ANTENNA TECHNOLOGIES

A teaching module for the production of spirulina

Objectives

The installations and techniques described in this document are intended primarily for trial, demonstration and training purposes. However, they should permit a steady output of about 40 grams of dry spirulina per day (enough to provide a very valuable food supplement for 10-20 children).

This document aims to ensure that satisfactory results are achieved, at the price of some extra cost (electric pump, "expensive" culture media, etc.). It does not describe the many possible variants and adaptations: once this "module" has been successfully tested, users should refer to the book of J.-P. Jourdan (see reference at end of text), which will enable them to move on to a real production unit adapted to local conditions.

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1. General: the key factors

1.1 The liquid medium (or culture medium)

The liquid used for the production of spirulina is a solution of mineral salts in water. This liquid has to supply the spirulina with all the chemical elements it needs. The pH of the culture medium (i.e., its level of alkalinity) should be between 8.0 and 11. There are various recipes for culture medium for spirulina. The one shown here is one of the most "friendly"; it is the best for ensuring an easy culture, even if it is far from being the cheapest (for composition, see Chapter 3 and Table 1).

1.2 Temperature

The temperature of the culture medium has a direct influence on the speed of growth of the spirulina: while fairly resistant to cold (down to 3-5°C above zero), spirulina only begins to grow appreciably above 20°C. Growth is fastest around 35-37°C. Above that temperature, there is soon a risk of destruction of the crop (which is inevitable after several hours above 43-44°C). Note that sharp changes in temperature are disastrous.

1.3 Light

A very strong light (full sunlight) can be dangerous in the following circumstances:

- on a cold culture (below 14-15°C), especially if exposed suddenly;
- on a very warm culture, because it becomes still hotter;
- on a culture that is very diluted (Secchi of more than 6 cm see Chapter 4);
- on a culture that is struggling (following an accident).

On the other hand, when concentration and temperature conditions are good, a culture can benefit from being exposed to a maximum of natural light. The light should be deliberately reduced by shading in any of the four circumstances mentioned above.

1.4 Agitation

It is essential, at least from time to time (2-4 times a day), to agitate a spirulina culture. This helps to ensure an even spread of the spirulina in the liquid and its exposure to the sun. If the agitation is too violent, it damages the spirulina (fragments visible under the microscope) and causes foam to appear. Certain types of centrifugal pump, as well as falling water with splashing, are particularly

harmful. The module proposed here provides continuous agitation of the culture using a small electric pump (see Chapter 2). For small volumes of culture (less than 100 litres), continuous agitation by air injection is possible with a small compressor used for aquariums. This last method is a very practical way of maintaining a culture on stand-by (see Chapter 10).

2. Description of the learning module

2.1 Equipment

- A flask of live spirulina;
- A covered tank with a total surface of 4 sq. m., consisting of:
 - 2 sheets of 0.2 mm polyethylene
 - 4 semicircular frames
 - 3 bars 4 m long
 - planks or bricks to build the walls;
- An electric mini-pump, aquarium type, 220V, 5-7 W (for agitation and harvesting);
- 1000 litres of culture medium (water, fertilizer and mineral salts according to the recipe used);
- Some simple measuring tools: a Secchi disk (see Chapter 4.1), litmus paper to measure pH, a thermometer.
- Harvesting equipment (see Fig. 2).

Possibly: An extruder and a dryer with removable trays (Fig. 4); A small air compressor, aquarium type (ca. 5W)

2.2 Installation of the covered tank (drawings in Fig.2)

The site selected should be a flat space of at least 2m by 8m, in an open situation (or lightly shaded in very hot climates). The tank should be built according to the drawings in Fig. 2, using bricks, breeze-blocks, planks or just banks of packed earth to build the walls. If termites are present, a layer of at least one centimetre of ash should be placed under the bottom sheet. If there are rodents, a metal grill of the type used in henhouses should be placed under the bottom sheet. It is prudent, but not indispensable, to use a double layer for the bottom sheet, especially if only plastic sheeting less than 0.2 mm thick is available. In all cases, the bottom of the tank should be carefully smoothed before placing the sheet in position.

The frames supporting the transparent covering of the pond can made from 6-8 mm iron reinforcement bars or bamboo. The frames can be triangular (A-shaped) instead of semicircular. The transparent cover (agricultural polyethylene, anti-UV if possible) should be held down on three sides with piles of earth or stones. One long

side is left unattached to allow access to the culture by lifting the plastic.

3. Preparation of the culture medium

The water used should be potable, and if possible with low calcium content (less than 100 mg of calcium per litre). Slightly salty water can be used (up to 4-5 g/litre of NaCl, in which case the salt in the recipe should be omitted). Table 1 gives the recipe to be followed according to the volume of culture medium required.

- N. B.: Ammonium nitrate, which is widely available, cannot be used here!
- Products shown in parentheses are not essential, at least in the short term or depending on the quality of water used. For example, calcium chloride (or lime) should be added if a very soft water is used (less than 10 mg of calcium per litre). Water that is even moderately rich in sulfate (more than 20 mg/litre) enables potassium sulfate to be omitted, provided that **potassium** nitrate is used as indicated (if **sodium** nitrate is used, the potassium sulfate becomes essential for its potassium).
- The infusion of green tea prevents iron precipitation: the development of a purplish colour after the addition of iron is normal.
- Dissolve the iron sulfate in a glass of water before adding it to the medium; then add the magnesium sulfate, which should again first be dissolved in a little water.
- This culture medium can be kept for several days before using it; in that case, it should be kept away from light.

4. Seeding of the tank and control of growth

4.1 Measurement of the concentration of a spirulina culture

The concentration of a culture can be gauged by the intensity of its colour, for which a Secchi disk is used: this is a graduated ruler at the end of which a small white disk is attached perpendicularly. This instrument is submerged in the water to the point where the disk stops being visible. The depth of the disk is then read off the graduated ruler. A culture is diluted if the Secchi disk remains visible beyond a depth of 5-6 cm; a value of 2-3 cm shows a culture ready for production. Values below 2 cm indicate that the culture should be diluted, or heavy harvesting should be undertaken. In good conditions, the amount of spirulina present in a culture doubles every 2-4 days, until it reaches a maximum concentration (Secchi < 1.5 cm). Between 1.5 cm and 3.5 cm, the Secchi scale is roughly linear, so that 1.5 cm \approx 0.5 g/litre (weight of dried spirulina per litre of medium) and 3 cm \approx 0.25 g/litre.

4.2 Growth phase: obtaining a 50-litre pre-culture

If only a small sample of spirulina culture (a few cubic centimetres) is available, take half of this quantity and dilute it in 0.25 litres of culture medium, e.g. in a well washed transparent plastic bottle. Keep the rest of the sample in reserve. The highly diluted spirulina is fragile; avoid exposing it to full light, or shaking it violently. Place the culture in a bright place but without direct sunlight; move it gently once a day and wait for it to become a very dense green (Secchi < 3 cm). Depending on the initial quantity of spirulina, this first phase of growth can take 1-4 weeks. Then proceed with further dilutions of the culture by doubling its volume with additions of new medium each time a density of less than 3cm on the Secchi scale is reached. In good conditions, these successive dilutions should take place every 2-4 days. The culture can then be placed in the sun, provided that care is taken to ensure that its temperature never exceeds 37-38°C; the culture should then be agitated manually at least four times each day. An electric pump can be used to agitate the solution as soon as a volume of about 30 litres is available (use a plastic or galvanized metal basin, or a basket made watertight with a plastic sheet lining). When the volume is less than 50 litres, the pump outlet tube* should be pinched together to reduce the outflow; the object is to obtain a gentle movement of the whole of the liquid. Continue the process of successive dilutions until 50 litres of concentrated culture have been obtained (Secchi < 2.5 cm).

*Or adjust the exit valve fitted on some pumps.

• Note that the pH value (= alkalinity) of the liquid tends to increase when the spirulina develops (check this value with litmus paper). From about 8.5 at the start (fresh medium), the pH can rise to 10 or even 11. This last value indicates that the culture medium should be renewed (or diluted) (see Chapter 6).

4.3 Seeding the tank with the 50 litres of pre-culture

In order to avoid a too strong dilution, one will start by sowing a quarter of the basin, that is to say a surface of 1 m^2 : one will create a temporary mini-basin by delicately posing a small beam (or any other adequate object) across the principal basin, one meter away from its small side. The surface of 1 m^2 thus delimited will be furnished with a plastic sheet of $1.5\text{m} \times 1.5\text{m}$. Be carefull not to damage the bottom plastic sheet from the main bassin! Test the thightness of the mini-basin by pouring 100 liters of water. Waiting a few hours. Then add fertilizers to this water in order to obtain 100 liters of culture medium and add 50 liters of dense spirulina culture; turn on the stirring pump and wait until this new culture becomes dense again (Secchi < 2 cm). At this moment, withdraw temporary separation (as well as the plastic of the mini-basin, alowing spirulina to invade the main bassin), add 250 liters of culture medium. If the depth of the liquid is lower than 10 cm, adjust it with water.Let spirulina grow as above. When the density of spirulina is again close to 2-3 cm Secchi, add * 600 liters of new medium: the basin contains now 1.000 liters of

culture $(4m^2 \text{ and } 25 \text{ cm deep})$.

* One can add water and then the corresponding fertilizers, directly in the spirulina culture. Stir up the culture thoroughly (e.g. using a broom).

4.4 Summary and sequence of installation of the module

Check the availability of the equipment, the fertilizers, and a suitable site for the module and its operation. Contact Antenna Technology (or a spirulina producer) in order to obtain a sample of living spirulina. On receipt of the living spirulina sample, prepare a few liters of culture medium (cf 3.1) and proceed with multiplication of spirulina (cf 4.2). When the culture contains more than 50 liters of dense spirulina, proceed with the construction of the covered basin (cf 2.2). Check tightness of basin, then put in place the mini-basin (cf 4.3). Test the thightness of the mini-basin (100 liters of water, a few hours). Add fertilizers to this water in order to obtain 100 liters of culture medium; then add 50 liters of dense spirulina culture (cf 4.3), turn on the stirring pump and wait until this new culture becomes dense again. Withdraw the elements of the mini-basin, add 250 liters of culture medium and let spirulina grow as above. Once spirulina culture is dense again, add 600 liters of fresh culture medium.

5. Harvesting

When the culture concentration goes below the 2-3 cm mark on the Secchi scale, harvesting should be undertaken, preferably in the morning (especially if the intention is to dry the spirulina, but note that it is simpler and preferable to <u>eat it</u> <u>directly</u>, like a sandwich spread or a cream cheese).

5.1 Filtering

Assemble the harvesting equipment according to the drawings in Fig.2. The electric pump should be placed on a support to avoid sucking in the bottom of the pond (possible presence of deposits and precipitates). Do not forget to attach a piece of double mosquito netting over the entry to the pump (pre-filtering). The pump output should be adjusted so that the filter is not under pressure, but just continuously fed. For this adjustment, pinch the flexible tube with a clamp, or raise the height of the filter in relation to the level of the tank (or adjust the exit valve, according to the pump model). The filter should be closed carefully: fold the end of the filter should be practically colourless. After 15-20 minutes of filtering, stop the pump and let the contents of the filter drain and settle for 2-3 minutes; if the filter is not at least three-quarters full, run the pump for another 15-20 minutes.

• Printing polyester (or possibly nylon) fabric with a mesh between 30 and 60 microns is ideal for making the filter. Ensure a double hem and close stitching. Other fabrics with very tight mesh (especially silk) can also be used, though they are much more fragile. After use, the filter should be carefully washed, as quickly as possible, and then dried away from direct sunlight.

5.2 Pressing

When the drained filter is at least three-quarters full, unscrew the clamp and remove the filter. Leave the closure clip in position and squeeze the spirulina paste by hand (keeping the other end of the filter securely closed) to extract as much liquid as possible. After being pressed in this way, the spirulina should have the consistency of cream cheese). Take care here: from this point the spirulina should be treated like milk or meat. Follow the rules of hygiene and storage time limits scrupulously! If the spirulina is not going to be dried, it must be consumed in the hour after harvesting (a maximum of two days in the refrigerator; it can also be frozen).

- N.B.: to be effective, pressing should always follow immediately after filtering!
- If the spirulina is not to be dried, the pressed paste should be weighed and the resulting figure divided by four to obtain the weight of dry spirulina. If need be, the length of the "sausage" of paste can be measured in the filter itself (after pressing); in a filter with a diameter of 4.5 cm, each centimetre contains the equivalent of about 4 g of dry spirulina.

5.3 Extrusion and drying (drawings in Fig. 3)

Drying is a good way of conserving spirulina over a long period (at least a year if it is correctly conditioned). This drying should be done as fast as possible (less than four hours), but if a heated dryer is used a temperature of 50°C should not be exceeded, in order not to destroy the vitamins and essential fatty acids. To help it to dry, the spirulina paste should be extruded in the form of spaghetti-like strands. For small quantities, this can be done by squeezing the paste through a large syringe. For more than 200 g of spirulina paste, a special extruder should be used (see illustration in Fig. 3). As they are squeezed out, these "spaghetti" are laid out on mesh trays, preferably in a single layer. When they are full, the trays are stacked in a ventilated cabinet covered with mesh to so that they can dry protected from direct light and insects. The "spaghetti" are easy to remove from the trays.

• After pressing, the spirulina paste contains about 25% of dry matter. Its

weight therefore shrinks by about three-quarters during the drying process.

5.4 Conditioning

Once they are really dry, the spirulina "spaghetti" are gathered on a clean cloth and roughly broken by hand, through the cloth. The fragments thus obtained should be conserved in a dry place, away from air and light (e.g. in tins, opaque jars, etc.). In such conditions the nutritional qualities of the spirulina are preserved for at least a year.

- N. B. The spirulina should never be touched by people's hands, even when dry; wear gloves or use clean kitchen utensils.
- One dry, the spirulina should never be rehydrated (except for immediate consumption).

6. Maintaining the culture

6.1 Adding replacement fertilizers

After harvesting, it is essential to replace the elements absorbed by the spirulina. This is done by adding a mixture of agricultural fertilizers **in proportion to the amount of spirulina harvested**.

Composition of the fertilizer mixture:

Potassium nitrate	1.4 kg
Monoammonium phosphate	50 g
Potassium sulfate	30 g
Magnesium sulfate	20 g
(If the water is calcium-deficient,	10 g of lime)

Mix these substances very thoroughly (the best method is to grind them together in a mortar) and keep the mixture in a dry place. After each harvest, weigh the spirulina harvested (either dry weight or a quarter of the weight of the spirulina paste after pressing). For each gram of spirulina harvested, add 1.5 g (about one level coffee spoon) of mixture to the tank. If just the length of the harvested "sausage" after pressing has been measured (see Chapter 5.2), 6g of mixture (about four level coffee spoons) should be added for each centimetre of "sausage".

Dissolve the mixture in 2-3 litres of water, add a pinch (about 0.1 g) of iron sulfate, dissolve it and then pour into the tank. Adding half a glass of green tea makes the iron more soluble (the purple colour is normal).

6.2 Additions of water and control of culture temperature

The tank level should remain constant. Measure its depth regularly and add the necessary amount of water as appropriate. In a very hot climate it may be necessary to leave the tank open, to allow some evaporation and hence cooling of the liquid (reminder: 35-37°C is optimal; never let the culture temperature climb above 40°C!). In such cases, protect the openings with mosquito netting.

The tank can also be shaded during the hottest part of the day, e.g. by placing cloth or matting over the structure. Shading of 50% has very little effect on productivity, but beyond this level the effect increases rapidly. Shading and openings should be adjusted periodically according to the maximum temperature of the culture at the hottest time of the day.

6.3 Purging

To prevent the gradual deterioration of the culture medium, and to compensate for the carbon absorbed by the spirulina, a small proportion of the culture liquid should be renewed regularly. In a period of good production, and depending on the pH measured, it may be necessary to eliminate several dozen litres of medium each day, to be replaced by the same volume of new medium (ingredients Chapter 3.1). The medium to be thrown away should be taken from the liquid coming out of the filter during harvesting, to avoid losing spirulina.

The pH of the culture should be checked daily with a strip of litmus paper. In stable conditions, this value should be between 10 and 10.5. Higher values indicate that more culture medium must be removed and replaced. On the other hand, pH values below 10.5 mean that purging can be temporarily halted (until a return to pH >10.5).

- Although very practical, this method wastes a fair quantity of fertilizer that is eliminated with the purges; other methods of adding carbon are described in the book by J.-P. Jourdan.
- Used medium should not be applied directly to the land (the salinity and pH are too high). It can either be diluted (in at least 5 times its volume of water) and applied, or be left to evaporate in settling ponds. Used medium can be recycled by stocking it in a deep pond (about 1 metre) in the bottom of which air in continually injected with a small aquarium compressor. The medium treated in his way can be reincorporated in the culture after 15-20 days.
- If the productivity of the system is not forced (by harvesting less than 6 g/sq.m. per day), and if the culture density is kept at a high level (Secchi < 2.5), it is practically unnecessary to purge. In fact in that case the carbon in the air (carbon dioxide) is enough for the spirulina's carbon requirements.

7. Summary of operations on a harvesting day (* = when the spirulina is dried)

- Measurement of the concentration (Secchi scale), pH and depth of the tank liquid;
- Filtering of about 200-300 litres (if Secchi reading is < 3);
- Removal of part of the filtered liquid (= purge, if pH >10.5);
- Pressing the spirulina, weighing and then *extrusion of the paste on the drying trays, *start of the drying;
- Preparation and addition of fresh medium (amount equivalent to the amount purged);
- Weighing (proportionate to the harvest) and addition of compensatory salts and of water needed to maintain the level;
- Cleaning the equipment;
- Checking the temperature at the beginning of the afternoon (aeration or shading if necessary);
- *End of drying: recovery and rough crushing of the "spaghetti", weighing and conditioning.

8. Quality control

8.1 Visual qualities

<u>Fresh</u> spirulina paste should be a very dark green, almost without smell and taste. A blueish-red tint indicates that it has been pressed too violently, or that it has been kept beyond its storage time limit (in the latter case, a smell of rotten eggs).

<u>Dry</u> spirulina of good quality should be very dark green, with a characteristic faint smell (alga or fungus) and weak taste. A blueish turquoise tinge indicates strong exposure to light (without danger, but greatly reduced nutritional properties).

8.2 Mini-tests for quality

When a pinch of powdered spirulina is left for a few minutes to several hours in a glass of water, a deep blue colour develops. This is a major protein of spirulina, phycocyanin. The absence of such colouring indicates poor drying (too hot), or, in a commercial sample, a counterfeit. Immediate colouring can indicate over-violent pressing or product degradation before or during drying (too slow).

9. Temporary stoppages: maintaining a culture without production

To "rest" a culture temporarily, its concentration should if necessary be returned to a moderate density (about 3 cm on the Secchi scale) by harvesting. The

pH should be below 10.5 (purge if necessary). Finally, the culture tank should be heavily shaded (by placing a white cloth, matting, palm leaves, etc., on the plastic cover). But not complete darkness!

In very hot periods, leave the openings protected by mosquito netting. Water should then be added from time to time (check the level).

10. Notes

- Do not forget always to keep a small quantity of culture (several litres) for safety's sake, in a protected place, without too much light and with occasional agitation (or better, agitation by bubbles, with a small aquarium compressor).
- Regularly remove any foreign bodies (insects, plant debris, etc.) from the tank with a sieve; take advantage of this maintenance to stir the corners of the tank (the parts least affected by the pump) manually.
- A culture that met with a temperature accident (overheating) can be recovered by placing it "at rest" (see Chapter 9), after a substantial purge. If the overheated culture changes colour (turns brown), possibly forming foam and giving off a smell of bad eggs, filter it as much as possible, wash the harvested spirulina with new medium in the filter itself, and re-suspend this spirulina immediately in further new medium (enough to obtain a Secchi reading of 4-5 cm). Leave "at rest" for several days. Begin without delay to increase the volume of your reserve crop, so that you can start again as soon as possible if your main tank turns out to be beyond recovery. If that happens, you will need to empty and clean the tank, then restart the seeding procedure described in Chapter 4.

J. Falquet Antenna Technology, June 1999

To find out more:

J.-P. Jourdan (1999). *Cultivez votre spiruline*. Geneva, Antenna Technology, pp. 126.

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Ingredient	1	2	3	5	10	25	100	500	
Sodium bicarbonate	16 g	32 g	48 g	80 g	160 g	400 g	1.6 kg	8 kg	
Potassium nitrate ¹	2 g	4 g	6 g	10 g	20 g	50 g	200 g	1 kg	
Salt ²	1g	2 g	3 g	5 g	10 g	25 g	100 g	0.5 kg	
Ammonium phosphate ³	0.1 g	0.2 g	0.3 g	0.5 g	1 g	2.5 g	10 g	50 g	
Strong green tea	1 ml	2 ml	3 ml	5 ml	10 ml	25 ml	1 dl	0.5 1	
Iron sulfate	10 mg	20 mg	30 mg	50 mg	0.1 g	0.25 g	1 g	5 g	
(Magnesium sulfate)	0.1 g	0.2 g	0.3 g	0.5 g	1 g	2.5 g	10 g	50 g	
(Potassium sulfate)	0.5 g	1 g	1.5 g	2.5 g	5 g	12 g	50 g	250 g	
(Calcium chloride, or lime, or plaster) ⁴	0.1 g	0.2 g	0.3 g	0.5 g	1 g	2.5 g	10 g	50 g	

Table 1. Composition of liquid culture medium for spirulina

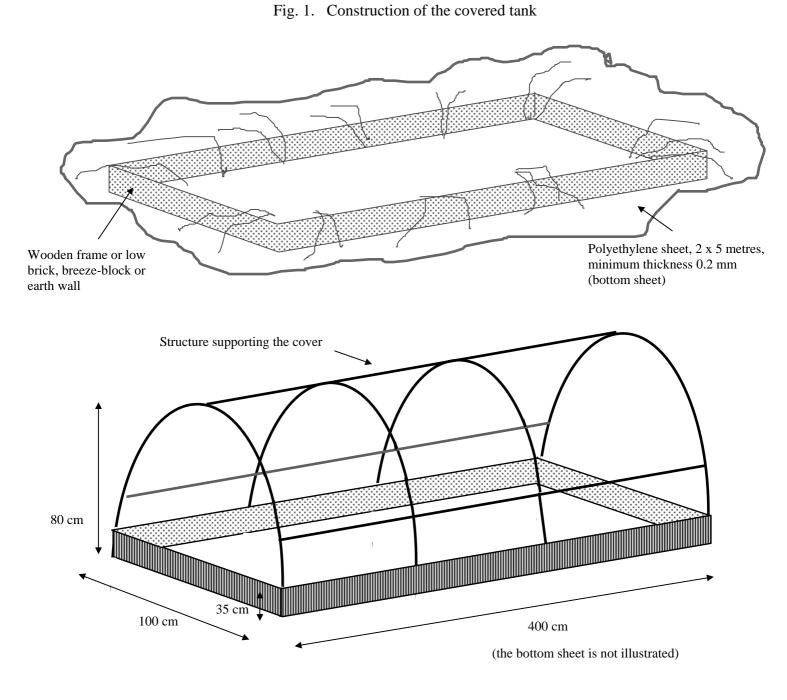
Volume of medium (litres)

¹ Or sodium nitrate (but in this case the potassium sulfate is indispensable).

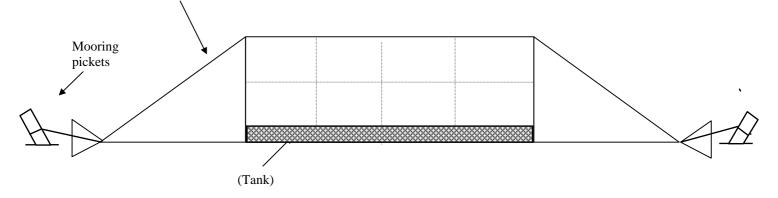
²Preferably coarse sea salt; failing that, modulechen salt.

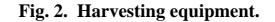
³Monoammonium phosphate (NH4)H2PO4, or diammonium sulphate (NH4)2HPO4, or monopotassium phosphate KH2PO4.

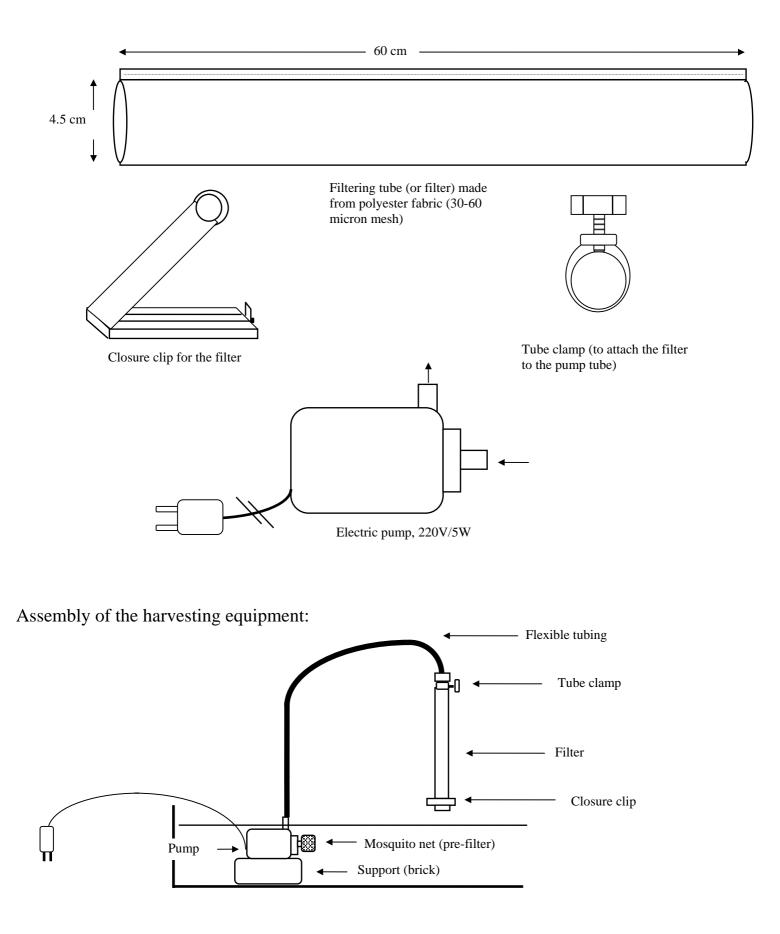
⁴Essential for very soft water (regions very low in calcium, granitic or silicious soils)

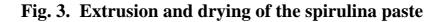


Cover (polyethylene sheet, 3 x 6 metres, transparent, anti-UV)









Extruder (modified mastic gun) for the production of spirulina "spaghetti"

