

# GROW YOUR OWN SPIRULINA

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## NOTICE

This is the condensed version of a "Manual of small scale spirulina culture" written in French and distributed by Antenna Technologies.

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 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, U.S.A. (1994), and "Spirulina, Production & Potential", by Ripley D. Fox , Editions Edisud, France (1996), or "Spirulina platensis (Arthrospira) : Physiology, Cell-biology and Biotechnology", edited by A. Vonshak, published by Taylor & Francis (1997)

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## **CLIMATIC FACTORS**

Temperature is the most important climatic factor influencing the rate of growth of spirulina.

Below 20°C, growth is practically nil, but spirulina does not die. The optimum temperature for growth is 35°C, but above 38°C spirulina is in danger.

Growth only takes place in light (photosynthesis), but illumination 24 hours a day is not recommended. 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 cannot stand a strong light when cold (below 15°C).

Light is an important factor but full sunlight may not be the best rate of 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 are not economical. Fluorescent tubes and halogen lamps are both convenient. Lamps can illuminate and heat the culture simultaneously.

## **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 non-toxic. Its shape is immaterial, although sharp angles should be avoided to facilitate agitation and cleaning. Its depth is usually 40 cm (twice the depth of the culture itself). It can be as small as 1 m<sup>2</sup> but 5, 20 or 100 m<sup>2</sup> 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 emptying. Two ponds are better than just one, for practical reasons.

The most economical ponds are made of U.V. resistant plastic film of 0.5 mm thickness or more (PVC or polyethylene), 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.

Concrete ponds are of course a good, durable solution where experienced labour 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 practically necessary.

Agitation can be manual, with a plastic broom, once every two hours. If electricity is available, aquarium pumps are practical to agitate the surface of the culture (one watt/m<sup>2</sup> is enough). "Raceway" ponds agitated by paddlewheels are standard in the industry, but somewhat outside the scope of this manual.

## CULTURE MEDIUM

Spirulina can live in a wide range of compositions of water ; the following is a convenient analysis :

Anions	Carbonate	2800	mg/l
	Bicarbonate	720	
	Nitrate	614	
	Phosphate	80	
	Sulfate	350	
	Chloride	3030	
Cations	Sodium	4380	
	Potassium	642	
	Magnesium	10	
	Calcium	10	
	Iron	0.8	

Total dissolved solids 12847

Density @ 20°C 1010 g/l

Alcalinity 0.105 N (moles strong base/liter)

pH @ 20°C 10.4

In addition, the solution contains traces of all micronutrients necessary to support plant life.

Such solution can be obtained by dissolving various combinations of chemicals ; here is one example convenient for many typical waters :

Fertilizer	g/l
Sodium carbonate (soda ash)	5
Sodium chloride, crude	5
Potassium nitrate	2
Sodium bicarbonate	1
Potassium sulfate, crystallized	1
Urea	0.02
Monoammonium Phosphate, crystallized	0.1
Magnesium sulfate, crystallized, (7 H <sub>2</sub> O)	0.2
Lime	0.02
Ferrous sulfate	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 muds which are 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. The make-up medium should best be as follows : carbonate is replaced by bicarbonate (8 g/l in total), urea is up to 0.07 g/l.

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 to supply nitrogen but is highly toxic at too high concentration. Spirulina can grow on either nitrate or urea alone, but using both together is advantageous.

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. This makes it possible to use potash extracted from wood ash to replace sodium carbonate/bicarbonate should these not be available (let the potash solution absorb CO<sub>2</sub> 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.

Micronutrients traces 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/l 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.

## **SEEDING**

Choose a spirulina strain containing a high proportion of coiled filaments (less than 25 % straight filaments, if possible none at all), 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 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 culture medium, about 0.3 g/l : the "Secchi disk" reading (see Annex 1) should not be above 5 cm, i.e. the color of the culture should stay clearly green (otherwise shading is mandatory). The rate of growth is about 30 % /day when the temperature is adequate and the make-up culture medium 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 (i.e. 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 it 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 is 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 fine net will accelerate somewhat the filtration and protect the cloth against rupturing, but a simple bag made from the cloth works well also.

The filter can 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  $\mu$ ) to remove any foreign matter such as insects, larvae, leaves and lumps of polysaccharide or mud.

When the spirulina floats, it is efficient to scoop out the "cream", using a straight edge pail. Harvesting the floating layer (generally richer in spiraled 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.

The filtration is accelerated by gently moving or scraping the filter. 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 spiraled forms and the culture medium is clean). Otherwise it may then 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/cm<sup>2</sup> 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 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 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 loss of valuable products; it may also introduce germs contained in the wash water. Washed biomass is a lot 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 (CO<sub>2</sub>), whenever the pH of the medium is above 10. However the air contains so little CO<sub>2</sub> that this absorption is a slow process, corresponding to a maximum productivity of 4 g spirulina/day/m<sup>2</sup>. This maximum rate is reached at or above pH = 10.5. Extra CO<sub>2</sub> can be introduced to increase the productivity, either pure CO<sub>2</sub> gas (from fermentation or from a cylinder) or dissolved organic matter that can be biologically oxidised to CO<sub>2</sub>, like sugar. The gas is bubbled into the medium, under a piece of floating plastic film (about 4 % of the total area of the pond).

Another popular, although costly, means of feeding carbon is bicarbonate. Adding bicarbonate is an easy and efficient way of reducing the pH, but it increases the salinity ; to maintain the salinity, it is mandatory to drain part of the culture medium from time to time and replace it by new medium rich in bicarbonate. Disposal of the drained medium may be an environmental problem and the cost may of the chemical can be uneconomical.

The amount of gas, sugar 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, but not dangerous, exopolysaccharide (EPS). A good practical dose of carbon feed is the equivalent of 40 % of the spirulina produced (i.e. about 0.8 kg of CO<sub>2</sub> or 0.4 kg of sugar per kg of dry spirulina harvested). Sugar may induce some degradation of the culture medium, so actually it is recommended to feed less than 0.3 kg/kg, and as regularly as possible..

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 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 muds.

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 and cost are the main criterions 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 were set.

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 nutrients apart from nitrogen. But very generally the cheapest source of nitrogen is urea. Urea, made up of ammonia and CO<sub>2</sub>, is an excellent nutrient for spirulina but its concentration in the medium must be kept low (below about 60 mg/liter). If sugar or other easily oxidizable organic materials are used as a source of carbon in the presence of nitrates they should not be fed in large concentration either, as they may reduce nitrates to ammonia that is toxic above 30 mg/liter. Excess urea can be 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 odour however indicates an overdose.

Here is a feed formula convenient in most locations, per kg of harvested spirulina (dry product) :

Fertilizer	weight	remarks
Urea	300 g	
Monoammonium phosphate	50 g	Concentrated pure phosphoric acid may replace the phosphate
Potassium sulfate	30 g	40 g if <u>no</u> potassium nitrate is used in the cultur
Magnesium sulfate	30 g	as the usual commercial product, cristallised with 7 molecules of water
Lime	10 g	
Iron sulfate	2.5 g	
Micronutrients solution	5 ml	supplied by Antenna Technologies, Geneva, upon request (the use of this solution is optional ; it is useful to make the biomass easier to harvest and also to reduce the need for renewing the culture medium)

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) at a dose of about 15 to 20 liters/ kg spirulina. Iron can be supplied by a saturated solution of iron in vinegar (use about 100 ml/kg) plus some lemon juice.

Fertilizers other than urea can be fed every month or so, but urea (or urine) has to be fed daily, 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 requisite. 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 ( $< 38^{\circ}\text{C}$ ) 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. 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).

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 muds is to provisionally transfer the culture into another pond and clean the bottom.

Accumulation of muds may cause "white skins" to float in the afternoon when the temperature of the culture goes above  $35^{\circ}\text{C}$ . These are not harmful as they will go back to the bottom again during the night, but their appearance is unpleasant : they can be removed using a net.

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 or on the addition of minor amounts of a concentrated solution of micronutrients as mentioned above.

Excessive production of exopolysaccharide (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 higher pH, nitrogen and iron contents 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. This applies to the organic muds 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 pH or salinity sudden 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 they will be in great danger of dying from suffocation. In order to facilitate their recovery, agitate the bottom often to give them more chance to disentangle from the muds.

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 reducing somewhat the productivity. Often they can be controlled by increased salinity, pH or temperature, or they disappear by themselves after a few weeks.

If the concentration of spirulina is too low, the culture may be invaded by chlorella (a unicellular, edible alga). Fortunately, chlorella sinks to the bottom of the pond : momentarily stopping the agitation will deprive chlorella from light and it will eventually die. The same applies to diatoms.

Toxic algae like anabaena, anabaenopsis arnoldii and microcystis do not grow in a well tended spirulina culture, but for safety's sake it is recommended to have the culture checked by a microscopic examination at least once a year. A culture of young artemias can be used to check the absence of toxic algae : add a little of the spirulina culture to be checked (10 % of the artemias culture) and observe the small animals ; if they retain their vitality for at least 6 hours, there is no toxic algae. Artemias eggs are sold by aquariophilic stores.

Usual pathogenic bacteria do not survive 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 most 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 the culture under shading.

## **STORING THE PRODUCT**

There is no question that freshly harvested, pressed biomass is superior to any other form 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.

Freezing is a convenient way to keep fresh spirulina for a long time. It also liberates the blue pigment, but it does not alter the taste.

Drying is the only commercial way to store and distribute spirulina. If suitably packaged and stored, dry spirulina is considered good for consumption up to five years. But drying is an expensive process and it very generally gives the product a different and possibly

unpleasant taste and odour. Dried spirulina is also not so easy to use.

## **DRYING**

The industrial type of spirulina dryer is the spray drier 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 artisanal 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 blueish.

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 vertically through the tray. The rod shape is theoretically 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 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 65°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 should 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 odour of spirulina most probably were once exposed to a low quality product. Good quality fresh spirulina is so bland it can replace butter on toasts 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 70°C the gorgeous green color often turns brown in the presence of water. So

you can choose your preferred color for soups and sauces.

## **A1) MEASURING THE CONCENTRATION IN SPIRULINA WITH THE SECCHI DISK**

The "Secchi disk" is a self-made instrument : a piece of white 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, after measuring the turbidity of the filtrate using a black Secchi disk, expressed in cm in the same way as the concentration.

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.

## **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 :

$$\text{If } D > 1.0155, \text{ SAL} = 1275 \times (D - 1) - 0.75, \text{ g/liter}$$

$$\text{Otherwise, SAL} = 1087 \times (D - 0.998)$$

## **A3) MEASURING THE ALCALINITY 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.

Alcalinity (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 at least once a week. 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

- pH 9.9 : 5.5 g sodium bicarbonate + 1.4 g caustic soda per liter water, or : 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 :

$$\text{pH at } 25^{\circ}\text{C} = \text{pH at } T^{\circ}\text{C} + 0.00625 \times (T - 25)$$

## A5) COMPARING SPIRULINA SAMPLES

Protein, iron, and gamma-linolenic acid 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.

The "pH test" reveals the degree of removal of the culture medium from the biomass. On fresh spirulina simply measure the pH : if near 7, the biomass is pure. For dry spirulina powder, mix a 4 % suspension in 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, take a one drop sample of the decanted solution and put it on a filter paper (for instance the "Mellita" filter paper for coffee making) maintained horizontal. The amount of blue color in the stain is proportional to the concentration of phycocyanin in the sample. Some spirulina samples require to be heated to 70°C before the test for the blue pigment to be fully released into the solution.

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  $\mu$  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 spiraled 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 glide on it.

Final dewatering of the biomass is accomplished by pressing the biomass enclosed in a piece of filtration cloth, either by hand or in any kind of press. The simplest is to apply pressure (0.15 kg/cm<sup>2</sup> 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 must then 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 breakage 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, which reduces the drying time. 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 aluminium 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 to 68°C, and drying time to 7 hours. With good ventilation and low charge (1 kg fresh rods/m<sup>2</sup> 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 as well as 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.

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