

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. You can download another version as a separate booklet (prepared by Robert Henrikson) by [clicking here](#).

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) :

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Physiology, 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.

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 m² but 5, 20 or 100 m² 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 U.V. resistant, food grade plastic film of 0.5 mm thickness or more, with sides supported by bricks or a wooden structure or

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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 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 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 surface of 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.

CULTURE MEDIUM

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

<u>Anions</u>	Carbonate	2800 mg/l
	Bicarbonate	720
	Nitrate	614
	Phosphate	80
	Sulfate	350
	Chloride	3030
<u>Cations</u>	Sodium	4380
	Potassium	642
	Magnesium	10
	Calcium	10
	Iron	0.8
Urea		< 50
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 soft waters :

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Sodium carbonate (soda ash)	5	g/l
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, MgSO ₄ , 7 H ₂ O	0.2	
Lime	0.02	
Ferrous sulfate, crystallized, FeSO ₄ , 7 H ₂ O	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 analysed 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, and nitrate can be 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 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 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.

Micronutrients traces contained in the water and in the chemicals are sufficient to support the initial growth.

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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. 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 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 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 (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 :

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- 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 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 μ) to remove any foreign matter such as insects, larvae, leaves and lumps of polysaccharide or mud.

When the spirulina floats, which is the normal case without agitation, 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 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

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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, either pure CO₂ gas (from fermentation or from a cylinder). 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 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 ; 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 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, 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 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 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.

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 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

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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 nutrients 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 60 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 odour however indicates an overdose.

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

Urea	300 g
Monoammonium phosphate	50 g
Potassium sulfate	30 g
(or 40 g if no potassium nitrate is used in the culture)	
Magnesium sulfate*	30 g
Lime	10 g
Iron sulfate*	2.5 g
Micronutrients solution**	5 ml

* as the usual commercial product, cristallised with 7 molecules of water (crude iron sulfate sold for treating lawns is unsuitable)

** supplied by Antenna Technology, 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).

Concentrated pure phosphoric acid may replace the phosphate.

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°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).

Accumulation of mud 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 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 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.

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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 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 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 pass 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 sterilised. The same procedure should be applicable 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 : 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. 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)

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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 aluminised heat sealed plastic bags, dry spirulina is considered good for consumption up to five years. 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 large scale plants.

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 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 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 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 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.

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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, aluminised 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.

.....
At his spirulina greenhouse at Le Castanet in the South of France in 2002, Jean-Paul Jourdan demonstrated how he grows, harvests and dries spirulina, producing a tasty food product. In his manual "Cultivez Votre Spiruline", he describes how to cultivate spirulina on a family scale.

ANNEX

A1) MEASURING THE CONCENTRATION IN SPIRULINA WITH THE SECCHI DISK

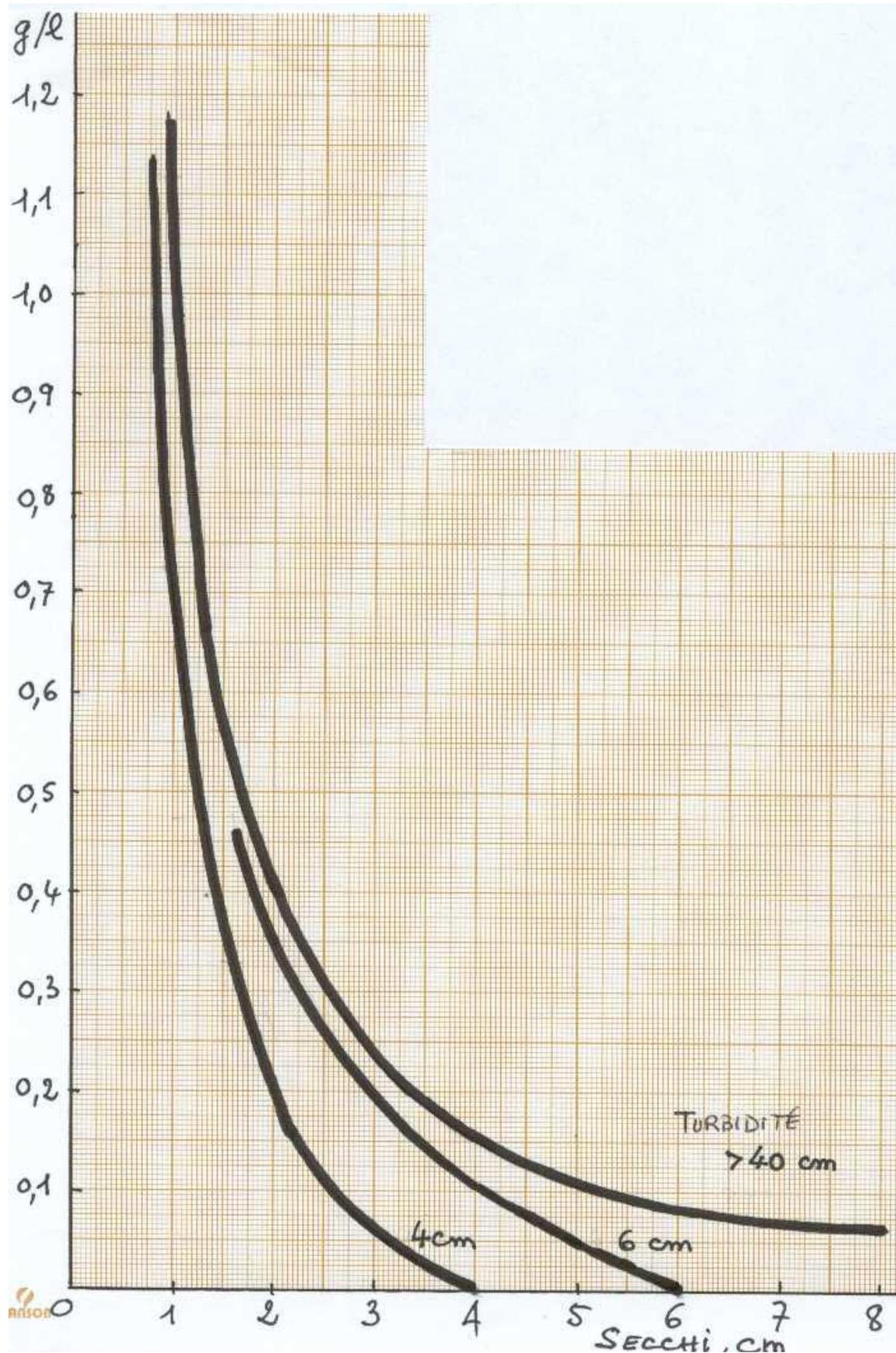
The "Secchi disk" is a self-made instrument : a piece of white plastic fixed at the tip of a graduated rod. Dip it vertically into the spirulina culture until you just cannot see the white piece ; the reading in centimeters gives an approximate value of the concentration. If the medium itself (the filtrate) 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.

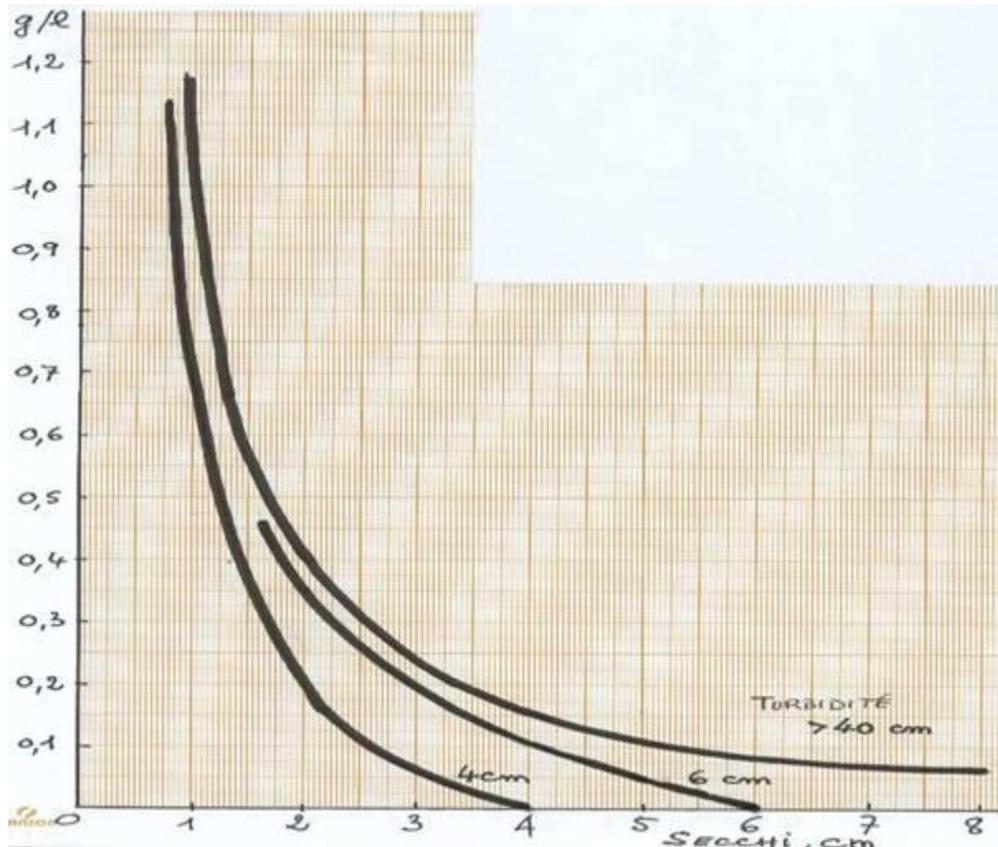
The following graphs were established by the author for the Lonar (coiled) and for the Paracas (loosely coiled, almost straight) strains. They can be used as approximations.

PARACAS



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LONAR



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

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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, 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, 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.

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-

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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 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/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 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.

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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/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 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.

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 (English version)

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, PERSO, IMPRIM 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).

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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 = $k \times f(\text{light}) \times f(\text{temperature}) \times f(\text{pH}) \times f(\text{stirring}) \times f(\text{salinity})$. 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 the 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 use.

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 :

$$\textit{photosynthesis} = k \times f(\textit{light}) \times f(\textit{temperature}) \times f(\textit{pH}) \times f(\textit{salinity}) \times f(\textit{stirring})$$

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 synonymous 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.

Ambiant air temperature and solar radiation are calculated hourly from meteorological data, latitude and altitude of the site, with formulas used commonly 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 harvest 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

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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 gmol/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 :

	<u>Medium,</u>	<u>Production,</u>
	<u>g/liter*</u>	<u>g/kg**</u>
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.

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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](#) show 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 comprehending 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, k_a , was assumed to be 18 gmole/hour/m²/atm]

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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 @ 10°C = 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

WEATHER 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

Table 2 Example of meteorological data

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RESULTS

Nutriments, 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 = 3.8

Final concentration in spirulina, g/l = 0.3

Final salinity of medium, g/l = 17.4

Final basicity of medium, moles/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

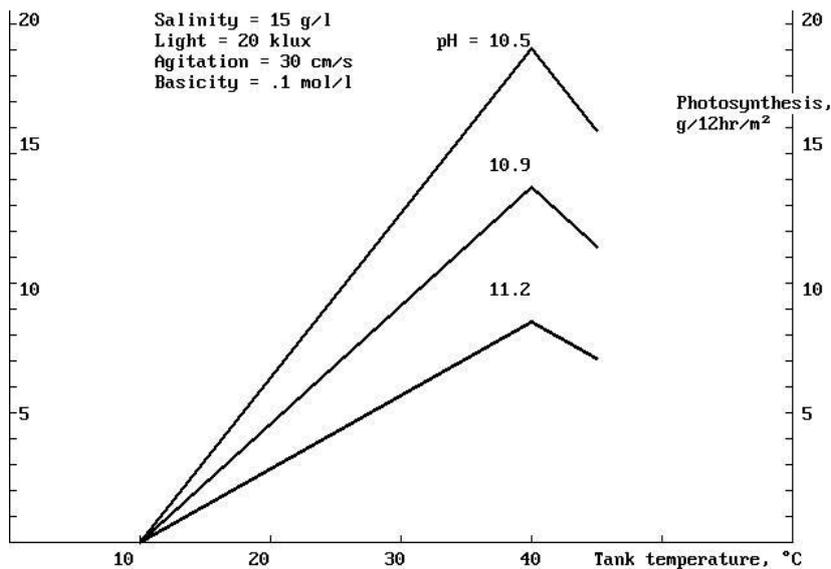
Maximum level in tank, cm = 10.0

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

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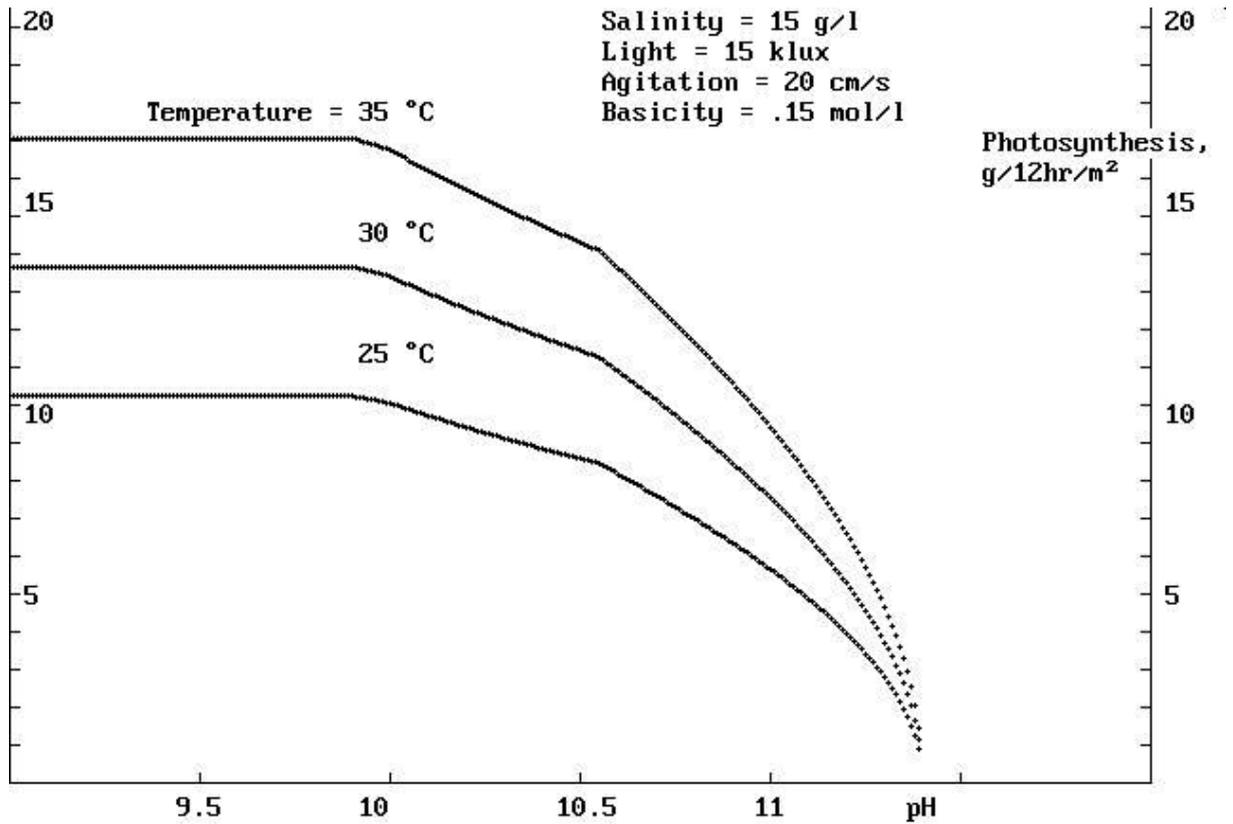


Fig. 2 Photosynthesis vs. pH

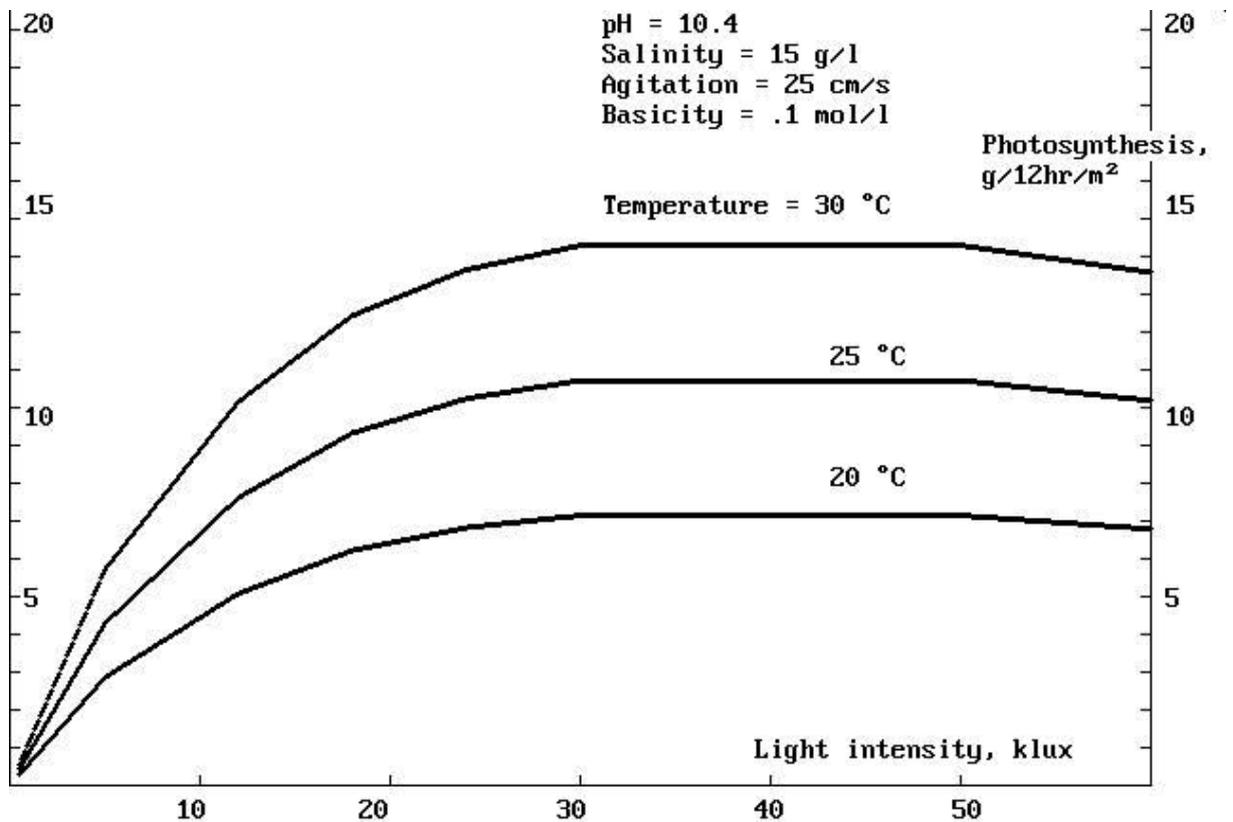


Fig.3 Photosynthesis vs. Light intensity

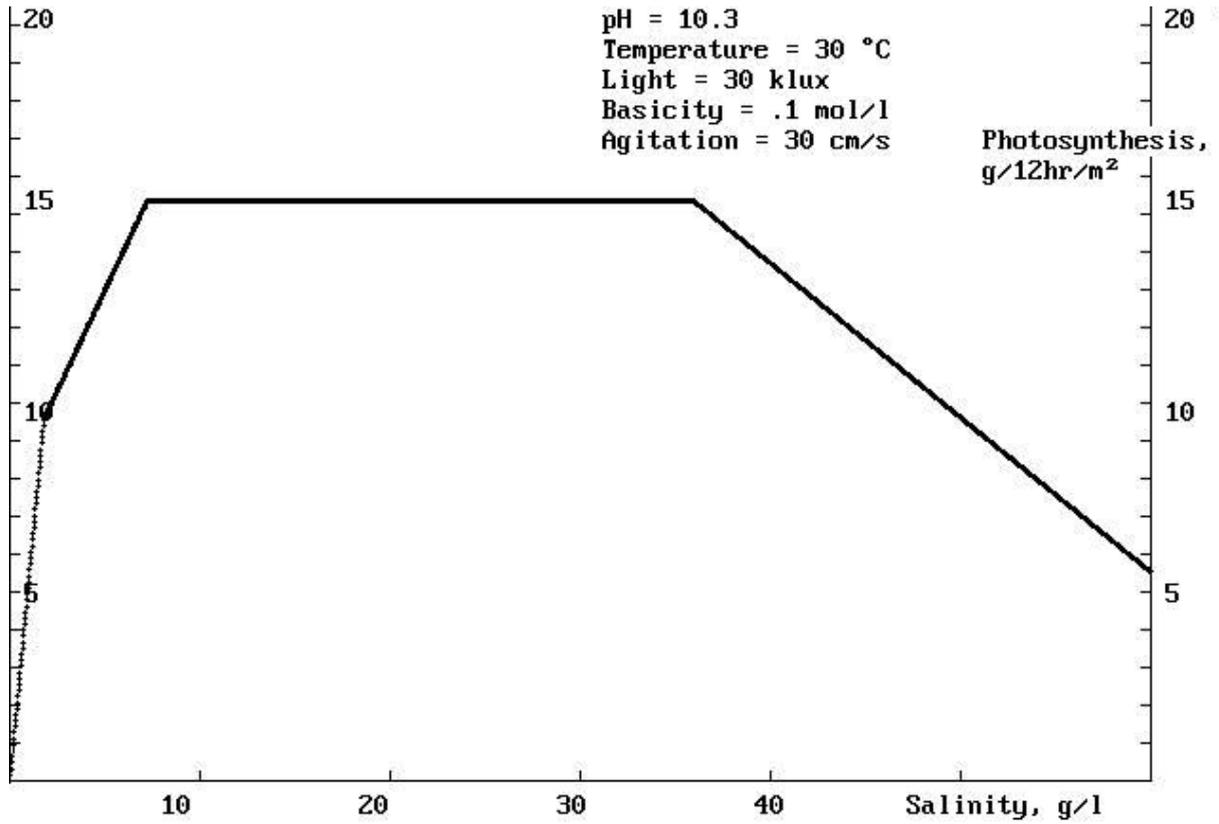


Fig.4 Photosynthesis vs. salinity

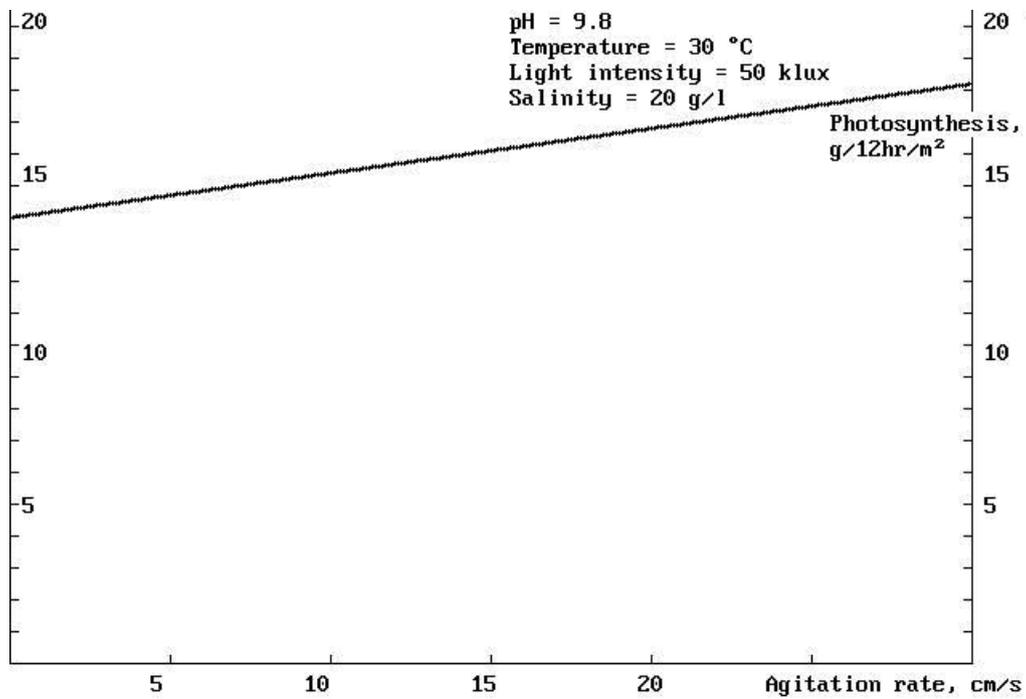


Fig. 5 Photosynthesis vs. stirring rate

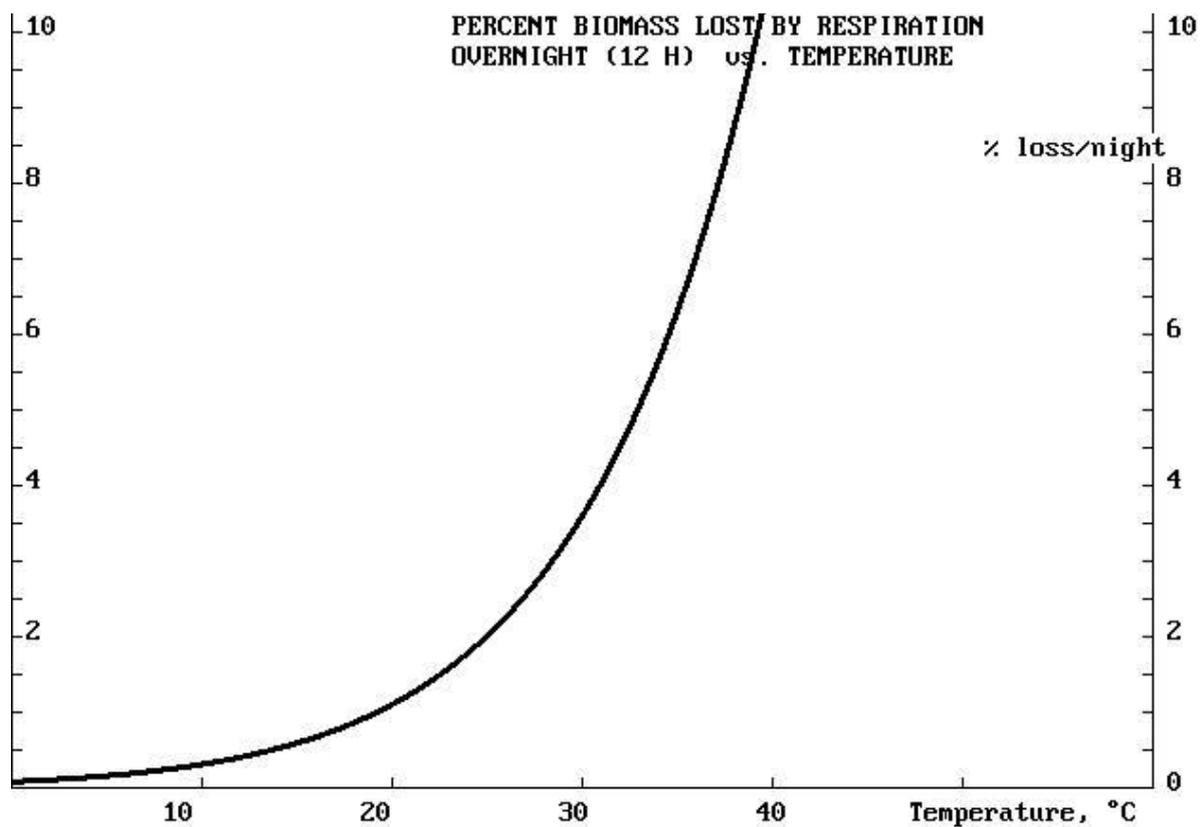


Fig. 6 Respiration rate vs. temperature

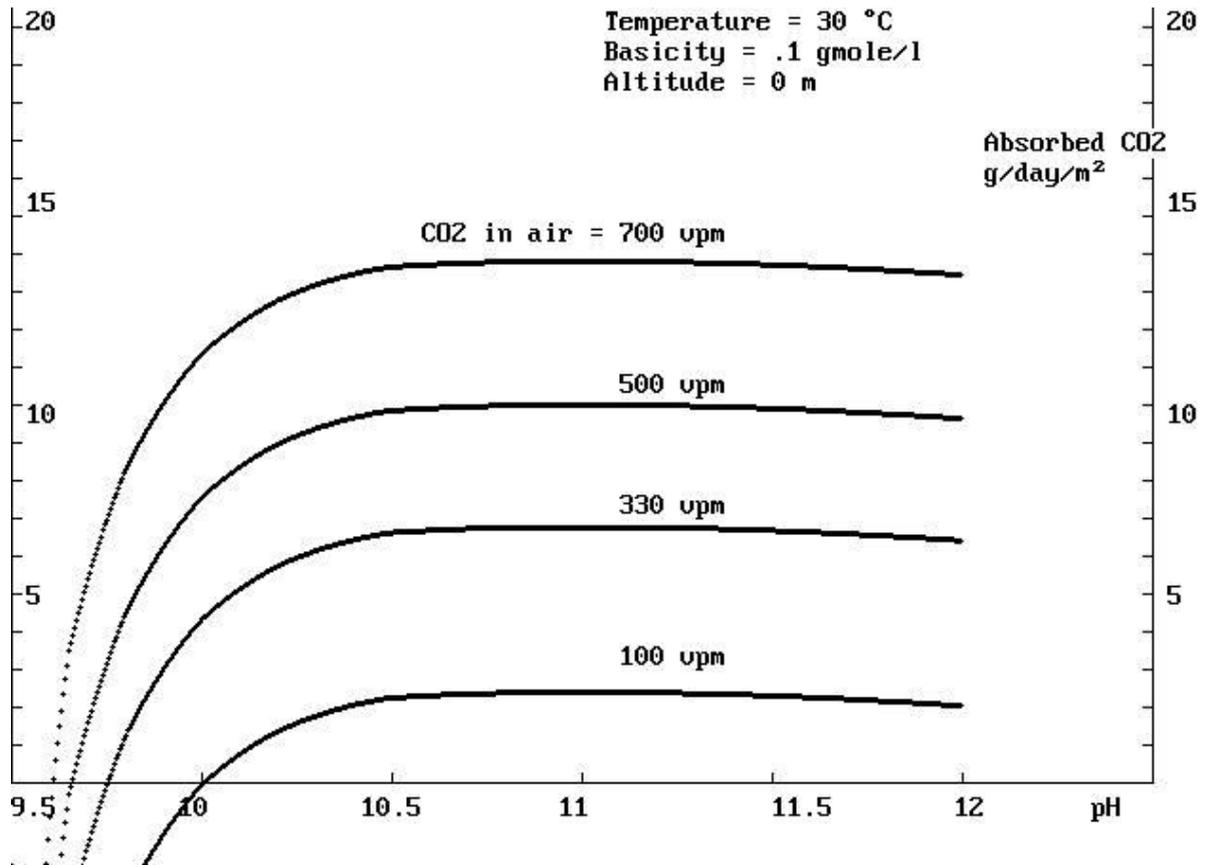


Fig. 7 CO2 absorption vs. pH

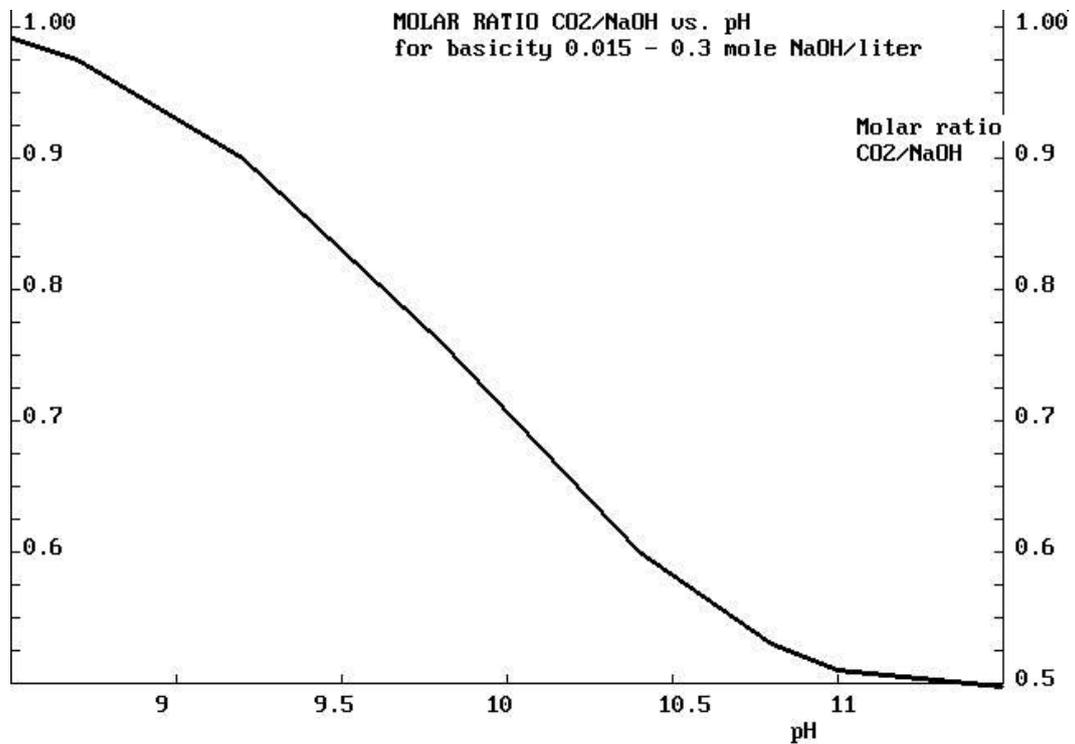


Fig. 8 CO₂/base molar ratio vs. pH

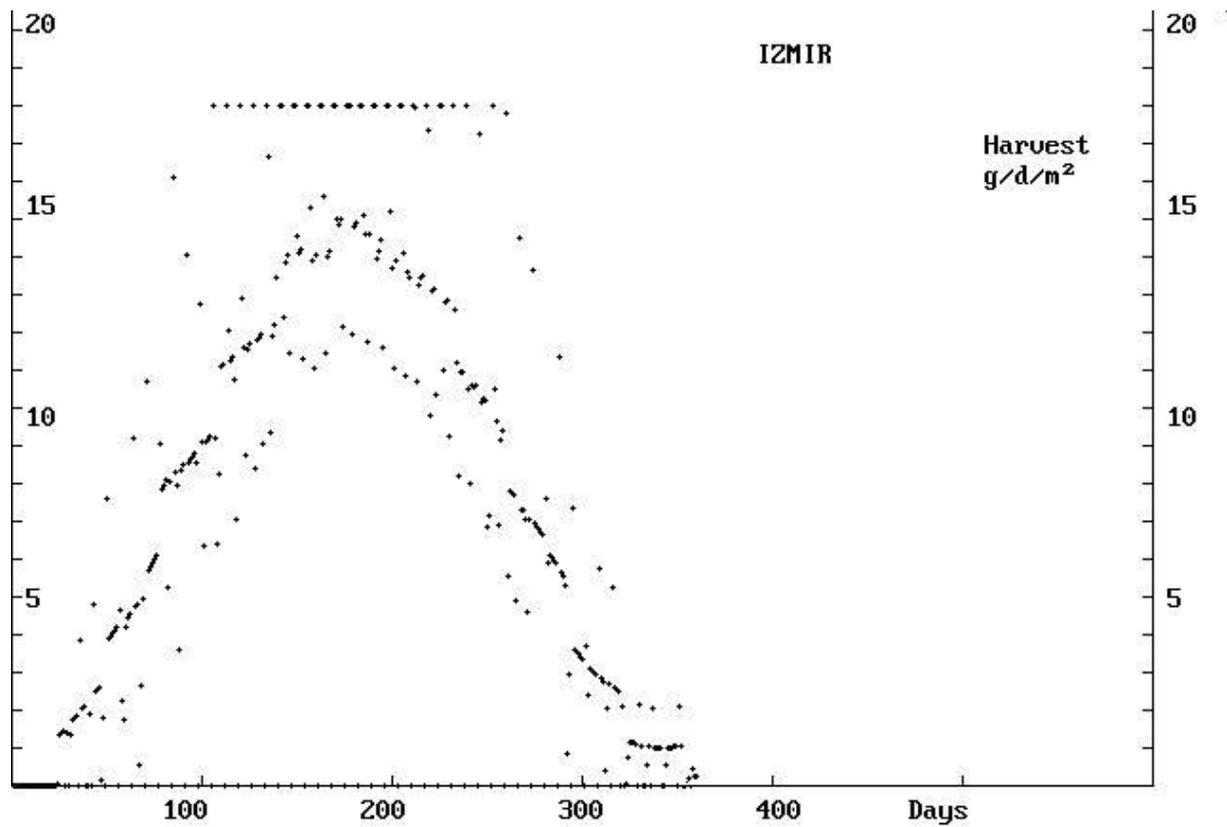


Fig. 9 Daily production

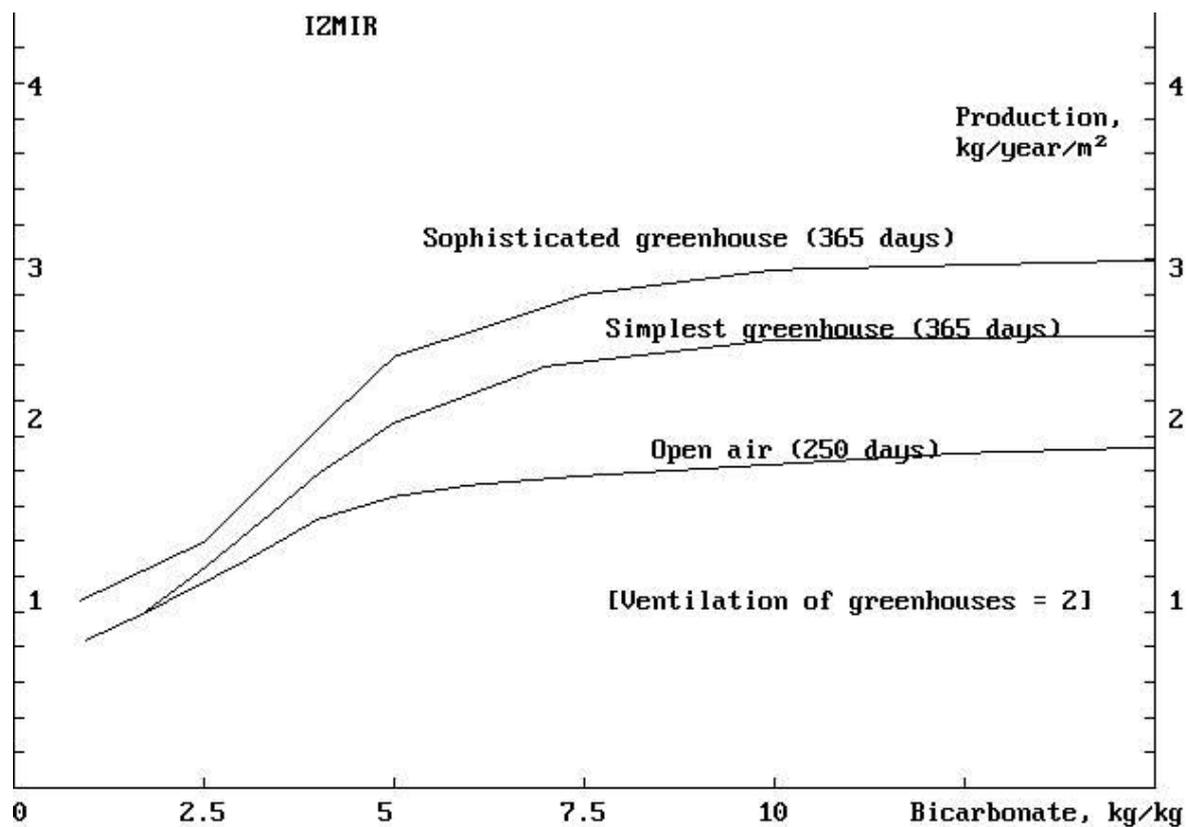


Fig. 10 Production vs. bicarbonate consumption

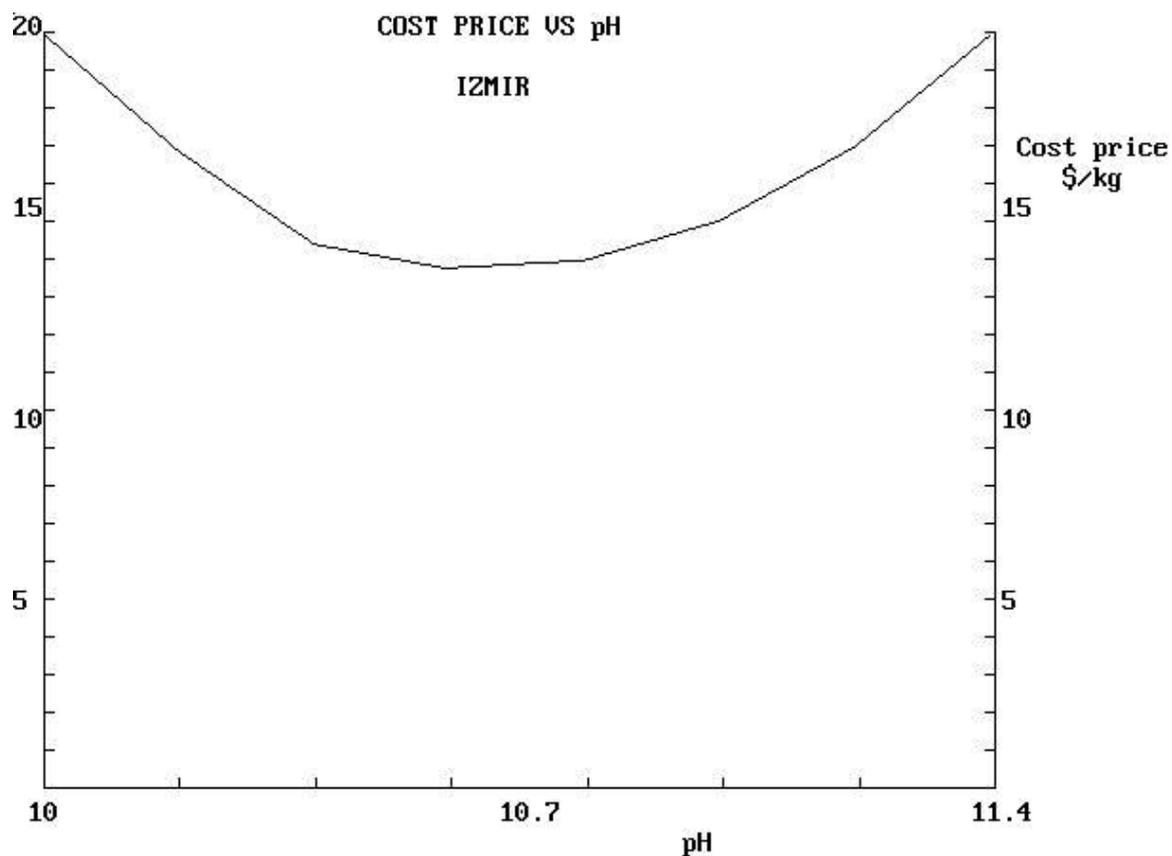


Fig. 11 Cost price vs. pH with bicarbonate (@ 0.8 \$/kg)

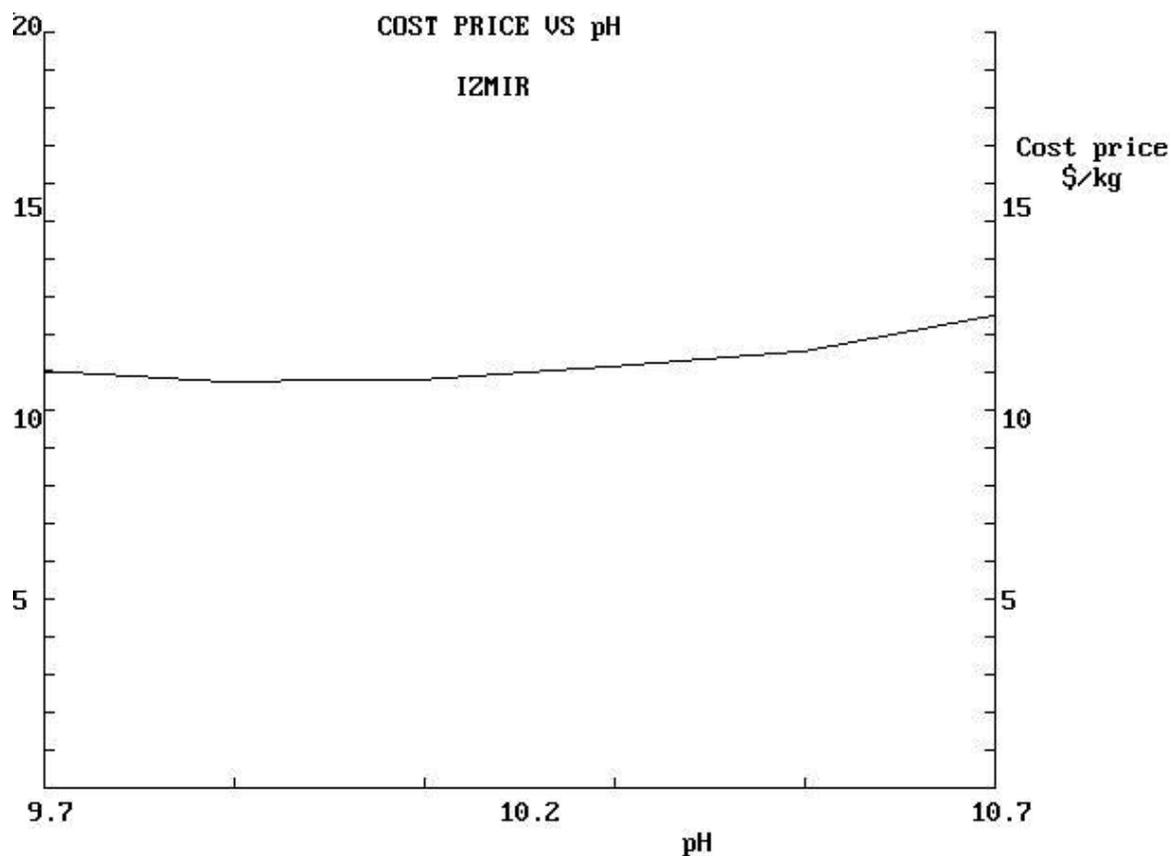


Fig.12 Cost price vs. pH with CO₂ (@ 2 \$/kg)

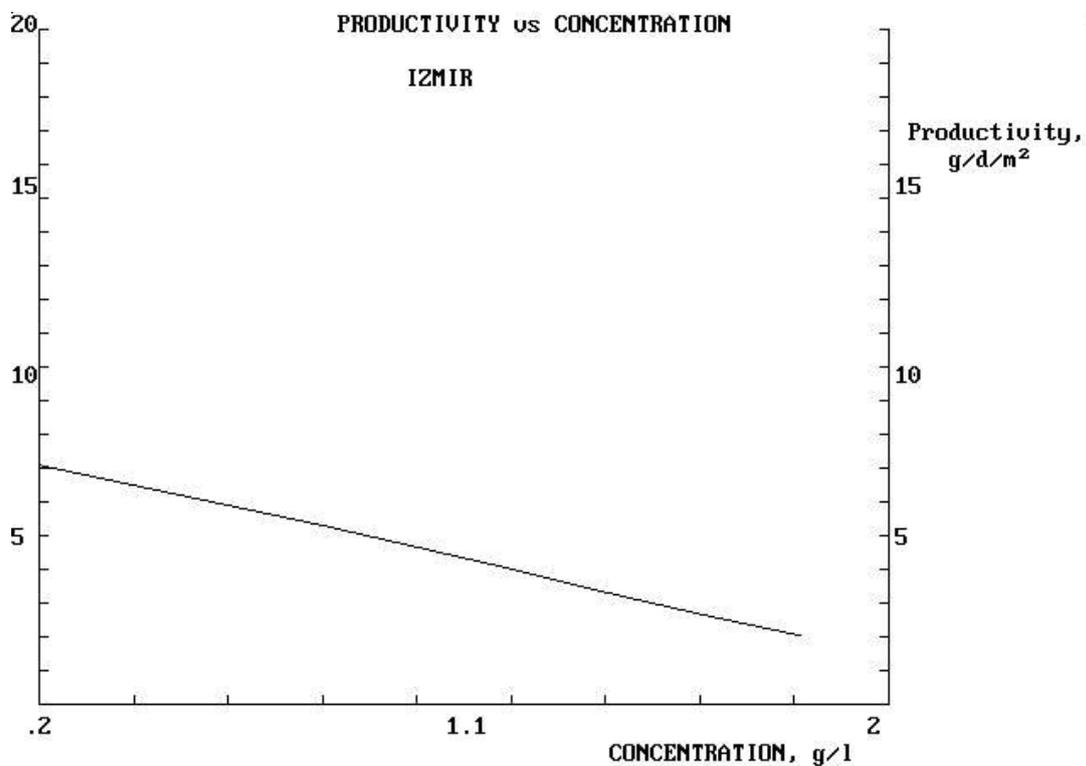


Fig. 13 Productivity vs. biomass concentration

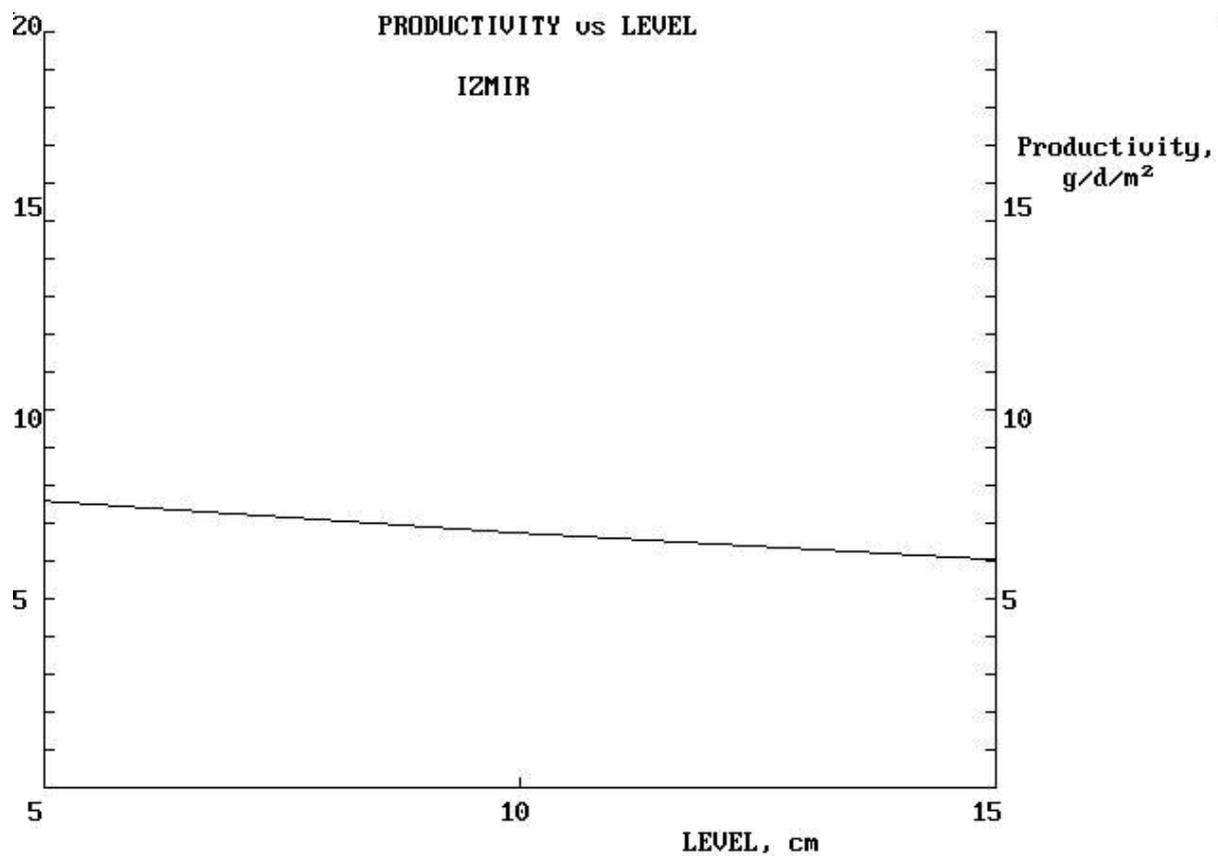


Fig. 14 Productivity vs. tank level

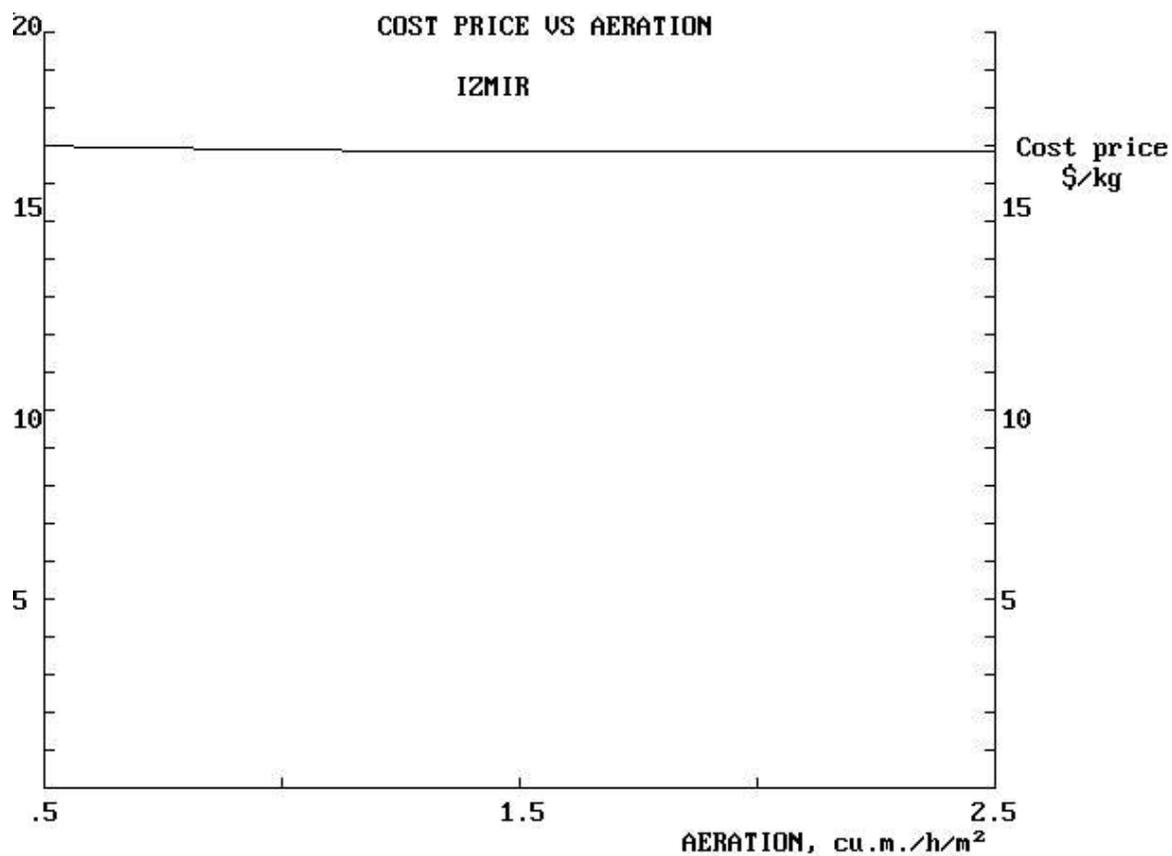


Fig. 15 Cost price vs. ventilation

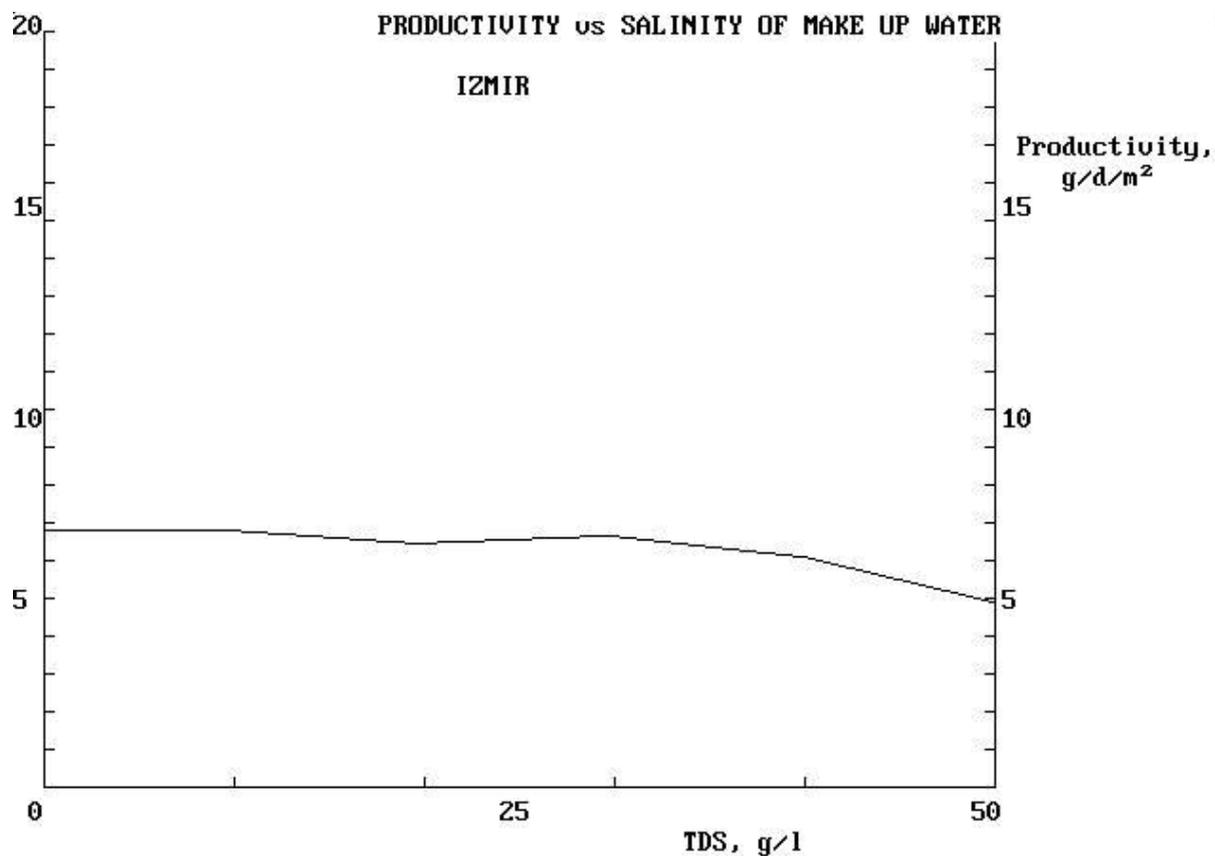


Fig. 16 Productivity vs. salinity of make-up water (maximum salinity = 40)

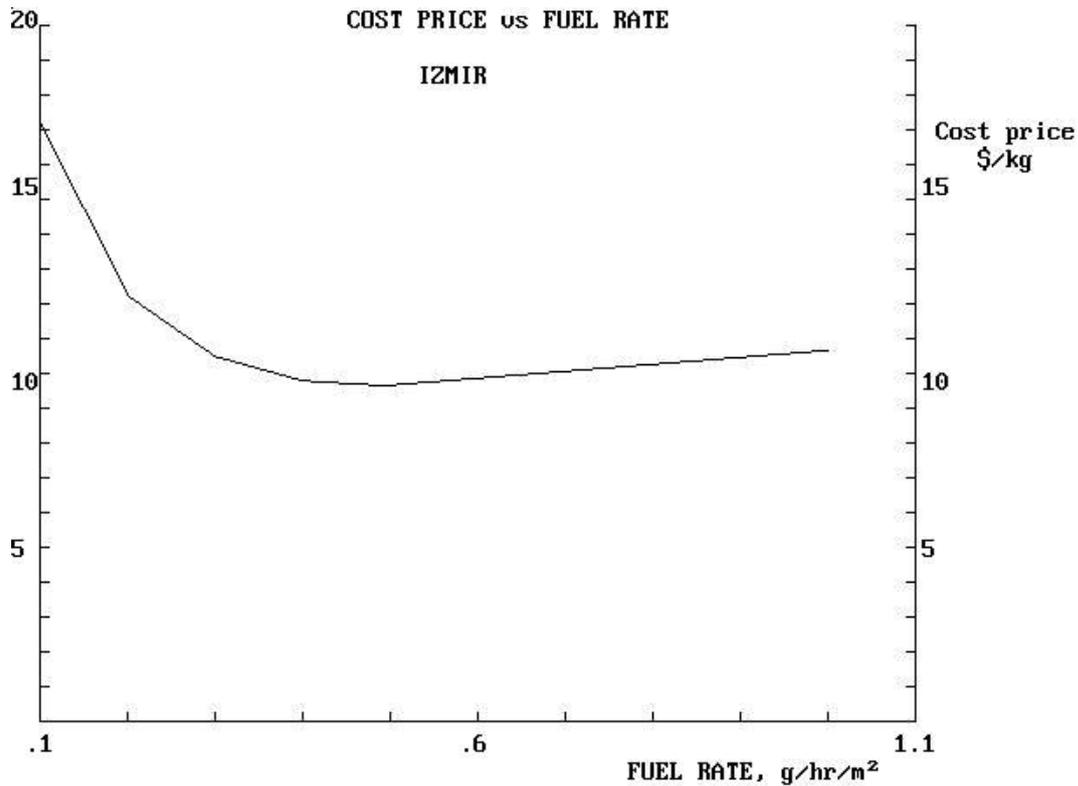


Fig. 17 Cost price vs. fuel rate
(propane @ 1 \$/kg as sole carbon source and ventilation rate = 0.1 m/h)

LEGENDS OF TABLES AND FIGURES

Table 1 Example of data

Table 2 Example of weather data

Table 3 Example of results

Fig.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 rate vs. temperature

Fig. 7 CO₂ absorption vs. pH

Fig. 8 CO₂/base vs. pH

Fig. 9 Daily production

Fig. 10 Productivity vs. bicarbonate consumption

Fig. 11 Cost price vs. pH with bicarbonate (@ 0.8 \$/kg)

Fig. 12 Cost price vs. pH with CO₂ (@ 4 \$/kg)

Fig. 13 Productivity vs. biomass concentration

Fig. 14 Productivity vs. culture depth or level

Fig. 15 Productivity vs. ventilation rate

Fig. 16 Cost price vs. salinity (total dissolved salts) of make-up water

Fig. 17 Cost price vs. fuel rate with propane @ 1 \$/kg as sole carbon source and ventilation rate = 0.1 m/hr