. The amount of blue color in the stain is more or less proportional to the concentration of phycocyanin in the sample. Some spirulina samples require to be heated to 65°C before the blue pigment be fully released into the solution. Phycocyanin is a protein that is the most important component of spirulina for heath; it amounts to around 25% of the total proteins.

To assay the carotenoids content, mix the dry powdered sample with twice its weight of acetone (or of 90% ethanol) in a closed flask, wait 15 minutes, and put one

drop of the decanted solution on filter paper. The intensity of the brown-yellow color of the stain is proportional to the concentration of carotenoids (and hence of beta carotene) in the sample. Old samples stored without precautions contain practically no carotenoids.

A6) HARVESTING AND DRYING SPIRULINA

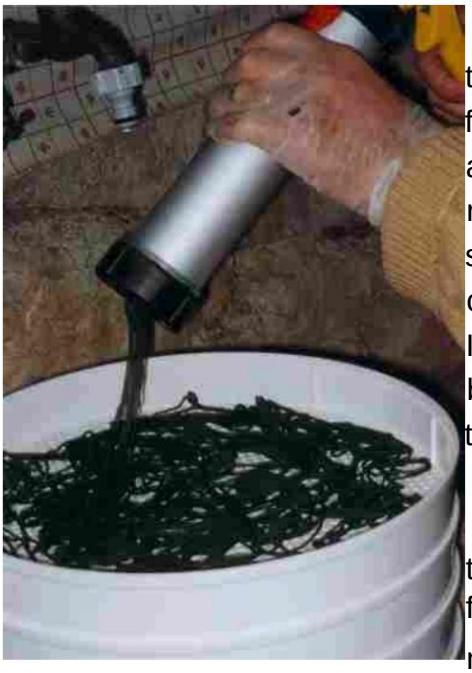
Filtration is done on a 30 µ mesh cloth.

When most of the water has filtered through, the biomass will agglomerate into a "ball" under motion of the filtering cloth, leaving the cloth clean (this desirable condition happens when the biomass is richer in spiralled forms and the culture medium is clean). At this stage the biomass contains 10% dry matter and it has a soft consistency; it will not stick to plastic materials but rather glide on it.

Final dewatering of the biomass is accomplished by pressing the biomass collected on cloth, either by hand or in any kind of press. The simplest is to apply pressure (0.15 kg/cm² is enough) by putting a heavy stone on the bag containing the biomass. The "juice" that is expelled comes out clear and colorless, and the operation can be discontinued when no more liquid drops out. For the usual thickness of cake (about one inch after pressing), the pressing time is about 15 minutes. Practically all the interstitial water (culture medium) is removed. The pH of the pressed biomass is near 8 and may even be brought below due to breaking of some spirulina cells, but it is not advisable to bring it too low.

This pressing operation effects a more efficient separation of the residual culture medium than washing the biomass.. Washing with fresh water may cause rupture of the cell wall of the spirulina due to osmotic shock, leading to loss of valuable products; it may also introduce germs contained in the wash water.

Pressed biomass contains twice as much dry matter as unpressed biomass. It has a firm consistency (can be cut by a knife like cheese). It can be eaten as is.



The biomass to be dried must be thin enough to dry before it starts fermenting. It is extruded into fine rods ("spaghetti") of a diameter of 1 to 2 mm onto a plastic perforated tray (or nylon mosquito net). The rods must be sturdy enough to maintain their shape, so this type of drying is restricted to biomasses that can be dewatered by pressing into a firm consistency. In India the "indiappam makker" kitchen instrument can be used for extruding (the wooden type is preferred to the aluminum one).

During the drying process as well as afterwards the product must be protected against contaminations from dust and insects and should not be touched by hands.

Drying temperature should be limited 42°C but can be briefly increased to 68°C near the end; drying time should be no more than 7 hours. With good ventilation and low charge (1 kg fresh rods/m² of tray) the drying time may be reduced to 2 hours. The final % water should be less than 9. The dry product detaches itself easily from the tray.

Incipient fermentation during drying can be detected by smelling during the drying process afterwards.

The dry rods are usually converted to powder by grinding in order to increase their apparent density. The best storage is under vacuum in heat sealed, aluminized plastic bags.

A7) A SIMULATION MODEL FOR THE CULTURE OF SPIRULINA

[This section deals with an old version of the simulation model which is no longer supported. However it is left here as an illustration. The present model is described in the French version only.]

Instructions for use of the simulation **model**

The models presented here are freely available for non-commercial uses. They can be run on any PC with DOS. Create a new folder on your local disk (C) and name it SPIRUL. In SPIRUL create 4 subfolders and name them SITES, PERSONAL, PRINT and EXE. Download BSI.EXE, METEO.EXE into the folder named EXE and run METEO.EXE once before using the models. The main model is SPIRU-E.EXE. The models can be downloaded into EXE or they can be run directly from their link (in this case, to the question input path?, answer C:/SPIRUL/EXE).

If you ask for a printout of the results, go to the file SPIRU-E.DOC automatically generated in the folder IMPRIM, and print it. To print graphs use Print Screen.

Other models can be run the same way: SPITFIX.EXE for simulating laboratory cultures at constant temperature under constant light, and PRIXSPIR.EXE (French) for the calculation of spirulina cost prices.]

[Part of the following reproduces a paper given at the First ALGAL Technology Symposium, Ege University, Izmir, Turkey, October 24-26, 2001]

A PRACTICAL SIMULATION MODEL FOR SPIRULINA PRODUCTION

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Abstract

A model was written to simulate the operation of a spirulina *(Arthrospira platensis)* culture under a greenhouse or in the open air. The rate of photosynthesis is assumed to be directly proportional to five functions when biomass concentration is above 0.1 g/l: photosynthesis = kxf(light) xf(temperature) xf(pH) xf(stirring) xf(salinity). The rate of respiration is assumed to be a function of the temperature. The solar illumination is calculated from the sun's position and from local meteorological data; an artificial lighting may be provided. The culture temperature is calculated from a thermal balance and the pH from a CO2 balance around the tank. The calculations are carried out for each hour for a period of up to 600 days on end. The results include a graph of the daily production and a cost price analysis. In order to optimize the production about 80 technical parameters can be varied at will, including the temperature control means (inflatable double plastic roof, air circulation, shading, night cover, artificial heating). Various fuels are available either for heating or as a source of CO2. Make up water may be saline and/or alkaline. Purified culture medium may be recycled at a lower pH to increase growth.

The model appears to correctly predict the operation of a spirulina culture. It is useful to predict trends, optimize operating conditions, make technical and economic analyses, and as a tutorial aid.

Keywords : *Arthrospira platensis,* culture, simulation, model.

INTRODUCTION

The software SPIRU-E.EXE containing the mathematical model presented here is freely available for non-commercial uses. The program itself contains all necessary information for worn.

The model is based on data from the literature plus data obtained by the author in the course of ten years of experiments with spirulina *(Arthrospira platensis)* culture. It makes use of basic equations from the solar energy and chemical engineering fields. It applies to any spirulina culture in an open air tank or under a greenhouse, in any climate. It also applies to the case of cultures flowing on inclined planes.

In addition to technical aspects, the model also calculates a simplified cost price for the product.

MATERIALS AND METHOD

Starting from a given set of initial conditions, the growth of spirulina is calculated hourly for the desired duration of the culture (up to 18 months), as a batch culture or rather as a semi batch culture because of harvesting. The basis is one square meter of illuminated tank area. Temperature and pH of the culture are obtained by heat and CO2 balances around the tank and are the basis for the calculation of growth. The main hypothesis on which the model is based is that the rate of photosynthesis is assumed to be directly proportional to five functions when the biomass concentration is above 0.1 g/l:

photosynthesis = kxf(light) xf(temperature) xf(pH) xf(salinity) xf(stirring)

with the proportionality factor k chosen to best fit experimental results. This hypothesis may not be scientifically justified, but it makes the calculation much simpler and gives acceptable results. This equation assumes that photosynthesis is not limited by nutrients other than bicarbonates, and that it is independent of spirulina concentration (which is largely true as the biomass concentration is maintained above 0.15 g/liter). The functions of light, temperature, pH and salinity are based on Zarrouk 1966, adapted to better fit experimental results when necessary. Figs. 1 to 5 (Fig1 , Fig2 , Fig4 , Fig5) show these functions as used in the model. The function of the stirring rate is largely hypothetical (note: stirring and agitation will be consistent in this paper).

For biomass concentrations below 0.1 g/l the photosynthesis is exponential and is calculated by multiplying the above equation by the factor (concentration/.01).

The net growth is calculated as the difference between photosynthesis and respiration. The assumed influence of temperature on the rate of respiration is illustrated in Fig6, based on Tomaselli *et al.,* 1987 and Cornet 1992, for homogeneous cultures maintained in contact with air.

Ambient air temperature and solar radiation are calculated hourly from meteorological data, latitude and altitude of the site, with formulas commonly used in the solar energy field. The average percent cloudiness is assumed to be concentrated each month in three series of days evenly distributed within the month, which are the rainy days of the month. Dew point and wind velocity are assumed to be constant within each month.

Greenhouses used may be equipped with various devices to control their internal climates: inflatable double plastic roof, adjustable ventilation, adjustable shading, fixed shading, infra red reflectors (night screen) and night insulation. Various additional options for greenhouses in cold climates are available including heating by fuel combustion, night insulation and artificial lighting.

Adjustable and/or fixed shading and night screen may also be mounted on open air tanks.

Harvesting is done every day (except on 0 to 3 days on end without harvesting per week) at a given time of the day, reducing the spirulina concentration down to a given fixed value, but is limited by the harvesting capacity. There is no harvest as long as the pH is below a limit (generally 9.6) in order to minimize the pathogenic germs. At the end of the culture period a

final harvest reduces the concentration down to the initial value. The average productivity is based on the total duration of the culture period from inoculation to restarting a new culture, including the idle days.

The pH of the culture is controlled by daily feeding of CO2 or CO2-evolving compounds (bicarbonate, sugar) or (for greenhouses) CO2-containing combustion gases. The CO2 contributed by the urea and by the ventilation air is taken into account in the carbon balance. The absorption coefficient of CO2 from the air into the culture medium was experimentally determined as 20 gmoles/hr/m²/atm; this figure may be changed (in the following examples a figure of 18 was taken). The vapor pressure of CO2 over the medium is calculated using the formula given in Kohl and Riesenfeld 1960. The resulting rate of CO2 absorption from the air is illustrated in Fig7. The experimentally determined graph in Fig8 is used to relate the amount of CO2 contained in the medium to the pH of the medium. The CO2 consumption assumed in the examples given below is 1.8 gram per gram of spirulina grown, but the model allows it to be adjusted to take into account variations in the exopolysaccharide and other by products according to the strain and the culture conditions.

The tank level is allowed to fluctuate between a minimum and a maximum value, and is controlled either by draining part of the medium or by adding water (plus the salts needed to maintain the quality of the medium) depending on the needs. The salinity and alkalinity of the make-up water are taken into account, but its hardness is neglected. The salinity and the basicity of the medium are controlled below given maximum values by replacing part of the medium by water (plus the required salts).

Purified, low pH culture medium may be recycled with no change in basicity, salinity, level nor temperature in the tank..

The cost price calculated by the model is based on the following formulas for chemicals usages:

	Medium, g/liter*	Production, g/kg**
Monoammonium phosphate	0.08	50
Dipotassium sulfate	1.00	40
Epsom salt	0.16	30
Urea	0.02	300

(*plus the required sodium bicarbonate, carbonate and chloride corresponding to the initial basicity, pH and salinity)

(**plus the required amounts of C containing compounds).

The cost price also includes an adjustable fixed costs contribution.

The model does not take into account the cost of treatment of the spent culture medium, but it

allows recycling of the treated medium.

RESULTS

The site of Izmir, Turkey was chosen to give a series of examples of results obtained using the model with 6 harvesting days per week.. To facilitate comparison of the various cases, the same set of data were used in all cases, except the parameters being varied. A greenhouse of the simplest type is used in all cases, with no inflatable double roof, no shading, no night insulation nor night screen, but with adjustable ventilation. The standard duration chosen for the culture is one year. Table 1 and 2 (Table1 , Table2) show the printout of the standard data used.

The daily results of each simulation come out both as a graph (Fig9) and as a table (not shown here), while average results over the period of culture come out as a table (Table3).

The search for the minimum cost price or the maximum production is effected by varying parameters.

Fig10 gives the relationship between the bicarbonate consumption (used as the sole pH controlling agent) and the productivity when using the simplest type of greenhouse (standard case for the examples given here) and when using a fully equipped, modern greenhouse. At low pH the simplest greenhouse is 25% better than without any greenhouse.

Fig11 shows typical results obtained by varying the pH control value while using bicarbonate as the sole pH controlling agent and Fig12 shows the same using liquid CO2. The optimum pH obviously depends on the cost of the carbon source.

Fig13 shows the negative influence of high biomass concentrations on the productivity, due to the effect of respiration.

Fig14 shows the negative influence of a high depth of culture on the productivity, due both to the reduction of the maximum temperature and to higher respiration.

Fig15 shows the minute influence the air circulation rate has on the productivity. When an artificial carbon source is used, the influence is negative due to lower temperatures. Without an artificial carbon source, it becomes positive due to more CO2 available from the air, but it remains negligible.

Fig16 shows the influence of the salinity of the make-up water on the productivity.

Fig17 gives an example where propane fuel is the sole artificial carbon source. The cost price can be quite low provided the air circulation rate is kept minimal.

Another use of the model is to evaluate the economic penalty due to shorter periods between changing the culture medium. For three changes per year instead of one, the penalty comes out to be 4% on productivity and only 1% on cost price in the example given here. So, as a new culture is easier to harvest, it is recommendable to renew the medium several times a year.

DISCUSSION

In spite of taking into account around eighty parameters, the model is far from understanding the totality of the factors, notably biological, that influence the growth and quality of the spirulina produced in artificial conditions.
Although results from the model fit actual data generally well, the model has yet to be fully validated. It would be extremely desirable to compare a number of calculated and observed results, but for such comparisons to be valid, the data used should closely match the experimental conditions. Such a close match actually is beyond the scope of this work, but could constitute interesting thesis subjects for students. It is suggested that comparisons with actual results be communicated to the author for further validation or modification of the model.

Laboratory studies are often conducted as batch cultures under constant illumination twelve hours a day. A variant of the model was developed to facilitate validation from such laboratory studies.

As it is, this model can be useful to predict trends, optimize operating conditions, make technical and economic analyses, and as a teaching aid.

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TABLES AND FIGURES

[Note: in this paper, the coefficient of absorption of CO2, ka, was assumed to be 18 gmole/hour/m²/atm]

SIMULATION OF PRODUCTION OF SPIRULINA BY PROGRAMM SPIRU-E
IZMIR Date : 10-11-2001

HYPOTHESES

Notice : if variables were modified during the simulation,
these modifications are reproduced here

1. bicar added = 150	2. CO2 added= 0	3. sugar added = 0
4. depth = 10	5. thermal equiv. = 5	6. days = 360
7. no carbon days = 5	8. inter days = 5	9. pH control = 10.2
10. initial c = 1	11. harvest cap. = 20	12. wind coeff. = 1
13. shad. coeff. = .3	14. night cover = 0	15. max temp. = 41
16. initial b = .1	17. max. b = .2	18. fixed salts = 5
19. max. sal. = 20	20. water tds = 0	21. ventilation = 2
22. vent. coeff = 1	23. fuel type = 2	24. biogas % CO2 = 0
25. fuel flow rate = 0	26. electr. gener. = 0	27. heat used = 100
28. gases used = 100	29. insulation = 0	30. insul. coeff = 1
31. double roof = 0	32. lamps = 0	33. lamps control = 0
34. lamps heat = 0	35. % drainage = 10	36. initial day = 15
37. initial mo. = 1	38. CO2 outside = 340	39. yield = 90
40. spir. conc. = .3	41. harv. time = 8	42. stirring rate = 20
43. adjust. coeff = 1	44. kg CO2/kg spi = 1.8	45. interest rate = 0
46. azimuth = 0	47. slope = 0	48. altitude = 120
49. latitude = 39.2	50. fixed shad. = 0	51. Reference = IZMIR
74. b (eau) = 0	75. pH (eau) = 0	76. klux max @ 10øC = 30

Prices, $/kg (except otherwise mentioned)

60. carbonate = .8	61. bicarbonate = .8	62. salt (NaCl) = .17
63. urea = .17	64. liquid CO2 = 1	65. sugar = .7
66. sulfate (Mg) = 3	67. sulfate (K) = 3	68. phosphate = 3
69. water = .1	70. kWh = .13	71. fixed costs = 15

Table 1 Example of input data

WHEATHER DATA for IZMIR
(Average monthly values)

Month	Temp max	Temp min	Dew point	% cloud	Wind	Haze	Rain
1	11.1	3.3	3.3	18.7	2	.26	87
2	12.2	4.4	4.4	20.7	2	.26	72
3	15	6.1	6.1	15.3	2	.26	63
4	20.5	9.4	9.4	8.8	2	.26	38
5	25.5	13.3	13.3	8.5	2	.26	29
6	30.5	17.7	17.7	3.3	2	.26	12
7	33.3	20	20	0	2	.26	0
8	32.7	19.4	19.4	3.2	2	.26	8
9	28.8	15.5	15.5	6.7	2	.26	21
10	24.4	12.2	12.2	6.5	2	.26	20
11	18.8	9.4	9.4	13.2	2	.26	52
12	14.4	7.2	7.2	22.9	2	.26	110

Note : Temperatures in deg C
Wind = velocity in meters/second
Haze scale : 0.5 = very polluted, 0.26 = normal
0.17 = very clear
Rain = rainfall in liters/sq.m./month

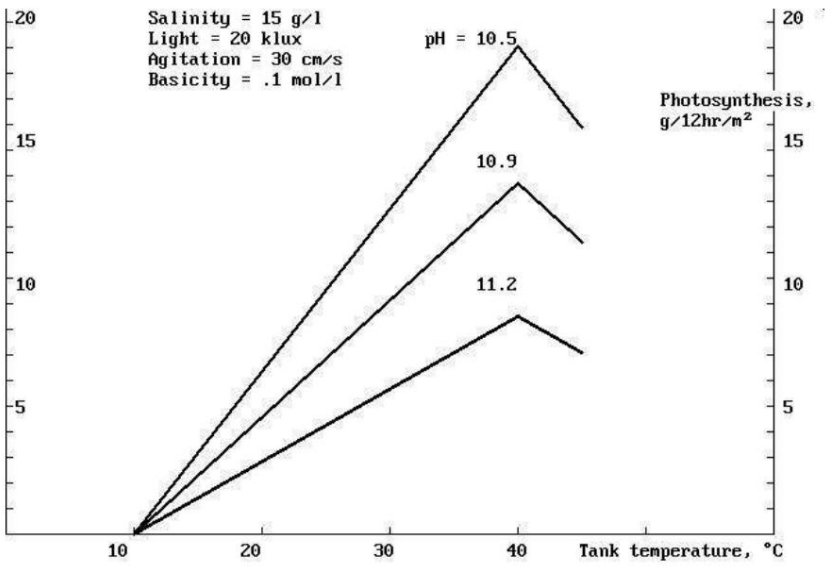
Chart 2 **Example of meteorological data**

RESULTS

Nutrients, kg/kg of harvested spirulina:
bicarbonate (initial medium and drainages included): 9.17 bicarbonate (excluding initial medium): 8.85 carbonate = 0.00 sugar: 0.00 liquid CO2: 0.00 Water consumption (including medium, drainages, evaporation), l/kg = 710

Total rainfall on area equal to tank area, l/kg = 192 Drainages, average %/day = 3.35 Fuel consumption, kg/kg = 0.00 Surplus electricity (sold), kWh/kg = -3.8 Electricity consumption by lamps, kWh/kg = 0.0 Electricity consumption by agitation, kWh/kg = Final concentration in spirulina, g/l = 0.3 Final salinity of medium, g/l = 17.4 Final basicity of medium, milimoles/l = 0.20 Maximum pH (before days without carbon feed) = 10.32 Maximum tank temperature, °C = 38.2 Minimum tank temperature, °C = 4.4 Maximum CO2 concentration in internal air, vpm = 398 Minimum CO2 concentration in internal air, vpm = 302 Maximum level in tank, cm = 10.0 PRODUCTIVITY, gram per day per m² = 6.79 PRODUCTION, kg per m² = 2.48 COST PRICE (present value at day 1), $/kg = 16.86

Table 3 Example of results



ig. 1 Photosynthesis vs. temperature

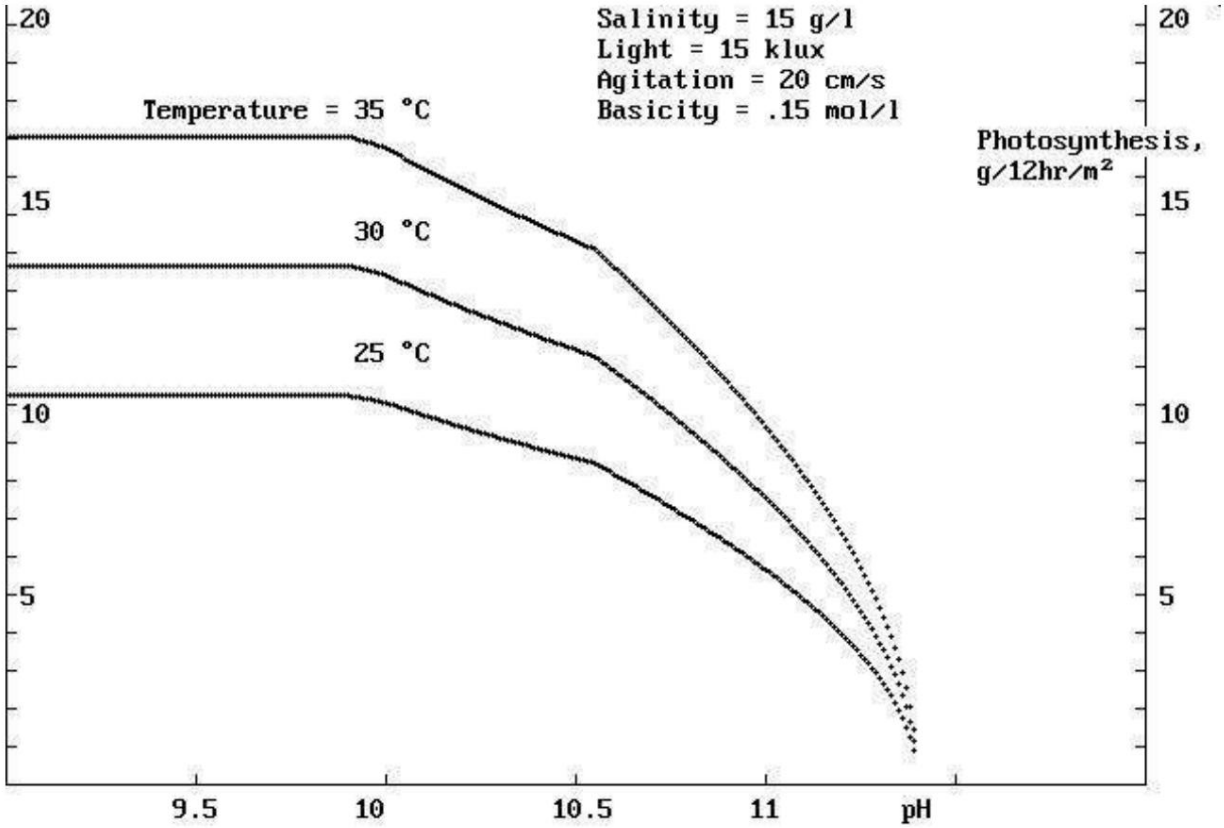


Fig. 2 Photosynthesis vs. pH

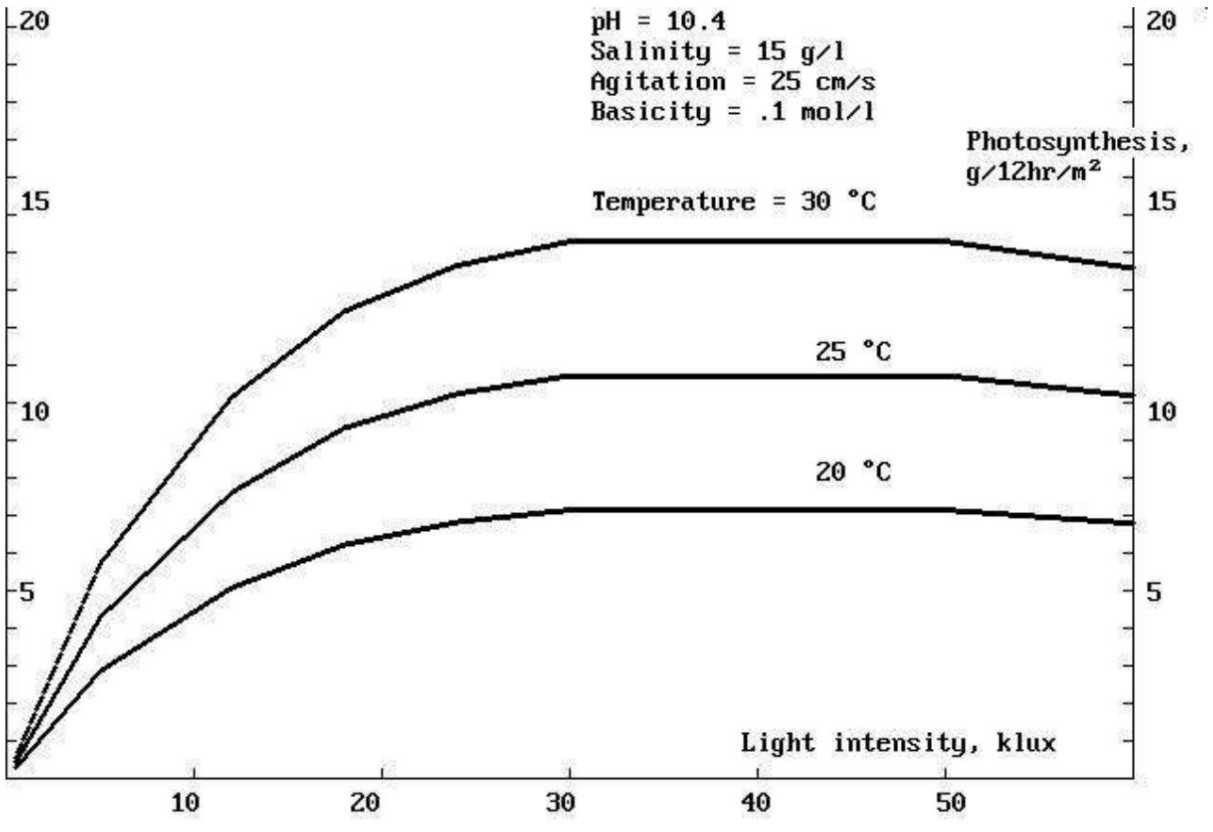


Fig.3 Photosynthesis vs. light-intensity

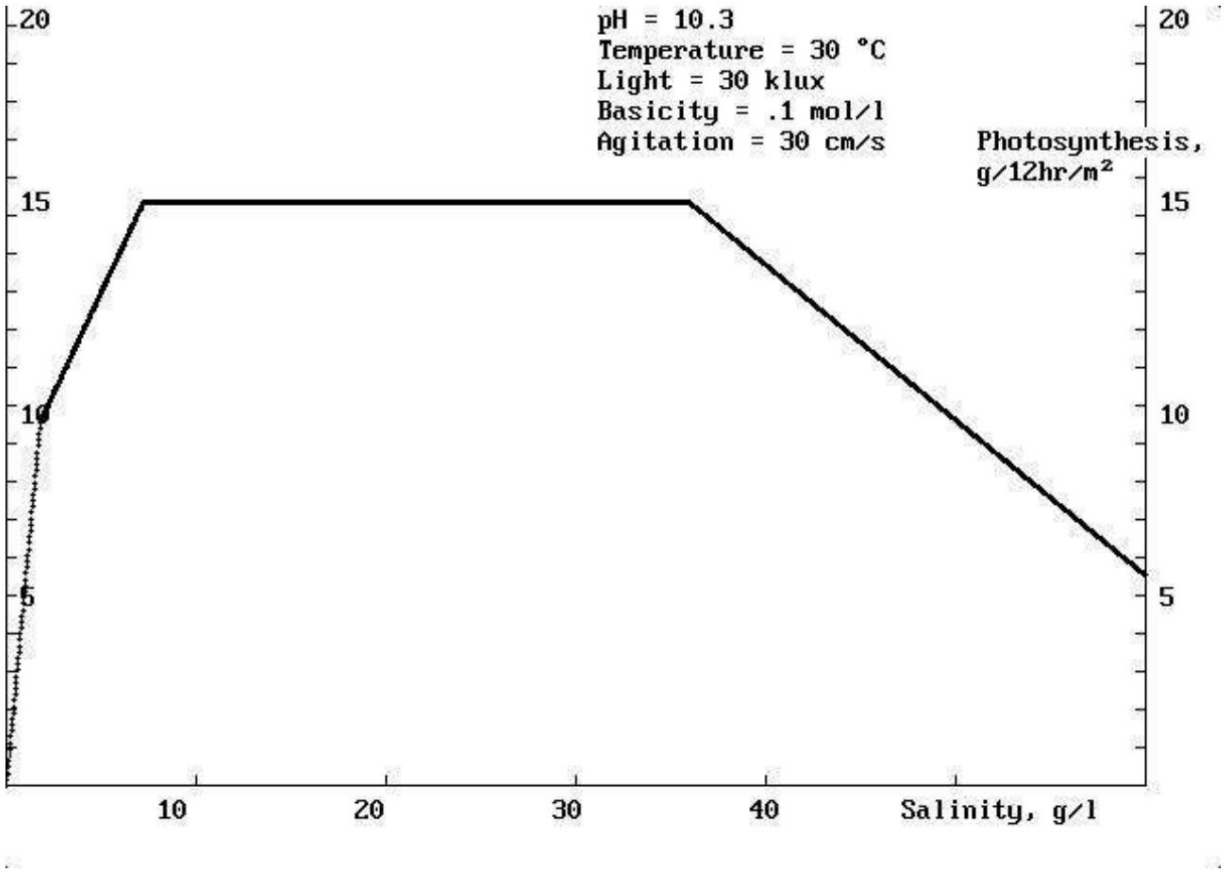


Fig.4 Photosynthesis vs. salinity

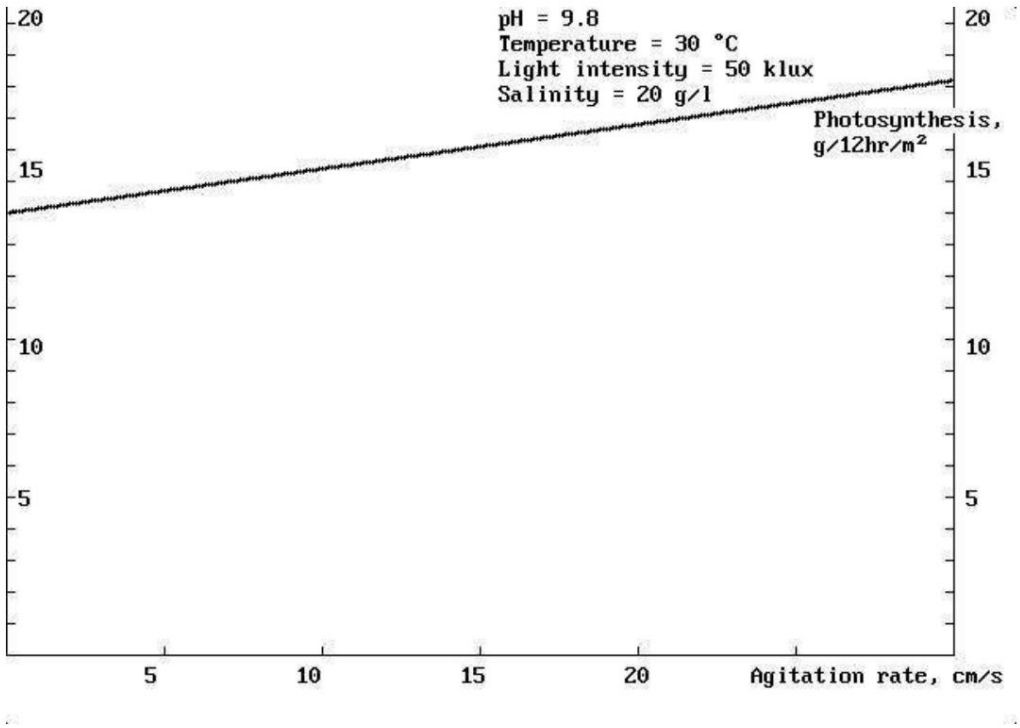


Fig. 5 Photosynthesis vs. stirring rate

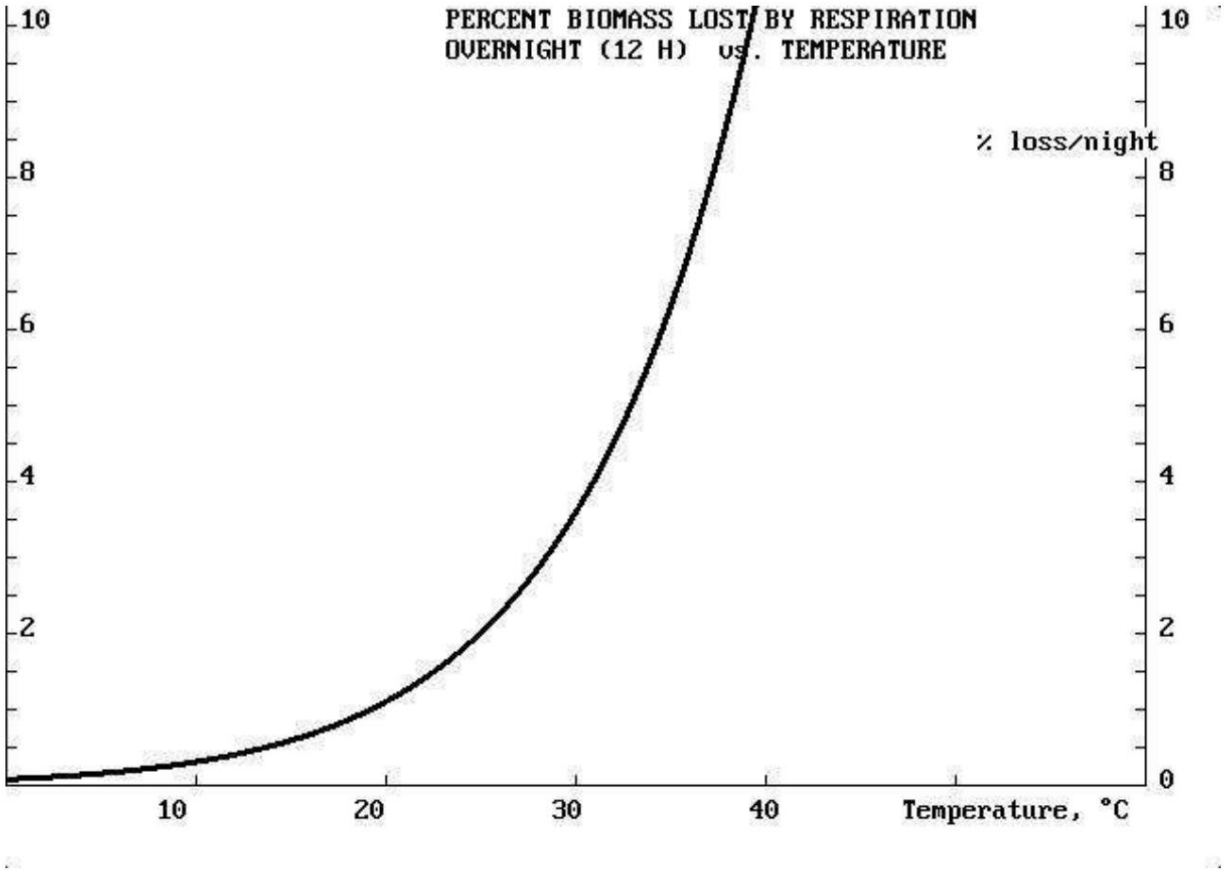


Fig. 6 Respiration spleen vs. temperature

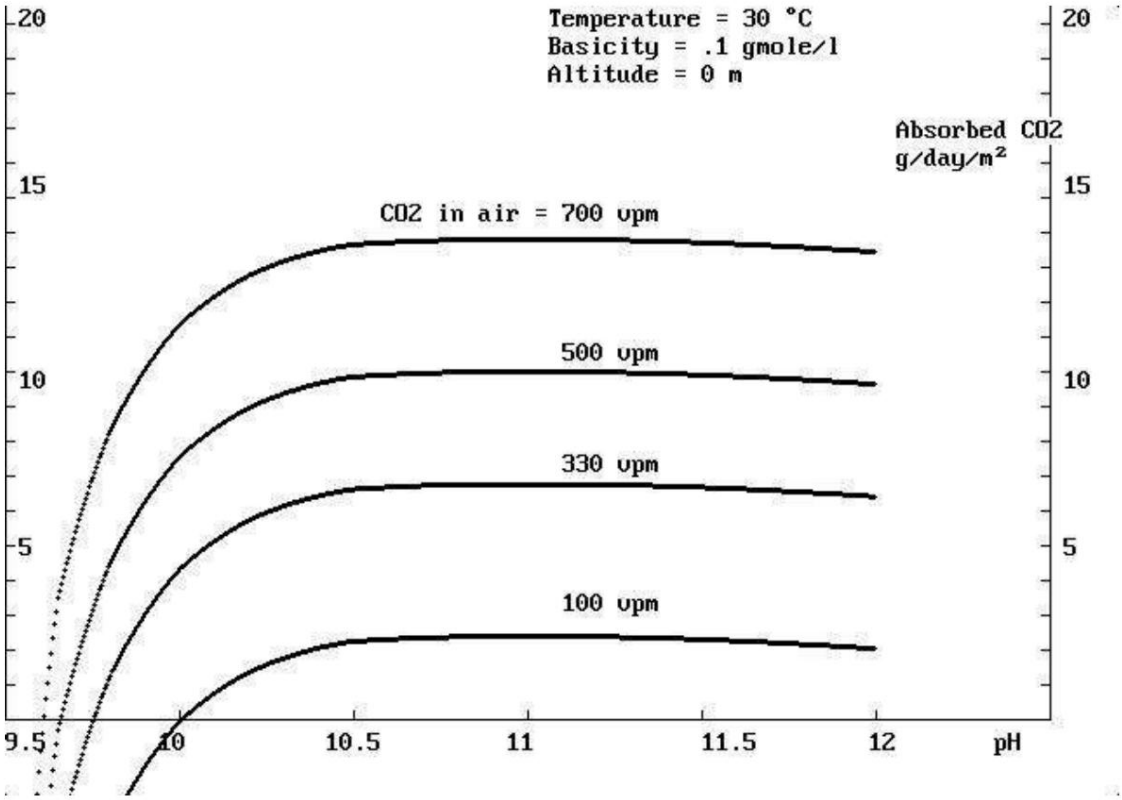


Fig. 7 CO2 uptake vs. pH

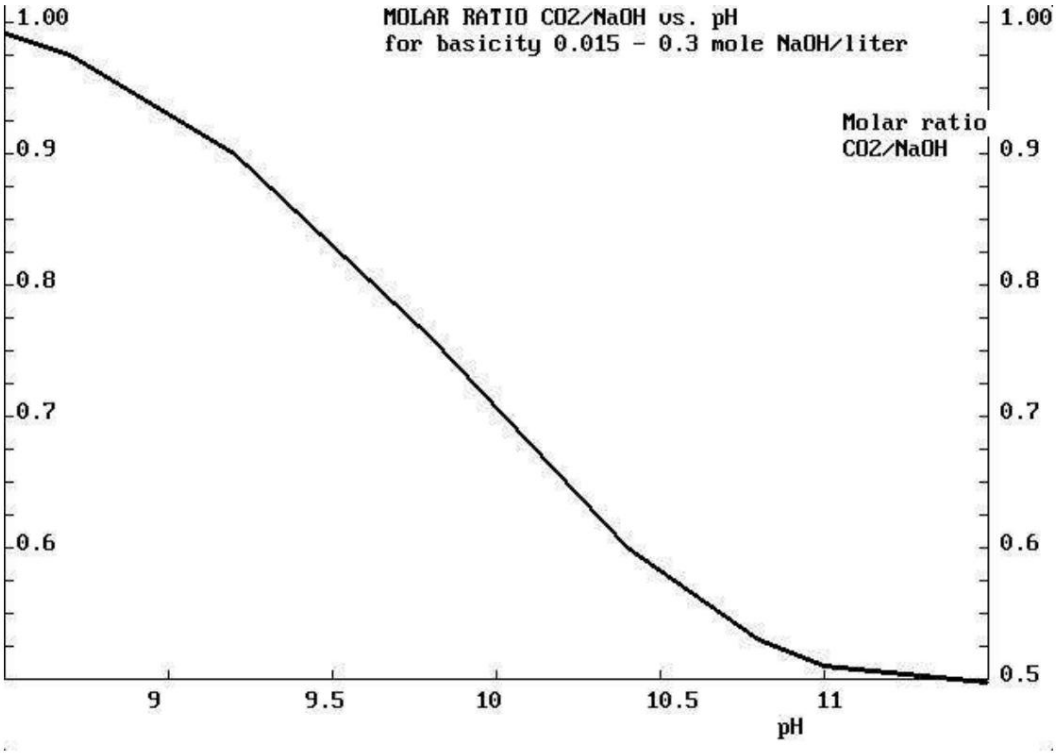


Fig. 8 CO2/basic molar ratio vs. pH

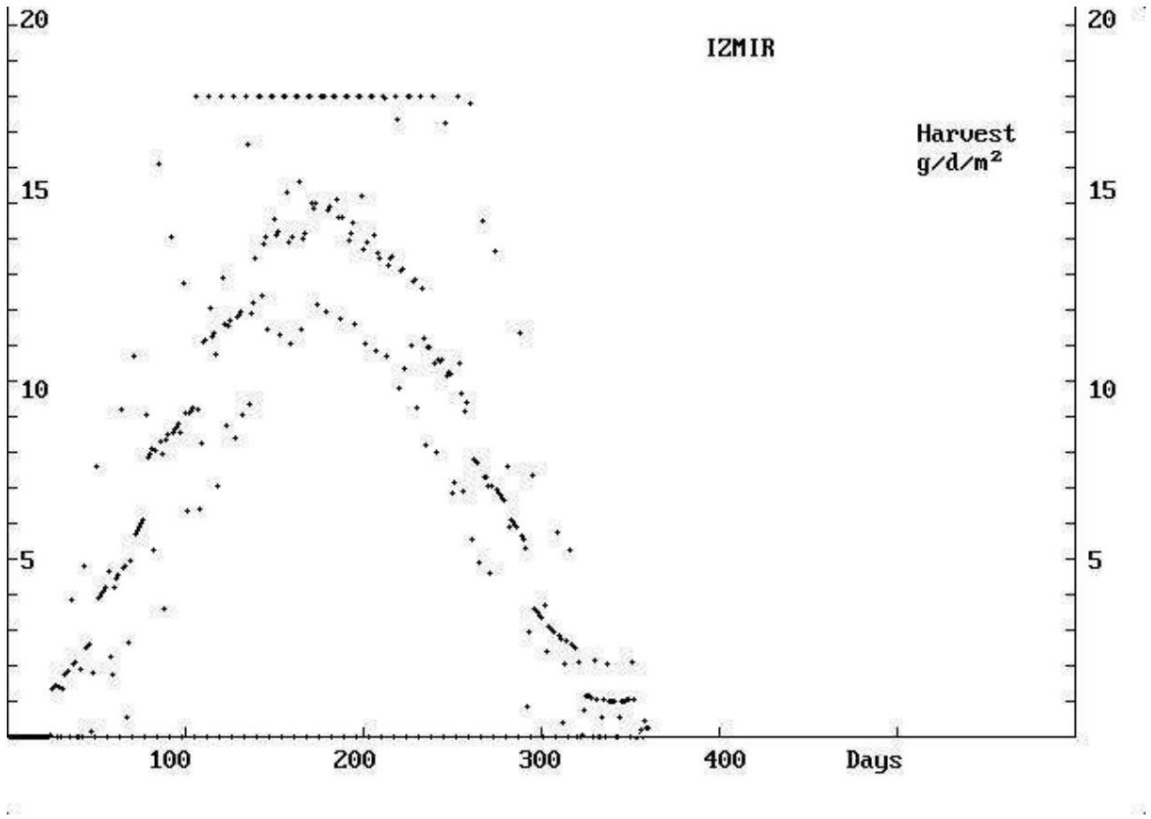


Fig. 9 Daily production

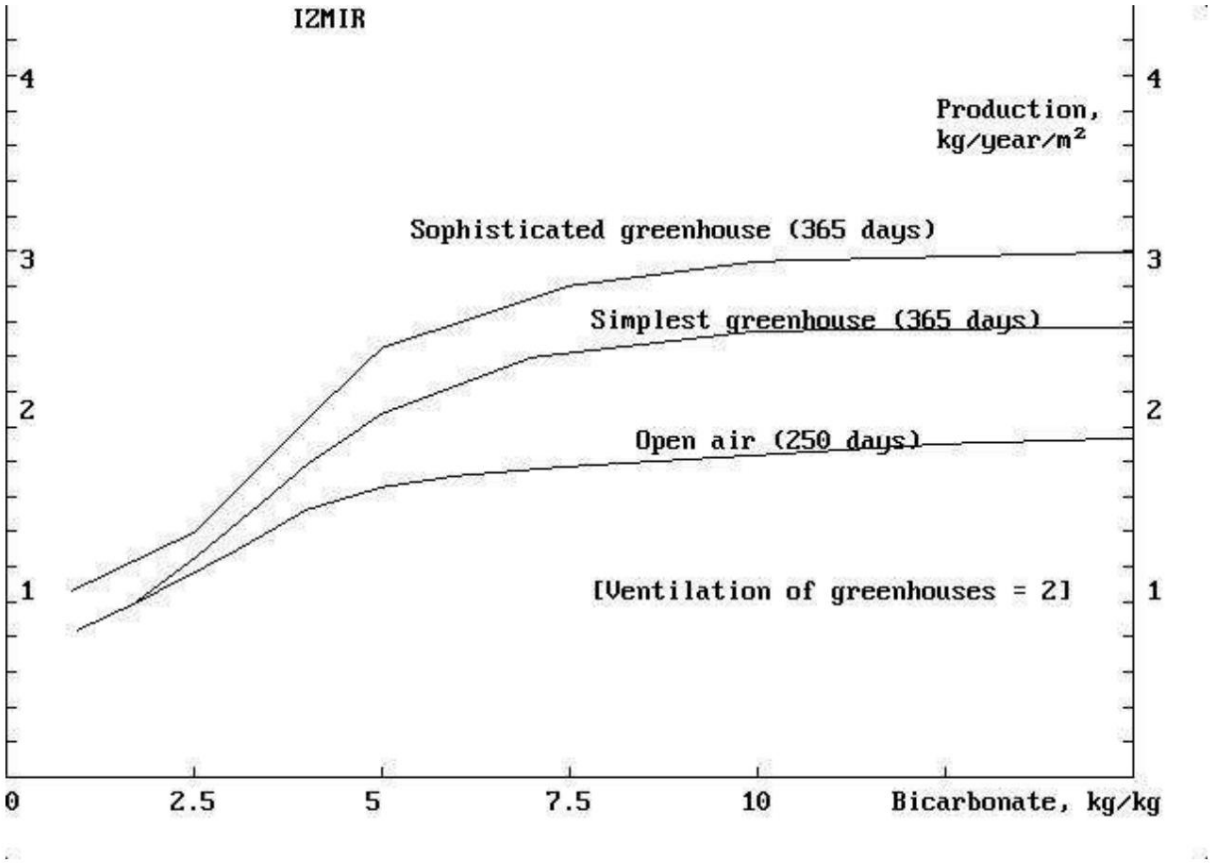


Fig. 10 Production vs. bicarbonate consumption

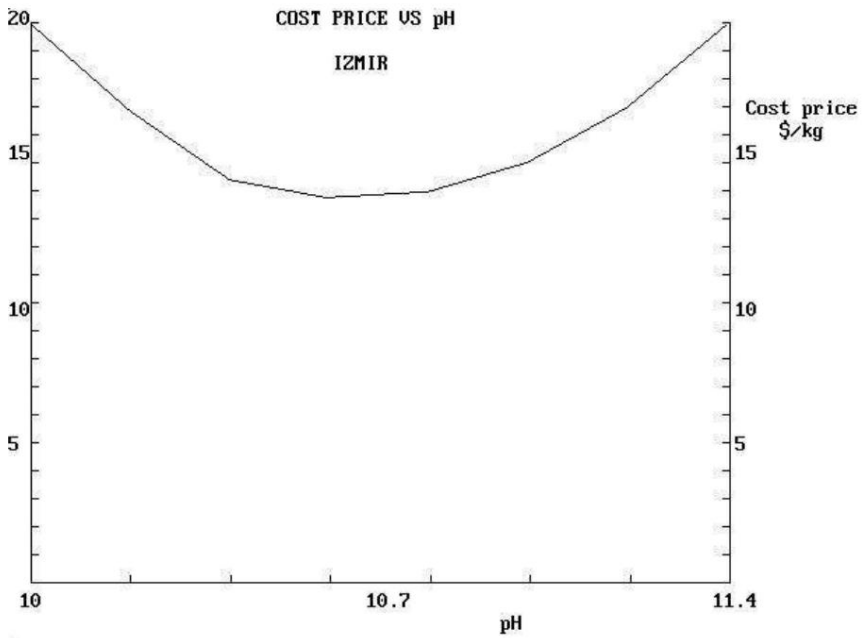


Fig. 11 Cost price vs. pH with bicarbonate (@ 0.8 $/kg)

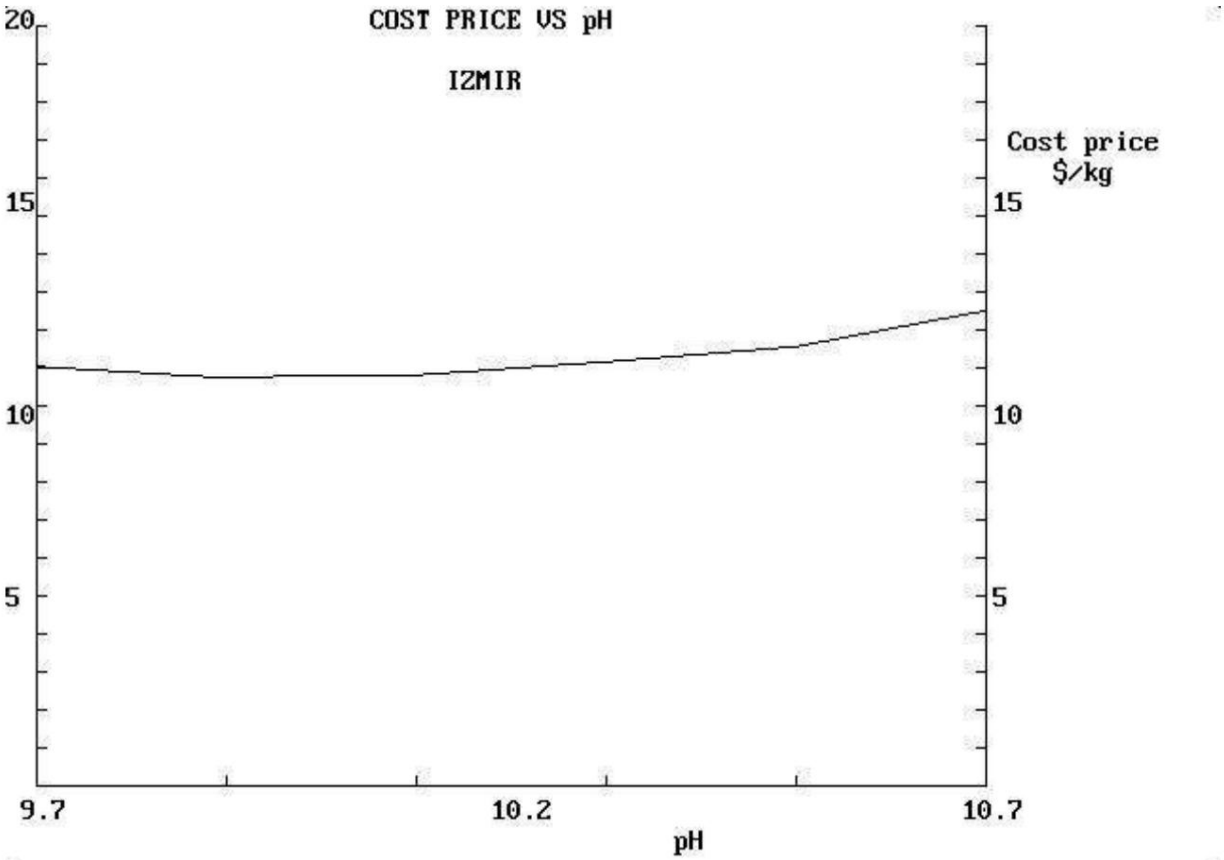


Fig.12 Cost price vs. pH with CO2 (@ $2/kg)

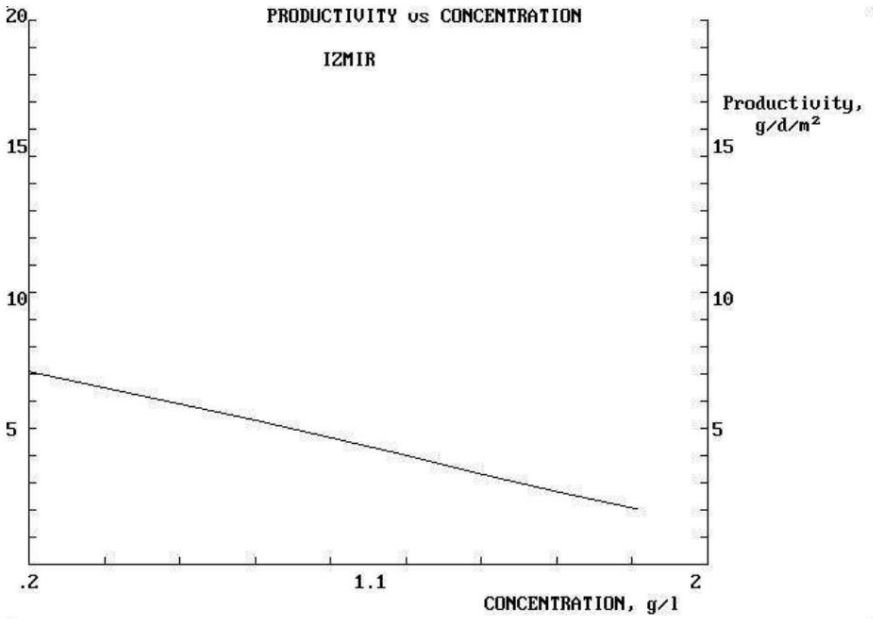


Fig. 13 Productivity vs. biomass concentration

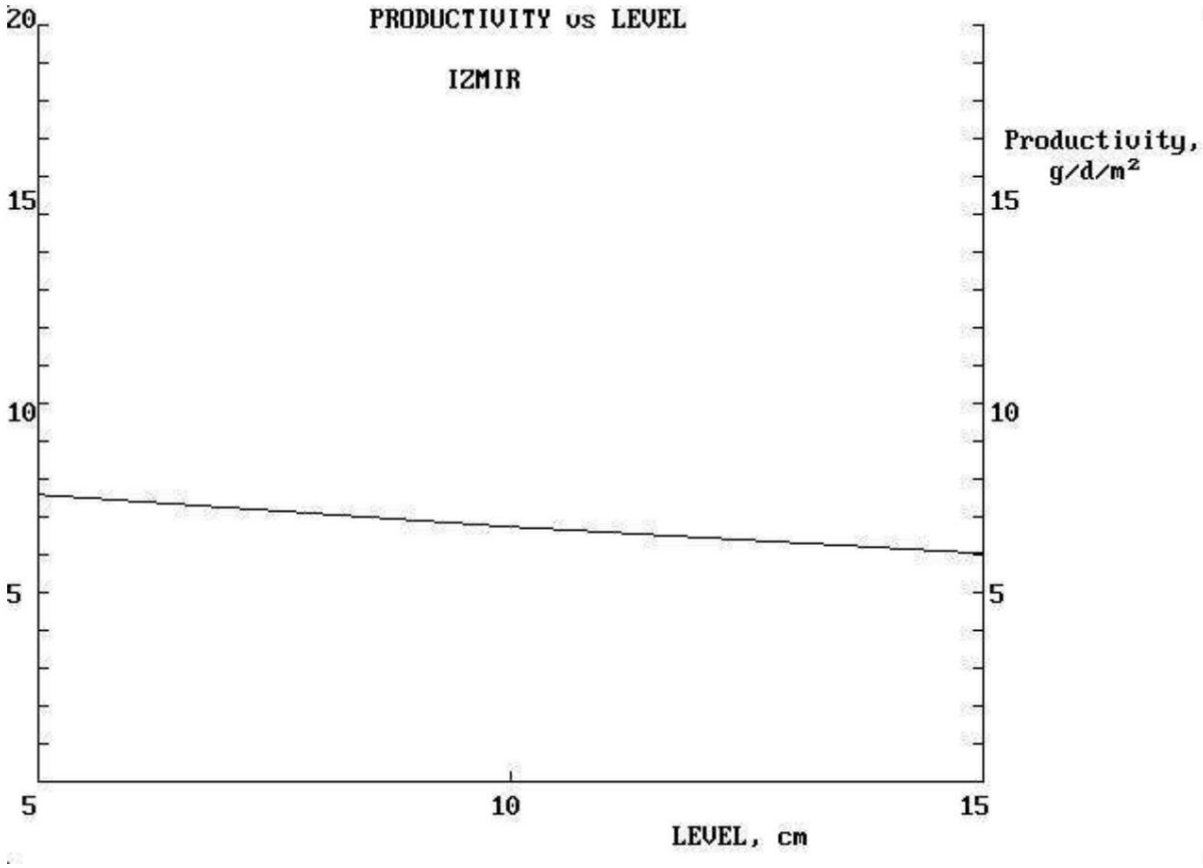


Fig. 14 Productivity vs. tank level

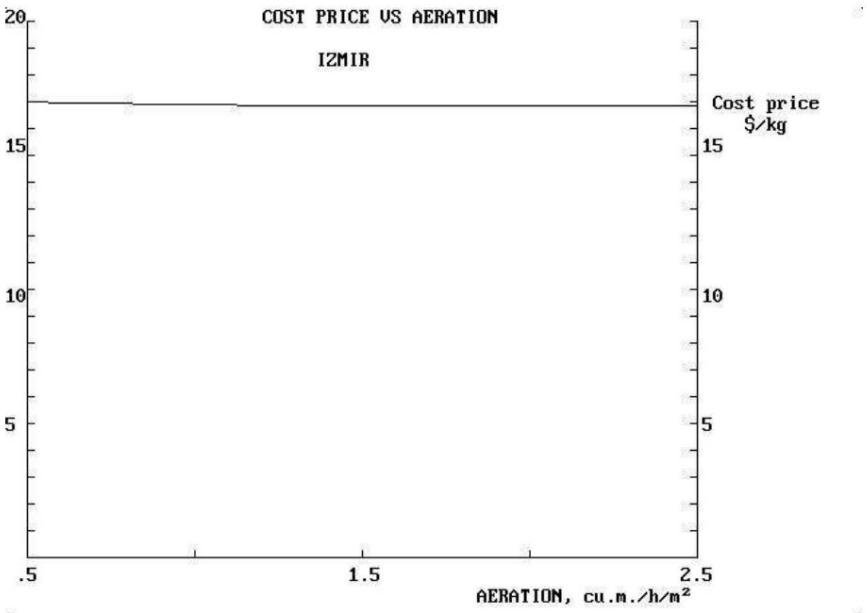


Fig. 15 Cost price vs. ventilation

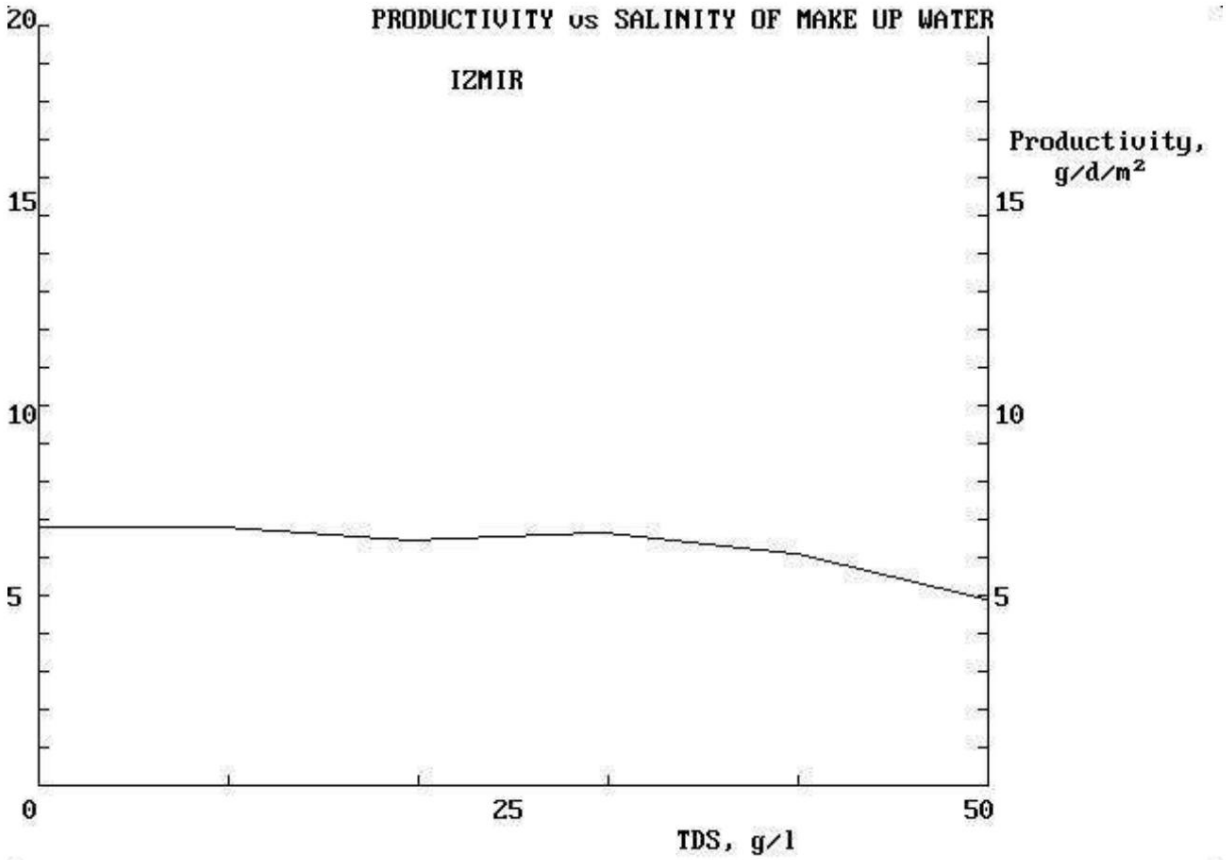


Fig. 16 Productivity vs. salinity of make-up water (maximum salinity = 40)

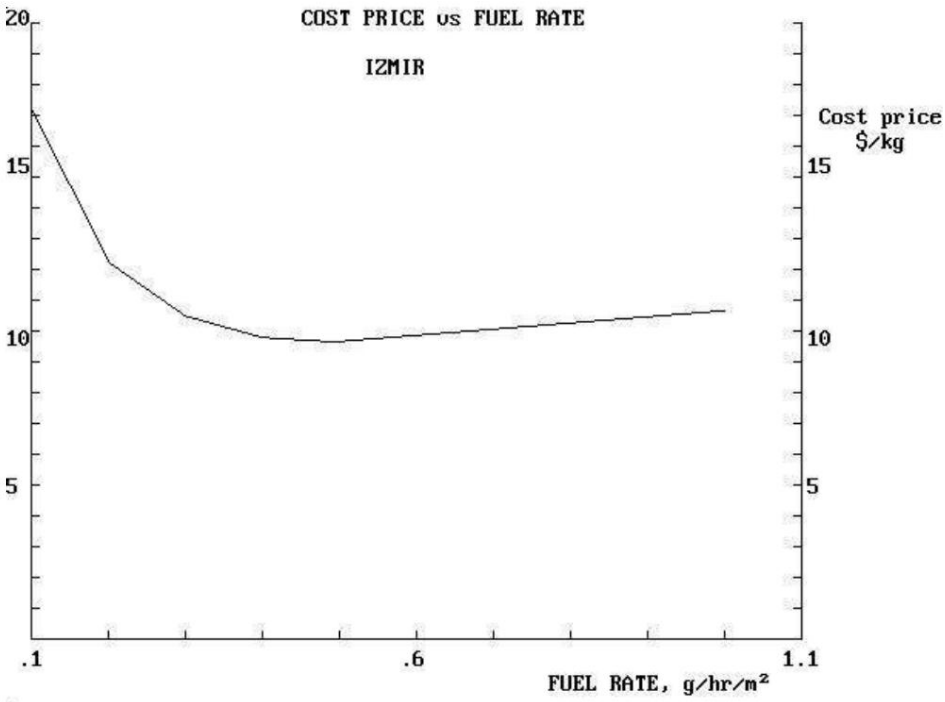


Fig. 17 Cost price vs. fuel rate (propane @ 1 $/kg as sole carbon source and ventilation rate = 0.1 m/h)

CULTIVATION

CRAFTSMANSHIP

OF SPIRULINA

(Summary of the inglesa version)

08/28/2005

INDEX

PROLOGO

El presente no es un nuevo libro sobre la spirulina; hay excelentes libros para responder a las siguientes preguntas:

- What is spirulina (Arthrospira platensis)? -
¿ dónde vive naturally? - ¿ cómo fué discovered
in los años 1960? - ¿ ¿ cuál es su composition
nutritiva? - ¿ a qué normas de calidad debe
responder? - How is the puede produced
industrially? - ¿ porqué se le predice a brilliant
futuro?

Consult por ejemplo: "Microalga Spirulina, Superalimento del Futuro", by Robert Henrikson, Ediciones Urano (1994).

El único objetivo de este manual summarized es de aportar mi experiencia práctica en el cultivo de la spirulina en pequeña escala a quienes así lo necesitan.

If algunos términos técnicos parecen difícil de compreerer, ustedes can consult a manual de quimica para alumnos de colegio que podrá aclararlos.

In practice, cultivar spirulina is no more difficult than cultivar tomatoes!

STANDS

The spirulina lives in agua a la vez salada y alkaline, contained in a container (o estanque) resistant to corrosion; Poco importa su forma, salvo los ángulos que deben ser redondeados para facilitar la agitation y limpieza de los rincones. Generally utilizamos estanques con bordes de 40 cm (el double de la profundidad normal del cultivo).

Los estanques pueden tener una surface de 1 m² - es lo que corresponds to the need for spirulina of a persona - pero los de 5, 10, 20 hasta 40 m² son más económicos. The dimensions of its sobretodo limitadas por la necesidad de agitar el estanque.

El fondo del estanque debe tener un hoyo y una ligera pendiente

to facilitate the desaguë.

Es preferible tener dos estanques que uno sólo grande por razones prácticas (transvase de uno al otro para limpiarlo por ejemplo).

A method of realizing economically estos estanques utiliza plastics of 0,5 mm of thickness (PVC, EVA), of calidad alimentaria of preference; the lateral ones are supported by a wall of ladrillos or a structure of madera or metallic tubes or PVC. If there are termitas in the region, it is recommended to colocar bajo el plastic una delgada capa de ceniza y una capa de arena seca.

El hormigón es un buen material para los estanques, pero necesita albaniles experimentados. The calidad del revoke is very important.
Antes de agregar el medio de cultivo es recomendable pintar la surface del estanque con dos manos de pintura común a la cal.

An invernadero sobre los estanques offers muchas ventajas on condition that pueda ser aireado y sombreado.

La agitation de los estanques se puede hacer a mano con escoba, una vez cada hora o dos horas (mas frecuente if el sol es fuerte). If you have electricity, you can use pequeñas de acuario bombas para agitar los estanques (una potencia media de 1 W/m² es suficiente).

Los industrial estanques son agitados con paletas, pero esta es a técnica considered as a little difficult to employ for the pequeños artisanal estanques que son los que aquí nos interesan.

CLIMATIC FACTORS

The temperature of the medium of cultivation is the climatic factor of major importance for the rapidity of crecimiento and the heat of the spirulina. Por debajo de 20°C el crecimiento es prácticamente nulo, aunque muchas spirulinas no mueren incluso a 0°C. The optimum temperature for the increase is 37°C. At 42°C, the spirulina is in serious peligro.

Illumination is essential for the increase in spirulina (fotosíntesis), pero no se debe mantenerla 24 horas contínuas por dia.
During the night, biochemical reactions continue to produce spirulina as well as protein synthesis and respiration. The breathing decreases the mass of the spirulina (the "biomass") sobretodo cuando the temperature is raised. Desde este point de vista las noches frescas

son buenas, pero la spirulina no puede support una fuerte iluminación al frío (debajo de 15°C). Unless illumination is an essential factor, el pleno sol no es ideal par la spirulina: una sombra media es preferible.
If the ground is the only source of heat to legar a buena temperature, is a problema: por eso a high ambient temperature is preferable.

Un filamento individual de spirulina no puede soportar una exposición prolongedada al sol: es destructido por fotolisis. De aqui la necesitad de agitar el cultivo.

The lluvia is beneficial to compensate for the evaporation of the agua, pero es necesario vigilant que no overflow el estanque.

The come is also beneficial for the agitation of the area y para airear el cultivo, pero está el riesgo del aporte de polvo y hojas en el cultivo.

Notamos que la luminación y el calentamiento artificiales pueden ser utilizados para hacer crecer spirulina. Tubos de neón convienen para illuminar pero las lámparas ordinarias tienen la ventaja de calentar al mismo tiempo que illuminar.

CULTIVATION MEDIUM

El agua utilizada para hacer el medio de cultivo debe ser limpia o filtered to eliminate contaminating algae.

Drinking water is convenient. If it contains too much chlorine, debe air.

If el agua es muy dura, cause the formation of depósitos desagradables pero no peligrosos. La utilización de agua salobre can be interesting pero es necesario analizarla antes de utilizarla.

Algunas aguas contienen bastante o demasiado magnesio y/o hierro.

El agua de mar, muy rica en magnesio, puede ser utilizada pero con precauciones o tratamientos que no están incluidos en este documento.

El medio de cultivo puede obtentionerse disolviendo los products químicos siguientes en el agua:

	g/litre
Baking soda	
Sal	
Potassium nitrate (or salitre)	8 5 2

Sulfato Dipotásico	1
Fosfato monoamónico	0.1
Magnesium sulfate (MgSO4.7H2O)	0.2
Solución de hierro (10 g of Fe/l)	0.1
Cal (if el agua es muy poco dura)	0.02

Si se utiliza sal no refinada, no se necesita el sulfato de magnesio.

The solution of hierro is prepared dissolving 50 g of sulphate of hierro (FeSO4, 7H2O) and 50 ml of chlorhídrico acid concentrated in a liter of agua. You can also use a solution saturated with hierro (clavos) in vinegar with a little juice of lemon or carambola.

Este medio de cultivo se utiliza para iniciar nuevos cultivos o para complete the level of the estanques luego de vaciarlos partially.

The composition arrived mentioned may vary in amplifications proportions. Así, in addition to 8 g of bicarbonate, you can use a mixture of 5 g of sodium carbonate and 1 g of bicarbonate, obtaining a pH of 10.4.

Ciertos iones pueden ser introducidos en cualquier concentration, no limit for total salinity that does not exceed 25 g/l.
Se trata de los ions: sulphato, chloride, nitrato y sodio.

Loss of phosphate ions, magnesium and calcium will not be able to be used in highly elevated concentrations without provoking the formation of mineral deposits and imbalances in the formula.

The concentration in potasio may be increased a voluntad, salvo que ella no supere 5 veces la concentration de sodio (se trata de concentraciones en peso). Esto permite utilizar the potasa extraida de la ceniza de madera, con la lejía como reemplazante del bicarbonate/carbonato de sodio (es necesario dejar la lejía expuesta al aire suficiente tiempo para que ella se carbonate hasta que su pH baje debajo de 10.8 antes de utilizarla como base del medio de cultivo). La ceniza utilizada debe ser blanca.

In case of necessity (or situation of survival) is possible replace nitrato, phosphate and sulfate with the orina of personas or animals in good health and that no consuming drugs as antibiotics. The dose is 4 ml/ l of medio.

El nivel normal de medio de cultivo en un estanque es alrededor de 20 cm, any possible cultivar with 10 cm hasta 40 cm.

INOCULATION

Scoop out a simiente (cepa) of spirulina well espiralada, con pocos o no filamentos rectos (al menos 50% espiralada). A simiente concentrate is obtained easily from a cultivo en buena salud, tomándola de la nata or rediluyendo con medio de cultivo una masa de spirulina fresca cosechada pero no exprimida. At the maximum concentration of 3 g of spirulina (contada en sec) per liter, the simiente can be kept and transported during a week if it degrades, depending on the condition that the recipient sea medio lleno y ventilado al menos dos veces por dia. If the ventilation is continuous with bursts of air, the concentration may drop to 10 g/l.

The inoculation simply consists of mezclar la simente con el medio de cultivo. It is recommended to maintain a nuevo cultivo initially and in course of crecimiento (progressive dilution with medium de cultivo nuevo) with a concentration of spirulina alrededor of 0.3 g/l (very green).

You can expect a 30% increase by day if:

- the temperature is correct, -
the medium of cultivation is based on bicarbonate,
- the surface of the pond is increased, maintaining the depth of the cultivation at low level (no superando 10 cm) and the concentration of spirulina alrededor of 0.3 g/ I.

When the final surface of the estanque is the excess, increase the level and the concentration of the cultivation hasta the level excess and the optimum concentration of 0.4 g/l before initiating the cosecha.

COSECHA

El mejor momento para la cosecha es temprano en la mañana, por muchas razones:

- the low temperature hace el trabajo más pleasant, -
habrá más horas de sol par secar el producto, - el
porcentaje de proteínas está su máximo en la mañana, - la
filtration está mas rápida.

The cosecha essentially includes these steps: -
Filtration to obtain a biomass at 10% dry matter (1 liter = 100 g of dry matter), - Exprimido para eliminar el medio de cultivo residual y to obtain the "spirulina fresca", lista a ser consumida o secada, containing alrededor of 20 to 25% of materia seca según las cepas y la salinidad del medio.

The filtration is simply carried out by engraving through a synthetic trunk (polyester or polyamide) of approximately 40 µ (0.04 mm) of aperture. El filtro puede ser un saco colocado encima del estanque para recciclar directamente lo filtrado. Antes de ser filtrado el cultivo debe pasar por un colador or un tamiz de malla 0.3 mm para eliminar los cuerpos extraños como insectos, trozos de vegetales, etc.
It is possible to use a container with rectos borders by receiving the capa flotante (if hay), avoiding moving the bottom of the deposit. Filtración can speed up filming or smoothing the malla. Whenever the mayor parte del agua es colada, the spirulina (the biomasa) se junta formando como una "bola" gracias al movimiento de la malla. With veces, the bola no puede formarse bien o se pega.

El expressido final se hace simplye a presión: the biomasa se pone como una torta de unos centímetros de espesor en una malla (la misma que sirve para la filtración es buena (preferablemente doubled por una tela fuerte de algodon) entre dos placas ranuradas con pesos encima (piedras, ladrillos, bloctas, etc.) or en una prensa o un lagar.
A pressure of 0.2 kg/cm² lasting a quarter of a hour is enough to eliminate the interstitial water, but with the pressure y/o the temperature increases more slowly to obtain a firm enough pressure.
To hold the pressure cuando el "jugo" is seen too much green.
This system is more adequate than the washing with agua to eliminate the restos of the medium of cultivation without destroying the spirulina, except that the expression is very difficult or impossible to debido a biomasa of inferior quality (100% of filamentos rectos por ejemplo). In this last case el lavado debe hacerse de preference con agua potable lightly salada y acidificada.

COMO ALIMENTAR EL CULTIVO

El principio consists of replacing, luego de cada cosecha, los elementos nutritivos tomados del medio de cultivo por la spirulina cosechada, in order to maintain the fertilidad del medio de cultivo. En la práctica los nutrientes se pueden añadir regularly cada día según la productivity media.

The main nutrient element is the carbon, that the medium of cultivation spontaneously absorbs from the area low the form of carbon anhydride (CO2) when the pH is higher than 10. If the area contains very little CO2, the absorption of this corresponds to a maximum productivity (when the pH reaches 11) of 4 g of spirulina per day and per m² of estanque. It is possible to inject additional CO2 to increase productivity, reduce the form of alcoholic fermentation gas or a liquid CO2 bottle: the gas burst in the medium of cultivation debajo of a plastic with its support of madera (with surface area alrededor of 4% of la del estanque) que lo retaine como una campana pendante el tiempo que tarde en disolverse. O mejor el CO2 can be introduced in a flujo de cultivo in a tube or a manguera. A convenient CO2 dose is 1 kg per kg of spirulina producida.

The azúcar can replace the CO2 as a carbon leak (medio kg of azúcar = 1 kg of CO2).

Adema del carbono spirulina consumes the usual nutrients in agriculture: N, P, K, S, Mg, Ca, Fe and trace elements. In the mayor parte de casos los oligoelementos y el calcio son aportados por el agua y las impurezas de los dirty utilizados. In certain casos el agua contains too much calcium, magnesium or hierro, lo cual produce mineral deposits that have inconvenient veces.

Do not use granulated agricultural fertilizers of slow release ("slow release") that contain much impurities. Use crystallized soluble fertilizers for horticultural nutrient solutions.

In Chile el salitre potásico is the preferred source of nitrogen pero in the mayoría of countries the úrea is the source of nitrogen but economic. The úrea is excellent for the spirulina with the condition of limiting its concentration in the middle to 50 mg/litre. La úrea en exceso puede

transform into nitrate or ammonia. Si en el cultivo se siente un poco el olor de amoniaco no hay peligro pero si el olor es fuerte al menos una parte de la spirulina morirá.

He has a classic food formula per kilogram of spirulina (seca) cosechada:

Urea 300g
Fosfato monoamónico 50 g
Sulfato dipotásico 40 g
Magnesium sulfate (SO4Mg,7 H2O) 40 g
Cal 10g
Solución de hierro (10 g/l) 50 g

In case of necessity all the nutrients save the hierro pueden ser proporcionados por la orina de personas o animals en buena salud y que no consumen medicamentos como antibiotics. The dose to be used is alrededor of 17 ml/g of spirulina cosechada.

CULTURAL ATTENDANCES

Ademas de la cosecha y alimentación, a cultivo of spirulina requires careful attention to maintain it in good condition.

La agitation es necesaria pero no continuamente. Una vez por día, justo después de la cosecha, es bueno agitar el fondo del estanque para evitar la fermentation anaerobic de los depósitos orgánicos. La agitation superficial debe realizarse una vez cada dos horas o más frecuentemente si hay gran iluminación.

If the spirulina decanted al fondo del estanque (caso abnormal pero that can be produced by example of a sudden dilution for the water) is obvious that it is necessary to stir it frequently to avoid that it becomes asfixed.

The photographic capacity of spirulina is saturated by a luminosidad corresponding to a third of full soil. A darkening is beneficial for the health of the spirulina and also useful for reducing the evaporation of the water, the temperature (< 40°C) or the pH (< 11). In practice it is very rare that the sea temperature becomes too high in ponds in the open air, pero the pH may suffer very high if the carbon supply is insufficient.

El nivel de agua en el estanque debe mantenerse alrededor del

level deseado. The evaporation can compensate for agregando agua. Un exceso de lluvia puede ser rectificado vaciando una parte del medio para luego agregar los nutrientes contenidos en el volumen del medio arrojado.

Si se acumula mucho depósito al fondo del estanque, podemos reducing the mediante bombeo o sifón del medio de cultivo cerca del fondo, allí donde encontramos el depósito en mayor cantidad. Luego agregar el medio de cultivo nuevo en cantidad igual al del volumen arrojado. Another method, more radical, to sacar los depósitos consists of transvasar el cultivo en otro estanque para limpiar el fondo.

In the large industrial enterprises producing spirulina the content of the medium of cultivation referred to this nutrient, and including the trace elements, is determined by análisis químico, teniendo así the possibility of aggregating the exact quantity of elements that faltan. Este método resulta demasiado costoso para pequeños cultivos; en estos lo adecuado es renovar parcialmente el medio de cultivo de vez en cuando (por ejemplo 10 % cada mes).

In order to avoid the formation of lumps with the Lonar cepa it is recommended to maintain the pH at the top of 10.2 así as a buen aporte de nitrogen bajo formed de úrea.

El cultivo es an ecosystem en el cual diversos organismos viven en simbíosis: adapted bacteria that feed on organic wastes and zooplankton (como paramecias) that feed on bacteria, transform andolas in mineral nutrients and CO2 by spirulina.
Bacteria and zooplankton also consume the oxygen produced by spirulina, local conditions are favorable for the increase in spirulina. Estos biológicos processes son bastante lentos, surerte que si el nivel del cultivo es bajo y/o si la productivity en spirulina es elevada, podría haber accumulation de deshechos resultando en alta turbiedad y dificultad de cosecha. To improve the medium of cultivo sucio, basta renovarlo parcialmente or bajar la productividad sombreando el estanque or dejando to undergo the concentration of spirulina; el improvement normally occurs in una o dos semanas.

El cultivo puede ser colonizado por small animals that live at the expense of spirulina, like larvae of moscas Ephydra or mosquitoes, rotiferas or amebas (normally not toxic). Según nuestra experiencia estas invasiones no producen otros efectos molestosos que una reduction de la productivity. To eliminate animal estos fisicamente podemos utilizar a colador (para larvas) o para eliminarlos biológicamente podemos to temporarily increase the salinity, el

pH or the temperature of the crop. El incremento de la temperatura hasta 42°C parece el más fácil a realizar (con un invernadero) y también muy eficaz. Frecuentemente estos predatores desaparecen ellos mismos al final de algunas semanas.

A crop that gives the salinity or the concentration in spirulina its muy bajas can be invaded by a single-celled alga verde (edible): the clorela; Felizmente la clorela cae al fondo del estanque cuando la agitation es apagada, quedando en la oscuridad donde ella muere al cabo de unos días. Lo mismo ocurre con las diatomas. To eliminate the clorelas se puede hacer una cosecha total y lavar la biomasa con un poco de medio nuevo para eliminar las clorelas de la biomasa, y luego utilizar la biomasa para inocular un medio nuevo.

Algas azul-verdes tóxicas como Anabaena, Anabaenopsis arnoldii y Microcystis no viven en un culture de spirulina bien atendido, pero por seguridades es recommended hacer verificar su ausencia con un microscopio profesional pour un microbiólogo una vez por año, y tambien hacer un an analisis de cianotoxinas . Un cultivo de larvas de artemia en agua salada (30 g sal por litro) can be used to verify the ausencia of algas tóxicas: agregar al cultivo de artemia un poco del cultivo de spirulina y observar el behavior de las larvas: si al cabo de seis horas o más ellas estan siempre llenas de vitalidad, no hay una concentration peligrosa de algas tóxicas. Podemos to acquire huevos of artemia in the tiendas of acuariofilia. Pero ese método no es tan bueno que una analisis de toxinas.

Normally the usual pathogenic bacteria no pueden survive in the middle of cultivation when the pH is above 9.5 where it is during production. Without embargo it is recommended to have bacteriological controls of spirulina cosechada al menos una vez por año o in case of epidemic (el vibrio del cólera can survive hasta pH 11).

CONSERVATION

Es cierto que la spirulina fresca (la biomasa prensada) es superior a toda otra forma de spirulina tanto del point de vista organoléptico como por su valor nutritivo y de costo. It can be kept dos días in the refrigerator at 7°C or diez días at 1°C. Además freezes easily.

If you don't have a refrigerator or a freezer, the salad can be a solution. 10% of sal fina is added to the prensada biomasa, asegurando una conservation como de mes, bajo una light capa de aceite. The salado modifies the product: its consistency

Vuelve más fluída, su color más dark (la ficocianina azul es liberada) y el gusto se parece al de la pasta de anchoas.

El secado es el único modo de conservation commercial.

Conveniently packaged and packed the dry spirulina can be preserved hasta cinco años; pero el secado es costoso y frecuentemente da al producto un gusto y olor que pueden ser juzgados desagradables por el consumidor.

SECADO

In the industry the spirulina is very dry for atomization in the area at muy alta temperature, during a tiempo muy corto; este process da un producto de extrema fineza, poco densidad aparente y mal olor. This process is impossible to use in pequeña escala.

The liofilización is an ideal process for the calidad, incluso en pequeña escala, pero de costo tremendo.

El secado solar is frequently used by pequeños producers, pero requires all precautions. If the exposure to the direct soil is used, that is more quickly, it will be very short duration if the clorofila will be destroyed in the surface and the product will appear gray or azulado.

Sea cual fuere la fuente de calor, la biomasa a secar debe ser puesta bajo la forma suficientemente delgada para secarantes de commenzar a fermentar. Dos formulas for ello: the pasta can be ser sparcida in capa delgada on a plastic film or puesto as tallarines in cylinders of small diameter ("spaghetti" of 1 to 2 mm in diameter) on a perforated plate. In the first formula, the heated area will pass horizontally soberly and the film will come in the second, it may undergo vertically through the perforated plate. The extrusion is theoretically and practically greater if the diameter of the tallarines frescos no sobrepasa 2 mm, pero al mismo tiempo hace falta que los tengan cilindros bastante mechanical resistence to keep su form pendante el secado y no "derretirse"; esto es lo que impide el uso de este proceso de secado cuando la biomasa prensada es de calidad inferior y no es bastante firm. De todas formed a buen flujo de aire es el factor mas important para evitar accidents de secado.

Durante el secado y después la spirulina debe ser protegida del polvo y de los insectos y no debe ser tocada por la mano.

The drying temperature will be limited to 65°C and the temperature of

secado at 6 hours. If it is dry at low temperature, like 42°C, it is preferable to finish for 15 minutes at 65°C to achieve a good degree of sterilization and also reduce the humidity of the product to 5% of water.

If the fermentation has started during the secado, the podemos detect por su olorduringe y después del secado.

Las escamas o dry tillers are generally converted into polvo o trozos finos por molido para increase su densidad aparente y facilitar su almacenamiento.

CONSUMPTION

Las personas que dicen no poder soportar el gusto ni el olor de la spirulina han estado expuestas, ciertamente un día, a seco producto de calidad mediocre. La spirulina fresca y de buena calidad es neutral, tal que can replace la mantequilla sobre las tostadas y can serve to enriquecer practically as food; delicious bebidas heladas pueden ser preparadas mezclando la spirulina, especialmente fresca, con jugo de frutas. The spirulina fresca is a pasta easy to dilute, mix or thin.

Hay literally miles of recipes possible to use spirulina fresca, congelada o seca, cruda o cocida.

Note that sober 70°C in the presence of agua el bello color verde of spirulina (clorofila) with veces se vuelve marrón.

ANEXO

COMPARISON OF MUESTRAS OF SPIRULINA

The main análisis necessary for juzgar la calidad de una muestra de spirulina (contenido en proteínas, hierro, ácido gamalinolénico, y análisis microbiológico) necesitan realizarse en un laboratorio, pero algunas pruebas muy simples pueden ser realizadas por el mismo productor, comparando muestras entre ellas . Una muestra de buena calidad can serve as a reference.

El examination del color, olor y gusto es revealador de diferencias important. El color verde debe tender más hacia el azul que hacia el amarillo.

Para hacer el examination del pH de una spirulina seca, mezclar 4 gramos de polvo en 100 ml de agua y medir el pH al cabo de dos

minutos y de 24 horas (agitar de tiempo en tiempo): the initial pH should normally be around 8 to go down to 6 o menos, pero ciertos commercial products están largamente fuera de estas cifras (generalmente pH superiores).

Luego de la prueba precedente podemos muy easily to obtain a comparative medida of the content in ficocianina (pigmento azul muy important, that constitutes a cuarto of the total proteins). Es suficiente poner una gota de la solución sobre un papel filtro blanco (filtro a café por ejemplo) y dejar secar la mancha: la intensidad del color azul es una medida del content en pigmento. If the pigment is not "dirty" well, it is possible that the sea debido has a secado of the spirulina at low temperature; start again the prueba luego de haber calentado la muestra seca a 65°C por un minuto.

El content in carotenoids (el betacaroteno constituted between 40 to 50% of total carotenoids) can be evaluated mezclando una muestra de spirulina sec en polvo con dos veces su peso de acetona or alcohol de 90° dentro de un frasco cerrado y agitado. Al cabo de un cuarto de hora, tomar una gota de la solución decantada y ponerla sobre un papel filtro para examine el color de la mancha formada. The intensity of the marrón-amarillo color is proportional to the carotenoid content. Notamos que el color de la mancha no se guarda más que unas horas y que en las muestras de spirulinas antiguas almacenadas sin precaución el contenta prácticamente nulo.

MEDIDA OF CONCENTRACION IN SPIRULINA

AL DISCO OF SECCHI

El "disco de Secchi" is an instrument made up of a bar 30 cm wide, graduated in centimetres (or concentracion despues de calibrar), teniendo en su extremidad inferior un disco blanco. Allows an approximate medida of the concentration in spirulina.

Antes de medir, agitar para homogeneizar, luego dejar decantar los depósitos algunos minutos y anotar la profundidad en centímetros, allí justo donde es imposible to distinguish el disco.

MEDIDA OF THE SALINIDAD DEL MEDIO DE CULTIVO

It is necessary with the addition of a densímetro para densidades superiores a 1 (urinómetro por ejemplo) and the following temperature correction is applied:

D20 = DT + 0.000325 x (T - 20)

wave:

D20 = Density at 20°C DT
= Density at T°C

expressed in g/ml or kg/l.

From the density at 20°C, calculate the total salinity (SAL, in g/l) of the medium of cultivation for the formulas:

If D > 1.0076: SAL = 1250 x (D20 - 1.0076) + 10
Sino: SAL = 1041 x (D20 - 0.998)

MEDIDA DE LA ALKALINIDAD DEL MEDIO DE CULTIVO

The prueba is hace por neutralización de una muestra del medio con ácido clorhídrico normal (ácido concentrado del comercio diluído diez veces); the final point will meditate at pH = 4.

Alkalinity (= basic molecules fuerte por litro) is the relationship between the volume of acid used and the volume of muestra del medio utilizada..

MEDIDA DEL pH DEL MEDIO DE CULTIVO

El pHmetro debe ser ajustado una vez por semana. Soluciones muestras pueden ser compradas, o preparadas como sigue (approximate pH at 20°C):

pH = 9.7 to 9.9 (según el contenido del aire en CO2): dissolve 3.3 g of sodium carbonate + 3.3 g of sodium bicarbonate in a liter of demineralized water; Keep the solution in contact with the atmosphere and regularly aggregate the water to compensate for the evaporation.

pH = 7.2: dissolve 5.8 g of diamónico phosphate + 11 g of sodium bicarbonate in a liter of demineralized water and mantenerlo in a cerrada bottle.

pH = 2.8: ordinary vinegar at 6° (density 1.01)

Temperature correction over pH: pH at
20°C = pH at T°C + 0.00625 x (T - 20)

MEDIDA DE LA HUMEDAD EN LA SPIRULINA SECA

Place in a transparent and airtight container (like a Tupperware) approximately the misty volume of spirulina and air space together with a thermo-higrómetro that is pueda leer de afuera sin abrir. Calentar or cool so that the sea temperature cools to 25°C. Hope for temperature and humidity balance.

Hay a correspondence between the % of relative humidity (HR) in the area and the % of agua in the spirulina, asi:

25% RH = 5% water
32% RH = 6
43% RH = 8
49% RH = 9

Because spirulina seca keeps well, su% de agua debe estar menos que 9% (es la norma). Los microbios mueren dentro de dos meses en una spirulina à 7% de agua..

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