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MELiSSA – Adaptation for Space

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REVIEW AND TRADE-OFF OF TECHNOLOGIES

Standardisation of *Arthrospira* medium

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1. Introduction

The general concept of MELISSA includes the use of the biomass generated in the two photosynthetic reactors, *Arthrospira* in compartment IV and *Rhodospirllum* in compartment II, as food supply. Previous studies have shown that both microorganisms can be used as supplement in the food diet of rats (Borowitzka and Borowitzka 1988). On the other hand, *Arthrospira* has been used as an important source of proteins in children suffering from malnutrition and different types of food and pills based on *Arthrospira* are commercialized widely. Also has been used as human food for centuries, and forms part of the diet of tribes of Lake Chad and was used as food by aztecs in Mexico (Becker 1991).
In order to be used as food supply, the biomass obtained in the photosynthetic bioreactors has to be first harvested. The process of cell harvesting has already been described in TN 37.3 and will be further developed in the actual Melissa Space Adaptation contract.

2. Objectives

In the MELISSA concept, liquid-solid separation is required in compartments I, II, III and IV. For compartment I, this issue is addressed in the project 'Engineering of the Waste Compartment'. In the remaining three compartments, different types of microbial biomass have to be separated from the liquid phase. As already mentioned in TN 37.3, it is not obvious that one type of separation system can cover the problem on liquid-solid separation for all compartments.
The *Arthrospira* compartment is currently the most developed compartment and a adequate harvesting unit is necessary for integration in the MELISSA Pilot Plant at Barcelona.
The general purpose of the present project is schematized in **Figure 1**. The tasks grouped under work package (WP) 4200 are aimed to the delivery of TN 7. The attributed tasks to this TN are highlighted in **Figure 1**. The TN was subdivided into TN 72.1, TN 72.2, TN 72.3.1, TN 72.3.2, TN 72.3.3 and TN 72.4.

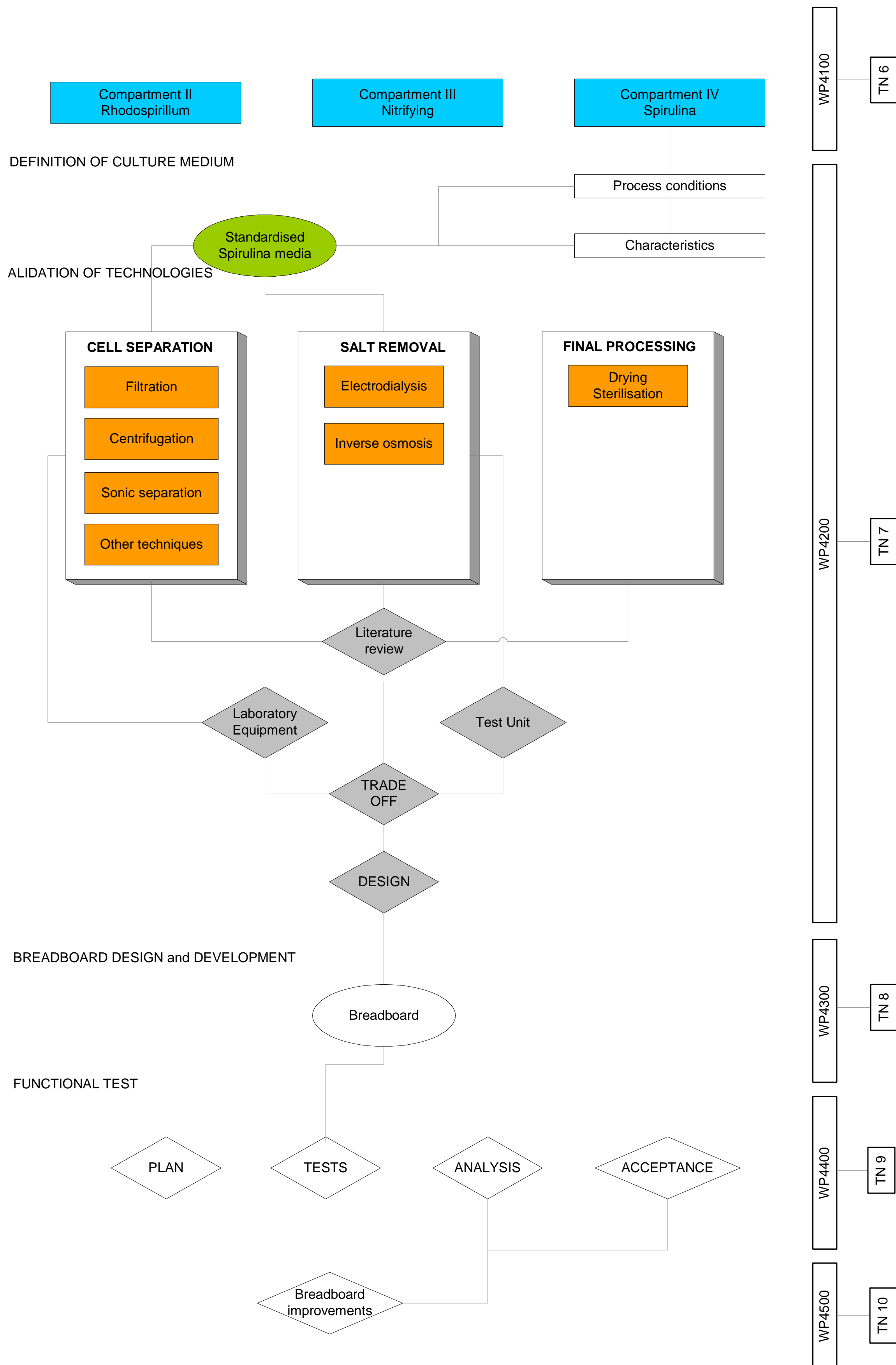

Figure 1. Requirements and performances for LSSS

Separation techniques on *Arthrospira* were already studied by different MELISSA partners under different contracts (TN 43.21, Morist et al., 1999). Centrifugation and ultrafiltration were already used, but the obtained results were not always reproducible (TN 37.30, Vernerey et al., 1998). The morphology and behavior of *Arthrospira platensis* seems not to be constant and is influenced by several culture conditions.

The aim of this work was to make a review of the growth conditions for *Arthrospira* and allocate the different factors that can influence its morphology. Moreover, based on the review, the most adequate culture medium will be selected for the culture of *Arthrospira* needed for testing the different concentration techniques.

Limiting conditions may be encountered in the closed MELISSA-loop. The flow originated from the nitrifying compartment and entering the *Arthrospira* compartment can be limiting in terms of salts concentrations. The decrease or increase in the concentration of one of the salts may induce changes in the quality or quantity of *Arthrospira* or may completely inhibit its growth.

3. Literature review: Arthrospira culture conditions

Blue-green algae are a diverse group of microscopic plants rarely used for human food. The exception is *Arthrospira*, which has become widely available as a food ingredient within the last twenty years. It is a nutritional food with rather unique phytonutrients and characteristics. *Arthrospira* can be used in a variety of healthy food applications. Wild *Arthrospira platensis* grows in sunny warm-water volcanic soda lakes throughout the world. This alga thrives in waters containing high amounts of sodium carbonate with pH values above ten. Where wild *Arthrospira* is found there are often flocks of flamingos and other animals feeding on it. *Arthrospira* has a distinctive spiral structure with filaments of cells being about 10 microns in diameter and up to 1,000 microns in length.

3.1 OUTDOOR ARTHROSPIRA CULTIVATION

Light level is one of the important variables for optimizing plant growth in general and *Arthrospira* in particular, others being light quality, water, carbon dioxide, nutrients and environmental factors.

3.1.1 Factors influencing Arthrospira growth

3.1.1.1 Temperature and light intensity

Temperature is the most important climatic factor influencing the rate of growth of *Arthrospira*. Below 20°C, growth is practically zero, but *Arthrospira* does not die. The optimum temperature for growth is 35°C, but above 38°C *Arthrospira* is in danger (Vonshak, 1997).

Growth only takes place in light (photosynthesis), but illumination 24 hours a day is not recommended (Vonshak, 1997). During dark periods, chemical reactions take place within *Arthrospira*, like synthesis of proteins and respiration.

Respiration decreases the mass of *Arthrospira* ("biomass"); its rate is much greater at high temperature so cool nights are better on that account, but in the morning beware that *Arthrospira* 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 (Laws et al., 1983)-.

Artificial light and heating may be used to grow *Arthrospira*, although they are not economical. Fluorescent tubes and halogen lamps are both convenient. Lamps can illuminate and heat the culture simultaneously.

3.1.1.2 Agitation

Individual *Arthrospira* 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. Wind is beneficial for agitating and aerating the culture, but it may bring dirt into it (Jourdan, 2001).

When stressed by a pH or salinity sudden variation, for instance by a heavy rain (more than 10% of the culture volume), the *Arthrospira* may sink to the bottom of the pond, where they will be in great danger of dying from suffocation (Jourdan, 2001). In order to facilitate their recovery, agitation at the bottom is often to give them more chance to disentangle from the mud.

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 (1watt/m² is enough). "Raceway" ponds agitated by paddlewheels are standard in the industry.

3.1.2 *Arthrospira* culture medium

Arthrospira can live in a wide range of compositions of water. The following Table 1 is a convenient analysis for mediums used in industry for the culture of *Arthrospira* at large scale:

Table 1. Culture medium of *Arthrospira* at large scale (according to Jourdan, 2001)

Component	Value
Anions Carbonate	2800 mg/l
Bicarbonate	720 mg/l
Nitrate	614 mg/l
Phosphate	80 mg/l
Sulfate	350 mg/l
Chloride	3030 mg/l
Cations Sodium	4380 mg/l
Potassium	642 mg/l
Magnesium	10 mg/l
Calcium	10 mg/l
Iron	0.8 mg/l
Urea	< 50 mg/l
Total dissolved solids (TSS°	12847 mg/l
Density at 20°C	1010 g/l
Alkalinity (moles strong base/litre)	0.105 N
pH at 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 in **Table 2**, is one example convenient for many typical waters :

Table 2. Microelements for life support of algae (according to Jourdan, 2001)

Component	Value (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, $MgSO_4$, 7 H_2O	0.2
Lime	0.02
Ferrous sulfate, crystallized, $FeSO_4$, 7 H_2O	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 analysed for its contents or tested. Seawater can be used under some very special conditions.
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 (Jourdan, 2001).

3.1.2.1 Influence of ions

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) (Zarrouk, 1966).
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. *Arthrospira* can grow on either nitrate or urea alone, but using both together is advantageous (Jourdan, 2001).
- Phosphate, magnesium and calcium cannot be increased much without precipitating magnesium or calcium phosphate, possibly leading to imbalances in the solution (Cornet, 1992).
- Potassium concentration can be increased at will, provided it does not become more than five times the sodium concentration to avoid toxicity (Jourdan, 2001). 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_2 from the air until its pH has come down to 10.5 before using it).

- Solutions of iron should preferably be introduced very slowly and under agitation into the medium.

If fertilizer grade chemicals are used, they should be of the "soluble" or "crystallized" type, not of the "slow release", granulated type.

3.1.3 Inoculation of *Arthrospira platensis*

The chosen *Arthrospira* strain should contain 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 (Vonshak, 1997).

3.1.4 Culture concentration

- Concentrated *Arthrospira* seed culture can be obtained either from the floating layer of an un-agitated culture, or by re-diluting a freshly filtered biomass (beware of lumps). A concentration of up to 3 g *Arthrospira* (dry) per litre is permissible if storage and transportation last less than a week time, and provided the seed culture is aerated at least two times a day. If aeration can be continuous, the concentration may be up to 10 g/l (weights of *Arthrospira* always refer to contained dry matter).
- It is advisable to maintain the growing culture at a fairly high concentration in *Arthrospira* after each dilution with culture medium, about 0.3 g/l: the "Secchi disk" reading should not be above 5 cm, i.e. the colour of the culture should stay clearly green (otherwise shading is mandatory). The rate of growth is about 30% per day when the temperature is adequate and the make-up culture medium based on bicarbonate (without carbonate) (Belay et al., 1994).
- 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, the *Arthrospira* concentration is let to rise to about 0.5 g/l (Secchi disk at about 2 cm) before harvesting.

3.1.5 Harvesting

When the *Arthrospira* is in good condition, separating it from the water is an easy operation, but when it gets too old and "sticky", mainly due to excess production of exopolysaccharides (EPS) at this stage of its life, harvesting may become a nightmare

The best time for harvesting is early morning for various reasons:

1. The cool temperature makes the work easier,
2. More sunshine hours will be available to dry the product,
3. The % proteins in the *Arthrospira* is highest in the morning.

There are basically two steps in harvesting:

(1) filtration to obtain a "biomass" containing about 10 % dry matter (1 liter = 100 g dry) and 50 % residual culture medium, (2) removal of the residual culture medium to obtain the "fresh *Arthrospira* biomass", ready to be consumed or dried, containing about 20 % dry matter and practically no residual culture medium.

Harvesting the floating layer (generally richer in spiraled *Arthrospira*) will tend to increase the % straight *Arthrospira* in the culture. Straight *Arthrospira* is more difficult to harvest. So actually it is not recommended to harvest the floating layer (Vonshak, 1997).

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.

3.1.6 Control of Exopolysaccharides production

Excessive production of exopolysaccharides (EPS) by the *Arthrospira* or its too slow biodegradation will cause "stickiness" of the biomass and/or a flocculation of *Arthrospira* 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 *Arthrospira*. 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 cleaning 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.

3.1.7 Contamination

The culture may become colonized by predators living on *Arthrospira*, like larvae of mosquitoes and Ephydra flies, or amoebas. In the experiences as reported by Vonshak et al; 1982, 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 *Arthrospira* 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 *Arthrospira* 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 *Arthrospira* 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 *Arthrospira* 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.

3.1.8 Culture colour

The colour 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.

3.2 INDOOR *ARTHROSPIRA* CULTIVATION

The morphology and physico-chemical characteristics of *Arthrospira platensis* are dependent on the culture conditions. For example the exopoplysaccharides content can vary and subsequently, will cause clogging problems if ultrafiltration technique is used for the concentration of the alga.

3.2.1 Improvement of algal growth

Mass cultivation techniques and extraction processes are actually investigated in some Asiatic laboratories (**D**ivision of **B**iotechnology at King Mongkut's University of Technology Thonburi (KMUTT) in order to obtain value-added compounds such as phycocyanin and gamma-linolenic acid (GLA). One of the goals is to enhance cell growth and maximize the production of value-added chemicals in *Arthrospira platensis***.** In the past one year, the Algal Laboratory has been very active in trying to find the right substrate and environmental conditions for cultivating different strains of *Arthrospira platensis.* For example, it was found that CO_2 could be used to replace $NaHCO_3$ in cultivating microalgae with substantial lower production costs (Pulz, 2001). Moreover, effect of light and temperature on the productivity and photosynthesis of the algae in an indoor environment were investigated (Pulz, 2001). In addition other research focuses on trying to understand the mechanism by which *Arthrospira* produces value-added compounds such as phycocyanin and GLA so that new strains with higher contents of such compounds can be developed. Different approaches were studied, including mutagenesis using EMS (Ethyl Methane Sulfonate) as a mutagen, the use of inhibitors, and the use of inducers/enhancers such as herbs.

3.2.2 Factors influencing *Arthrospira* cultivation

Most of photosynthetic microorganisms like *Arthrospira*, need light, CO_2, nitrogen source and minerals to grow. However, many factors are responsible for the good or wrong cultivation of *Arthrospira*. These factors are: the light intensity, the nitrogen source and its concentration, the carbon source and the macro-and microelements in the culture medium. **Table 3**. gives an overview of the factors influencing *Arthrospira* growth and their corresponding values for optimal cultivation to concentrations between 0.5 and 1 g/L.

Table 3. Optimal conditions for *Arthrospira platensis* cultivation to a concentration of 0.5-1g/L.

Constituent	**Value**	**References**
Light intensity	100 W/m^2	Cornet, 1992
pH	9.5 – 10.5	Boussiba, 1989
Nitrogen source	$NH_4^+ + NO_3^-$	Filali et al., 1997
Ammonium	< 1 mM	Boussiba, 1989
Carbon source	$CO_2 + HCO_3^- + CO_3^2$	TN 32.4

Salinity	< 0.2 M	Vonshak et al., 1987b
Macro-Microelements	NN*	Cogne et al., 2001
Temperature	34-36°C	Vonshak & Richmond,1988
Age of the inoculum	6-8 days	Pelizer et al., 2002

NN*: not necessitated

3.2.2.1 Influence of light and nutrients on the shape of *Arthrospira*

Some other factors can have effect not only on the growth of the alga, but also on the shape of the algal filaments. *Arthrospira platensis* seem to change in shape depending on two major factors: changes in light intensity and low salts concentrations mainly NaCl in the culture medium. They can change from S-coiled filaments into straight filaments when they are subjected to sudden changes in salts concentrations or in light intensities. **Figure 2** shows the two different forms adopted by the filaments when the NaCl concentration in the feed medium (Zarrouk medium) is 1 g/L and when halved.

(a) (b)

Figure 2. Microscopic view (400 x enlargement) of *Arthrospira platensis* cultivated in (a) Zarrouk medium and (b) half strength Zarrouk medium (images taken with light microscopy at EPAS n.v.)

The selection of the separation technique can depend on the type of *Arthrospira platensis*. According to Bai and Seshadri (1980), high light intensities and high nutrient concentrations cause transformations in the shape of the alga from the loose-coiled variant (S-type) to the tighter coiled one (C-type), and the latter showed increased coiling to the vary tight variant (H-type) at high light intensities and low nutrient concentrations. These changes may induce difficulties during the harvesting of the alga and decrease its gustative quality.

According to J.F.Cornet (PhD thesis, data not reported in MELISSA reports) some slight modifications in the phycocyanin content (pigment of photosystem II, linked to proteins) may occur when *Arthrospira platensis* is cultivated in low strengths Zarrouk medium, but this has no effect on the biomass growth rate. In his work, the results were summarized as shown in **Table 4**.

Table 4. Changes in phycocyanin and protein contents in *Arthrospira platensis* cultivated in different ionic strengths (according to Cornet, 1992).

Zarrouk medium strength	Ionic strength (mol/L)	Phycocyanin content (%)	Protein content (%)
2 fold concentrated	0.84	11	64
Standard	0.42	15	68
2 fold diluted	0.21	19	72
4 fold diluted	0.105	23	76

3.2.2.2 Nitrogen uptake

Cyanobacteria, in general, use inorganic nitrogen for growth, particularly nitrates and ammonium. Some of them, however, are also able to grow on organic nitrogen (Fogg et al, 1973). When nitrogen is taken up in its oxidised form as nitrate, it must be reduced before it is incorporated into organic molecules. It is then reduced to nitrite in the first step to be finally converted into ammonium. The latter is incorporated on glutamic acid and amino acid metabolism.

Arthrospira platensis is an alkalophilic strain, which can grow at pH above 10. At this high pH, it can be expected that the nitrogen form present in the medium is ammonia (NH_3) form and two components for the uptake of ammonia have been observed (Boussiba, 1989).

- Rapid initial uptake of ammonia from the medium immediately after application
- Subsequent slow phase

Regarding ammonia uptake and metabolic activities (measured by oxygen production), studies have shown no activity at pH 6, an optimum at pH 9 and approximately 80% of the optimum at pH 10.8 (Boussiba, 1989).

3.2.2.3 Use of various nitrogen sources

- Nitrate was mentioned as being the preferred nitrogen source by Zarrouk, 1996 and Cornet et al., 1998. Other authors (Guerrero and Lara, 1987, Boussiba 1989) plead for

ammonium, based on observations of ammonium being used first by *Arthrospira platensis* when it was growing on both nitrogen sources . However, toxicity of ammonia at higher concentrations may occur, which seems to be the reason for the predominant use of nitrate. The volatilization of ammonia at high pH values and thus its less accessibility to the alga, did not overcome its benefits since its low cost production out of urea. In the contrary, nitrate is less interesting in term of costs and it may be lost by denitrification as well (Vonshak, 1997).

- Combination of nitrate and ammonium has been yet investigated in detail. Cyanobacteria cultivated with nitrate and ammonium as nitrogen sources does not use nitrate until ammonium has been completely consumed (Ohmori et al., 1977). This observation is related to the regulation of nitrate assimilation. The uptake and reduction systems involved in nitrate utilisation are fully expressed when the latter represents the sole nitrogen source. The activities are suppressed, or at least decreased, when ammonium is present alone or in combination with nitrate (Guerrero and Lara, 1987). However, the activities are restored as soon as ammonium is exhausted in the medium. Furthermore, the degree of ammonium inhibition correlates with the rate of its assimilation by the cells. It was observed that also in the absence of exogenous ammonium, the rate of nitrate uptake is regulated by the amount ammonium produced during nitrate reduction. (Flores at al., 1980, Flores et al., 1983).It was observed that the combination of ammonium and nitrate caused higher growth of the algae than with ammonium as sole nitrogen source. The toxicity of ammonium was shown to be reduced (Filali et al., 1997).

Based on the above mentioned considerations, it can be assumed that although ammonium seems to be the preferred nitrogen source, nitrate is the more convenient one as it can be provided to the culture at high concentrations without effects caused by high pH values or toxicity.

3.2.2.4 Ammonia toxicity

It was demonstrated that *Arthrospira platensis* was resistant to ammonia (Peschek et al., 1985). At a concentration of 3mM and pH 10, the production of oxygen was inhibited by only 20%. At 20mM, nearly 40% of the maximum oxygen production was still observed. To try to explain this phenomenon, the dependence of two different parameters (ammonia uptake from

the medium and the internal pH) on pH was measured (Boussiba, 1991). The results showed that the uptake rate was constant at a given external pH, but increased with the increase of the internal pH values. At an external pH of 10, the internal ammonia concentration was nearly 300-fold more than in the medium. The proton gradient appeared to be maintained and this stability may be the reason that explains the ability of *Arthrospira platensis* to produce oxygen at high pH values in the presence of ammonia. Indeed, one of the possible mechanisms by which ammonia toxicity may be prevented in *Arthrospira platensis* is the maintenance of high internal pH values (Peschek et al., 1985, Boussiba, 1991).

Another effect, which was observed as well, is that ammonium exhibits a negative effect on the cellular activity levels of both nitrate reductase and nitrite reductase enzymes in *Cyanobacteria* in general, acting as nutritional repressor of their synthesis (Herrero et al., 1981, Herrero and Guerrero, 1986, Flores et al., 1983).

Very few is reported about the levels of toxicity. Some studies suggest that the ammonium concentration must be less than 7mM (Richmond, 1988), others reported that the uptake rate saturation was not reached at 1mM NH_4^+ in the medium at pH 10 (Boussiba, 1989), and that at ammonia concentration of 3mM, the oxygen production was inhibited by 20%. In more recent study (Filali et al., 1997), it was concluded that ammonium was toxic at concentrations higher than 2mM but the organisms may sustain long duration growth if maintained at lower concentrations.

3.2.2.5 Carbon limitation

- At high pH values ($8<pH<11$), the total dissolved carbon ($C_T = CO_2 + HCO_3^- + CO_3^{2-}$) is essentially composed of bicarbonate (HCO_3^-) and (CO_3^{2-}) ions. These concentrations are about 2000 times higher than CO_2 concentration at pH of 9.5. This is an important specificity because it creates a large buffer reserve of the total dissolved carbon in the culture media.
- The main feature of the limitation of *A. platensis* growth by the carbon source have clearly shown the coupling between light and carbon limitation (TN.32.4) as previously stated in the literature (Miller and colman, 1980; Coleman and Colman, 1981). The fact that no direct proportionality was found between C_T and rates showed the coupling between the mechanism of intracellular carbon concentration and photophosphorylation, directly dependent of the available radiant light energy in the medium.

From the results obtained in the technical note TN 32.4, it was observed that at the opposite of O_2 limitation for heterotrophic microorganisms, the purely physical limitation by CO_2 transfer does not exist (at least at pH of 9.5), because, even in the more limiting conditions (input CO_2 molar fraction in gas phase relative to an input in the reactor Y_{CO2}^E = 350 mg/L, and incident flux = 210 W/m^2), the CO_2 concentration in the liquid phase is very low (5.10^{-8} mol/L), but C_T is equal to 10^{-4} mol/L. The metabolic deviations occurring in bicarbonate limitation are:

1. Inhibition of the exopolysaccharides synthesis (the total sugars mass fraction remains equal to 12-13%, which corresponds only to the cell wall sugars.
2. Progressive decrease of the pigments mass fraction (phycocyanins and chlorophylls).
3. Decrease in total proteins.

3.2.2.6 Salinity

Cyanobacteria inhabit environments, which vary drastically in their saline levels. The last 20 years, many studies were published on the response of Cyanobacteria to different saline environments: the specific role of organic compounds as osmoregulants, modification in photosynthesis and respiration activity, and variations in the protein synthesis pathway. Exposure of *Arthrospira* cultures to high NaCl concentrations results in an immediate cessation of growth and decrease in biomass as shown in **Figure 3** ((Vonshak et al., 1987b).
After a lag period, a new steady state is established. The new growth rates after adaptation are slower and inversely correlated to the increased NaCl concentration in the medium (Vonshak et al., 1988). The lag period in many cases is associated with a decline in chlorophyll and biomass concentration in the culture.

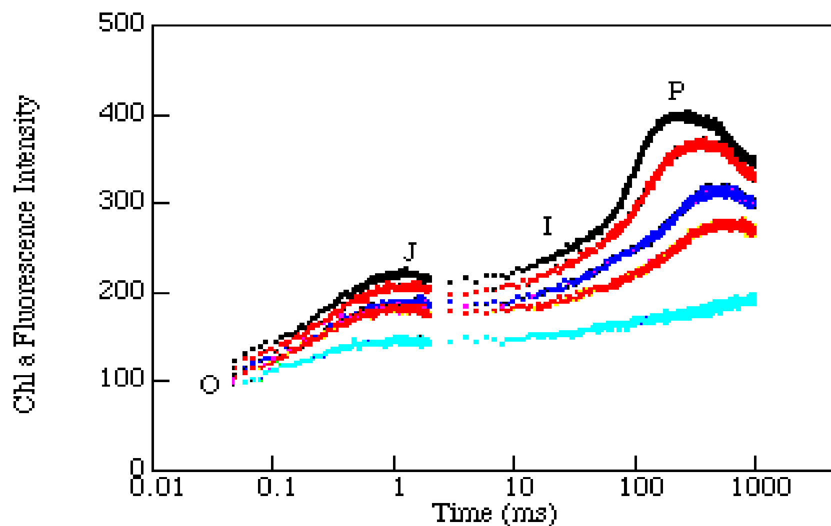

Figure 3. Chlorophyll a fluorescence transients in *Arthrospira platensis* exposed to various salt concentrations plotted on a logarithmic time scale. From top to bottom, the transients represent control cells, and cells exposed to 0.20 M, 0.40 M, 0.60 M, and 0.80 M NaCl. (Vonshak et al., 1987b).

Photosynthetic activity of *Arthrospira* declines under stress even if the culture is grown continuously in the saline environment and adapt to the new osmoticum. This decline is associated with a modification in the light energy requirement, i.e. less light is required for the saturation of photosynthesis. The amount of light required to saturate photosynthesis increases significantly with the increase of NaCl concentration in the culture media, again indicating that photosynthesis operates at a significant lower efficiency at high salt stress. The dark respiration is increased by 2.5 fold at NaCl concentrations of 0.5mM, which may be how cells produce the extra energy required to maintain their internal osmoticum. The reduction in the ability to use light energy absorbed by the photosynthetic apparatus increases the sensitivity of the salt-stressed cells to photoinhibition. When salt-stressed cells of *Arthrospira* are exposed to high photon flux density (HPFD), a much faster decline in photosynthesis is observed (60 to 80% reduction), as compared with the control (40% reduction) (Vonshak, 1997). Moreover, salinity stressed cells have a lower rate of protein synthesis. Since recovery from photoinhibition is associated with the ability to synthesize specific proteins associated with photosystem II (PSII), a reduction in the level of the protein synthesis affects the repair mechanism (Vonshak, 1988a).

3.2.2.7 Macrominerals and trace elements

Batch and continuous cultures of *Arthrospira platensis* under photoautotrophic conditions, uptake of macroelements and traces elements have been characterised by Cogne et al., 2001. The modifications concerned mainly the trace elements solutions, where concentrations of some compounds were reduced to lower levels, and trace elements (B, Mo, V, Cr, Ni, W and Ti) were removed from the culture medium.

Batch cultures of *Arthrospira platensis* were carried out with and without trace element solution B_6 in Zarrouk medium. No changes in growth rate were observed when trace elements solution was not added in the culture medium. Subsequently, the remaining investigations were performed without B_6 solution. Under these conditions, in light limiting conditions of 88 W/m^2 and batch experiments, the growth rate was 0.0134 kg/m^3.h. Moreover, the results showed clearly that Zn, Fe, Mn and Mg were fixed by *Arthrospira* alga and incorporated in biomass proteins. However, no evidence of bore and copper uptake has been demonstrated.

In continuous mode and high photon flux density (PFD) of 122 W/m^2 and 150 W/m^2, the assimilation and incorporation into biomass of some elements by *Arthrospira* was evident like Zn, Fe, Mn and Mg. However, other elements like B and Cu seemed not to be indispensable or limiting factors for the growth of the alga.

From element yield coefficients, further simplifications of the trace element solution A_5 from the Zarrouk medium could be made. Considering the literature dealing with the subject (Campanella et al., 1998), Mo is absent from *Arthrospira* biomass suggesting that this element is not required by this alga so its is the case of B. A minimum of 32 mg/L chlorine concentration is required for a normal growth of the culture of *Arthrospira*. For this element, the proposed Zarrouk medium seems to be adequate (Zarrouk, 1966).

In their study, Cogne et al., 2001, suggested the minimum medium allowing *Arthrospira* growth up to a concentration of 3.6 g/L, which is the maximum concentration attained in batch culture for 0.2 mol of carbon source. The proposed medium is shown in **Table 5** and **Table 6**.

Table 5. Minimum medium for growth and maintenance of *Arthrospira platensis*

compound	Kg/m^3	compound	Kg/m^3
NaCl	1.0	$NaNO_3$	2.5
$CaCl_2$	0.03	$NaHCO_3$	10.5
K_2SO_4	1.0	Na_2CO_3	7.6
$MgSO_4,7H_2O$	0.08	EDTA	0.08
K_2HPO_4	0.5	$FeSO_4,7H_2O$	0.01

Table 6. Trace element solution (1 ml per litre medium)

compound	Kg/m^3
$MnCl_2$, $4H_2O$	0.23
$ZnSO_4,7H_2O$	0.11
$CuSO_4,5H_2O$	0.03

3.2.2.8 Effect of light

Light is the most important factor affecting photosynthetic organisms. Due to the prokaryotic nature of *Arthrospira*, light does not affect the differentiation or development processes. Nevertheless, *Arthrospira* , like many other algae grown photoautotrophically, depends on light as its main energy source.

3.2.2.8.1 Effect of light on growth

Most of the laboratory studies on the response of *Arthrospira* to light were performed under photoautotrophic growth conditions, using a mineral medium and bicarbonate as the only carbon source. From data obtained in some laboratories, growth of *Arthrospira platensis* became saturated at a range of 150-200µmol.m-^{2}.s^{-1}. This is about 10 to 15% of the total solar radiance at the 400-700 nm range. This value is highly dependent on growth conditions and correlates with the chlorophyll to biomass concentration. Another experimental parameter which determines this response is the light path of the culture. Therefore, it is highly recommended that when attempting to establish the maximal specific growth rate (µmax), a tubidi-ostat system should be employed (spectrophotometer in most of the cases).

In other studies (Cornet, 1992), it was shown that light saturation for *Arthrospira platensis* occurs at light radiation of 100 W/m^2 in available radiant light energy at 36°C and pH of 9.9. Photoinhibition begins at 300 W/m^2 and that the maximal photosynthetic rate was $7.1.10^{-3}$ kmol O_2/kg biomass.h.

3.2.2.8.2 Light stress-photoinhibition

Vonshak at al., 1988a, demonstrated that different strains of *Arthrospira* may differ in their sensitivity to the light stress. At least in one case, it was found that this difference was most likely due to a different rate of turnover of a specific protein, D1, which is part of the photosystem II. The different response of *Arthrospira* strains to a photoinhibitory stress may be a genotypic characteristics as well as arising from growth conditions.

3.2.2.9 Effect of temperature

Temperature is the most fundamental factor for all living organisms. It affects all metabolic activities and affects nutrient availability and uptake, as well as other physical properties of the cells-aqueous environment. The usual temperature for laboratory cultivation of *Arthrospira* is in the range of 35-38°C with an optimum at 36°C for *Arthrospira platensis* .
Photosynthesis and respiration are dependent on temperature, but only CO_2 fixation and O_2 evolution are both light- and temperature- dependent. The kinetics of recovery from low-temperature incubation in the light, indicate that some repair mechanism must take place before the original photosynthetic activity is reached. Moreover, *Arthrospira* cultures incubated at low temperature in the dark seemed to acquire their original photosynthetic activity as soon as they were transferred to 35-36°C without any lag period (investigated at EPAS n.v.).
Many other factors interact with temperature and probably affect the growth and productivity of *Arthrospira.* Solubility of gases in the medium and availability of nutrients are some of them.

3.2.2.10 pH control

The pH of the medium is one of the most important factors in culturing *Arthrospira*. Maintaining a pH over 9.5 is mandatory in *Arthrospira* cultures in order to avoid contamination by other algae. pH adjustment is generally, made by supplying CO_2 gas to the medium. It is rare that the pH falls below about 9.0. However, upward shifts in pH in excess

of pH 10.5 are quite common. When this occurs, it is accompanied by precipitation of $CaCO_3$, which is followed by flocculation and sedimentation of algae. Excessive deposition of such detritus contributes to light attenuation and thus more organic matter deposition. The decomposition of the organic matter results in the increase of bacterial population, ciliates and other undesired algae. Under such circumstances, the culture has to be replaced completely to comply with quality guidelines.

3.2.2.11 Influence of inoculum age and concentration

The influence of inoculum age in *Arthrospira platensis* cultivation was investigated recently by Pelizer et al., 2002. The study was performed in Erlenmeyers flasks and in mini-tanks. After the determination of the best inoculum age, the study of the inoculum concentration influence in mini-tanks was done. It was shown during this study that the inoculum age should be in the range of 6-8 days for the best cultivation of *Arthrospira platensis* and the recommended inoculum concentration should be 50 mg/L. With these results, the biomass chemical composition and chlorophyll contents obtained were similar to those of literature data.

3.2.2.12 Maintaining uni-algal culture

The maintenance of uni-algal cultures of *Arthrospira* in outdoors as well as in indoor production is a challenge to any commercial or local production facility. The economic production of this alga necessitates the continuous recycling of the nutrients after the biomass is removed. This constant recycling results in excessive accumulation of organic matter. This not only results in contamination by other algae (Richmond et al., 1990); but also in what appears to be the autoinhibition of growth of *Arthrospira*. The build-up of organic matter is also manifested at times in excessive foaming, which is a result of decomposition and death of algae.

The major contaminant algae in *Arthrospira* cultures, as reported by Vonshak and Richmond (1988), are chlorella, the unicellular green alga *Oocystis sp* and small species of *Arthrospira, Spirulina minor*. The losses in productivity due to contamination and subsequent discarding of culture were estimated to be in the order of 15-20%.

4. Culture conditions

4.1 RELATIONS CULTURE CONDITIONS AND MORPHOLOGY OF *ARTHROSPIRA PLATENSIS*

The morphology and physical–chemical characteristics of *Arthrospira platensis* seem to be dependent on the culture conditions. Based on the limited amount of literature data and preliminary experiments at EPAS it seems that limiting conditions of light and nutrients influence the shape of *Arthrospira*. For example, as already mentioned, the polysaccharides content can vary and this will cause clogging problems if filtration techniques are used for the harvesting of the alga.

A clear answer on the relationship cannot be given in the framework of this study, but it is assumed that when optimal growth conditions are used, the change on a normal growth and morphology of *Arthrospira* is the highest. The general scheme of these different interdependent relations is presented in **Figure 4.**

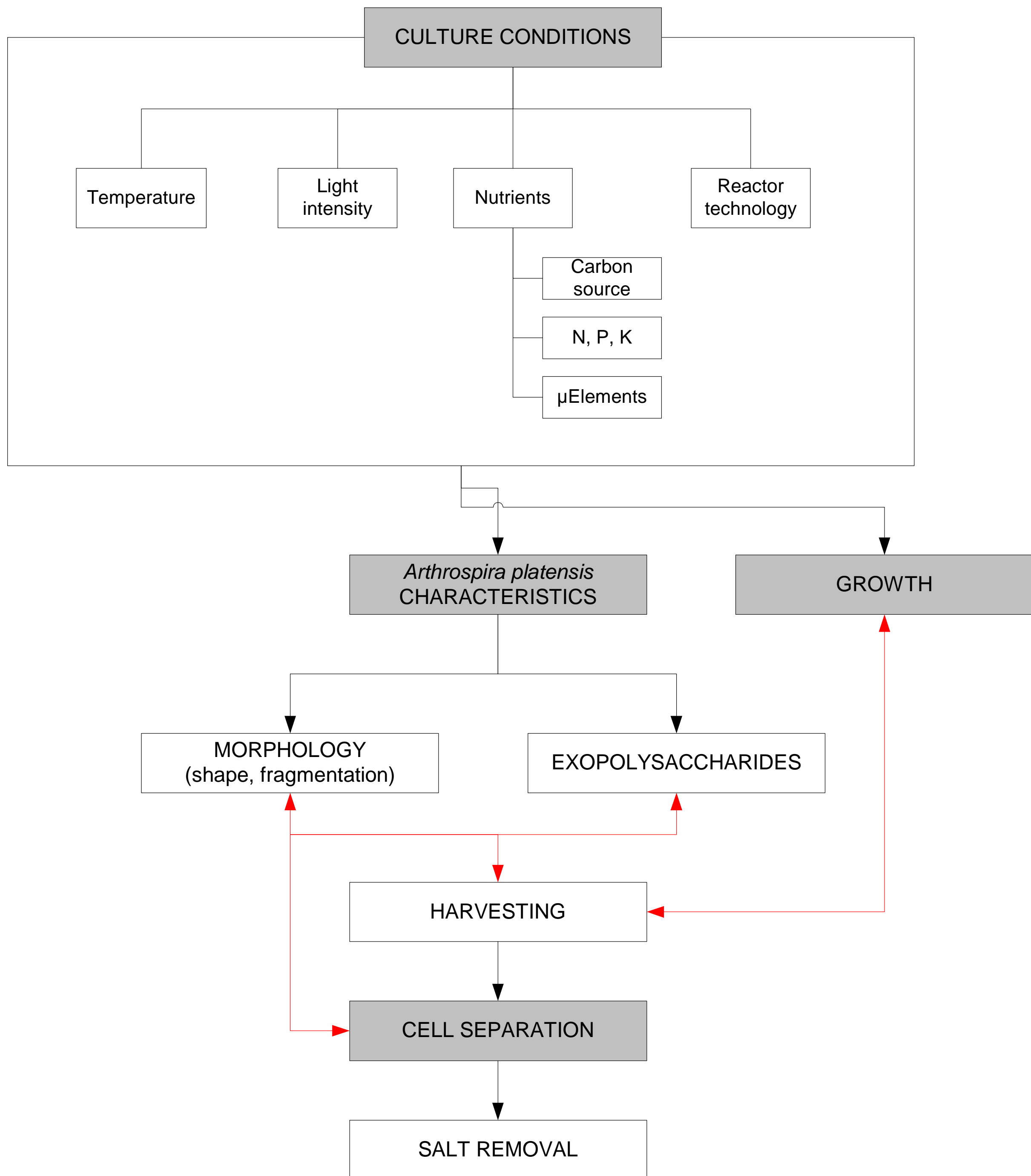

Figure 4. General view of the interdependent culture conditions, biomass growth, biomass characteristics and cell separation of *Arthrospira platensis*

4.2 SELECTION OF CULTURE MEDIUM FOR *ARTHROSPIRA* CULTIVATION

It was proposed to the MELISSA-partners to work with one culture medium, selected for its easy preparation, handling and conservation.

In this note a trade-off of the generally used culture media for *Arthrospira platensis* is made. If one considers the functioning of the photosynthetic compartment in the MELISSA loop,

limiting conditions will be encountered, since the flow originated from compartment III (nitrifying compartment) will not provide all the necessary elements for the growth of *Arthrospira platensis*. It is therefore recommended to select a culture medium, which can firstly, allow optimal growth of the alga without hindering the quality and secondly, does not necessitate the use of a lot of chemicals in high concentrations.

Different culture media are proposed in this note. The detailed composition of each culture medium is proposed in the addendum. The principal, common elements present in all media are shown in **Table 7,** the aim being to make differentiations related to their presence and content in the different proposed media. Some other elements, which are present but in less amounts in each medium are referred as trace elements and presented in details in the addendum.

The selection of the most indicated medium for *Arthrospira* cultivation was made by comparing the concentration of the crucial elements, indispensable for maintenance and growth of the alga of each culture medium with the minimum medium proposed by Cogne et al., 2001 (see **Table 5**).

Table 7. Common elements and their concentrations for *Arthrospira platensis* cultivation in different culture media

	Minimum medium (Cogne et al.2001)	**Zarrouk medium (Zarrouk 1966)**	**Schlösser medium (Schlösser, 1982)**	**Allen medium (Allen, 1968)**	**ES-enriched seawater medium (Provasoli, 1963)**	**BG-II + ASN-III (Pasteur- Institute France, Rippka, 1988)**
NaCl (g/L)	1.0.	1.00	1.00	NU	NU	**25**
$CaCl_2.2H_2O$	0.03	0.04	0.04	0.04	NU	0.53
K_2SO_4	1.00	1.00	1.00		NU	
$MgSO_4.7H_2O$	0.08	0.20	0.20	0.037	NU	**3.57**
K_2HPO_4	0.50	0.50	0.50	0.037	NU	0.06
$NaHCO_3$	**10.5**	**13.60**	**13.60**	NU	NU	
$NaNO_3$	2.5	2.5	2.5	1.5	0.35	2.25
Na_2CO_3	**7.6**	3.5	4.03	0.02	NU	0.08
Others	EDTA, $FeSO_4.7H_2O$					EDTA, Ferric ammonium citrate, citric acid, Mg $Cl_2.6H_2O$, KCl,
Trace elements	- $MnCl_2.4H_2O$ - $ZnCl_2.7H_2O$ - $CuSO_4.5H_2O$	A5 and B6* (4ml/L)	- PIV metal solution - Chu micronutrients-solution - Vitamin B_{12}	- Citric acid - PIV metal solution	- Fe-solution - PII metal solution - Vitamin B_{12} - Biotin - Tris buffer	- Trace metal mix A5 + Co - Na_2.EDTA-Mg
pH medium	9.5-10.5	9.5 - 11	9-10	7.8	7.8	7.4 – 7.5

*:May not be necessary in *Arthrospira platensis* growth conditions in MELISSA loop (see paragraph 3.2.2.7, Cogne et al., 2001

NU: not used

When Allen medium, ES-enriched medium and BG-II + ASN-III medium were definitely discarded from the selection, principally due to difficulties related to the preparation of the media and trace element solutions, neutral pH of the medium (niche for contaminations) and use of high NaCl concentrations (case of BG-II + ASN-III medium), two media, Zarrouk medium and modified SAG medium by Schlösser seemed to be closer to the composition of the minimum medium proposed by Cogne et al. 2001. The concentration of the principal elements is almost the same in most of the cases. The pH at which the alga could grow is high enough (> 9) to prevent contamination. To select a medium between the two, the composition of the trace elements was studied. Indeed, in the Schlösser medium, a lot of chemicals, in quite low concentrations have to be used to make the PIV metal solution and the Chu microelements solution (see Schlösser medium in addendum). This seems to be time consuming and may be un-necessary if one compares the composition of these solutions with the one of Zarrouk medium. In this case, the elements composing the trace solution are comparable with those of the minimum medium of Cogne et al. 2001. Nevertheless, the presence of some elements, which may not be necessary for the growth and maintenance of *Arthrospira* according to Cogne et al. 2001, might be used in the culture medium to prevent low efficiencies in compartment CIVa. Therefore, Zarrouk medium, seems to be more adapted for this situation. From this work, we could state that the most appropriate culture medium for *Arthrospira platensis* is the Zarrouk medium.

5. Addendum

5.1 ZARROUK MEDIUM COMPOSITION: (ZARROUK, 1966)

Table 8. Zarrouk growth medium for *Arthrospira platensis*

Composition	Amount (g/L)
Na_2CO_3	3.5
$NaNO_3$	2.5
$NaHCO_3$	13.6
K_2SO_4	1
NaCl	1
$MgSO_4.7H_2O$	0.2
$CaCl_2.2H_2O$	0.04
$FeSO_4.7H_2O$	0.01
EDTA-Na_2	0.08
K_2HPO_4	0.5
Solution A5 in ml	4
Solution B6 in ml	4

Table 9.Micro-elements for *Arthrospira platensis* growth (after Zarrouk 1966)

Solution A5	(g/L)	Solution B6	(g/L)
H_3BO_3	2.86	$KCr(SO_4)_2.12H_2O$	0.096
$MnCl_2.4H_2O$	1.81	$NiSO_4.7H_2O$	0.048
$ZnSO_4.7H_2O$	0.22	$(NO_3)Co.6H_2O$	0.049
$CuSO_4.5H_2O$	0.08	$Na_2Mo_4.2H_2O$	0.018
MoO_3	0.015		

5.2 SCHLÖSSER MEDIUM (SCHLÖSSER, 1982)

In order to prevent the formation of precipitates during autoclaving, two solutions A and B are prepared and autoclaved separately as shown in Table 10.

Table 10. Composition of *Arthrospira platensis* growth medium (Modified SAG medium by Schlösser, 1982)

Solution A		Solution B	
Glass-distilled water	500 ml	Glass-distilled water	500 ml
$NaHCO_3$	13.61 g	$NaNO_3$	2.5 g
Na_2CO_3	4.03 g	K_2SO_4	1.00 g
K_2HPO_4	0.50 g	NaCl	1.00g
		$MgSO_4.7H_2O$	0.20 g
		$CaCl_2.2H_2O$	0.04 g
		PIV metal solution	6 ml
		Chu micronutrient solution	1 ml
		Vitamin B_{12} (15 µg/100 ml H_2O)	1 ml

Both solutions are combined aseptically after cooling.

PIV metal solution		Chu micronutrient solution	
Glass-distilled water	1000 ml	Glass-distilled water	1000 ml
Na_2EDTA	0.750 g	Na_2EDTA	0.005g
$FeCl_3.6H_2O$	0.097 g	H_3BO_3	0.618 g
$MnCl_2.4H_2O$	0.041 g	$CuSO_4.5H_2O$	0.0196 g
$ZnCl_2$	0.005 g	$ZnSO_4.7H_2O$	0.044 g
$CoCl_2.6H_2O$	0.002 g	$CoCl_2.6H_2O$	0.020 g
$Na_2MoO_4.2H_2O$	0.004 g	$MnCl_2.4H_2O$	0.0126 g
		$Na_2MoO_4.2H_2O$	0.0126 g

5.3 ALLEN MEDIUM (ALLEN, 1968)

This is the modified M.M. Allen's medium for blue-green algae, suitable for axenic and xenic cultures; the marine Spirulina platensis LB 1928 (Medium: ES-enriched seawater) can be adapted to Allen medium. Table 11 shows the composition of the medium.

Table 11. Composition of Allen's growth medium for *Spirulina platensis*

Stock solutions	Amount
Glass-distilled water	963 ml
$NaNO_3$	1.5 g
K_2HPO_4 at 1.5 g/200 ml water	5 ml
$MgSO_4.7H_2O$ at 1.5 g/200 ml water	5 ml
Na_2CO_3 at 0.8 g/200 ml water	5 ml
$CaCl_2.2H_2O$ at 0.5 g/200 ml water	10 ml
$Na_2SiO_3.9H_2O$ at 1.16 g/200 ml water	10 ml
Citric acid at 1.2 g/200 ml water	1 ml

PIV metal solution (see Schlösser medium)	1 ml

The pH should be adjusted to 7.8 with an optional ingredient: agar at 10 g/l to solidify the medium and conserving it.

5.4 ES-ENRICHED SEAWATER MEDIUM (PROVASOLI, 1963)

This is the modified Provasoli's ES-enrichment medium for seawater (Bold and Wynne, 1978). Medium preparation is presented in Table 12.

Table 12. ES-enriched seawater medium

Compound	Amount
Glass-distilled water	100 ml
$NaNO_3$	0.350 g
Na_2glycerophosphate.$5H_2O$	0.050 g
Fe-solution	**25 ml**
PII metal solution	**25 ml**
Vitamin B_{12}	10 µg
Thiamine	0.0005 g
Biotin	5 µg
Tris buffer (Sigma Co.)	0.500 g

The medium is adjusted to pH 7.8, dispensed in 20 ml tube, autoclaved and stored at 10°C. One tube (20 ml) of the ES-enrichment is afterwards added to 1000 ml of pasteurized filtered seawater.

Fe-solution		PII metal solution	
Glass-distilled water	500 ml	Glass-distilled water	100 ml
$Fe(NH_4)_2(SO_4)_2 6H_2O$	0.351 g	Na_2EDTA	0.100 g
Na_2EDTA	0.300 g	H_3BO_3	0.114 g
		$FeCl_3.6H_2O$	0.0049 g
		$MnSO_4.H_2O$	0.0164 g
		$ZnSO_4.7H_2O$	0.0022 g
		$CoSO_4.7H_2O$	0.048 g

5.5 BG-II + ASN-III (INSTITUT PASTEUR-FRANCE, RIPPKA, 1988)

Two media are combined (1/1, v/v) for the culture of *Arthrospira platensis* in Pasteur Institute in France. The composition of both media, BG-II and ASN-III is presented in Table 13.

Table 13. Constituents of the growth medium for *Arthrospira platensis* (Pasteur Institute)

BG-II		ASN-III	
Glass-deionised water	1000 ml	Glass-deionised water	1000 ml
$NaNO_3$	1.5 g	NaCl	25.0 g
$K_2HPO_4.3H_2O$	0.04 g	$MgCl_2.6H_2O$	2.0 g

$MgSO_4.7H_2O$	0.075 g	KCl	0.5 g
$CaCl_2.2H_2O$	0.036 g	$NaNO_3$	0.75 g
Citric acid	0.06 g	$K_2HPO_4.3H_2O$	0.02 g
Ferric ammonium citrate	0.06 g	$MgSO_4.7H_2O$	3.5 g
EDTA (Na_2 -Mg)	0.01 g	$CaCl_2.2H_2O$	0.5 g
Na_2CO_3	0.04 g	Citric acid	0.03 g
Trace metal mix (A5 + Co)*	1 ml	Ferric ammonium citrate	0.003 g
pH after autoclaving and cooling: 7.4		Na_2 EDTA-Mg	0.0005 g
		Na_2CO_3	0.04 g
		Trace metal mix (A5 + Co)*	1 ml
		pH after autoclaving and cooling: 7.5	

* **Trace metal mix A5 + Co**	
Glass-deionised water	1000 ml
$H_3BO_3^-$	2.86 g
$MnCl_2.4H_2O$	1.81 g
$ZnSO_4.7H_2O$	0.222 g
$Na_2MoO_4.2H_2O$	0.390 g
$CuSO_4.5H_2O$	0.079 g
$Co(NO_3)_2.6H_2O$	0.0494 g

6. References

Bai, N.J. and Seshadri, C.V. 1980. On coiling and uncoiling of trichomes in the genus *Spirulina*, Archives of Hydrobiology Supplement 60, Algological studies, 26, 32.

Belay, Y. A., Ota, Y., Miyakawa, K. and Shimmatsu, H. 1994. Production of high quality Spirulina at Earthrise farms. In SEW Moi, P., Yuan Kun, L., Borowitzka, M. A. and Whintton, B. A. (Eds) Algal biotechnology in the Asia-Pacific Region, p. 92, Malaysia: Institute of Advanced Stidies, University of Malaya.

Borowitzka, M.A. and Borowitzka, L.J. (eds) (1988). Micro-algal biotechnology. Cambridge University press, Cambridge.

Boussiba. S. 1989. Ammonia uptake in the alkalophilic Cyanobacterium "*Spirulina platensis*". Plant Cell Physiology, 30(2) 303-308.

Boussiba. S. 1991. Resistance of *Spirulina platensis* to ammonia at high pH values. Plant Cell Physiology, 32(7), 953-958.

Cogne G., Lehmann B., Dussap C. G. and Gros JB. 2001. Uptake of macrominerals and trace elements by the Cyanobacterium Arthrospira platensis (*Arthrospira platensis* PCC 8005) under photoautotrophic conditions. Culture medium optimization. *Published in* Biotechnology and Bioengineering, Volume: 81, Issue: 5, Date: 5 March 2003, Pages: 588-593.

Coleman J. R. and Colman B. 1981. Inorganic carbon accumulation and photosynthesis in a blue-green alga as a function of external pH. *Plant Physiol*. 67: 917-921.

Companella L., Crescentini G., Avino P., Moauro A. 1998. Determination of macrominerals and trace elements in the alga *Arthrospira platensis*. Analusis 26: 210-214.

Cornet J-F. 1992. Etude cinetique et énergetique d'un photobioréacteur. Etablissement d'un modèle structuré. Application à un écosystème clos artificiel (Thèse n° 1989, Université Paris Sud, Orsay).

ESA/ESTEC P.O.: 161 081: Analysis of carbon limitation in the *Arthrospira* compartment (1998): MELISSA Technical Note 32.4.

Filali, Lasseur. C. and Dubertret. 1997. MELISSA: Nitrogen sources for growth of Cyanobacterium *Spirulina*. In Proceedings of the Sixth European Symposium on Space Environmental Control Systems, ESA SP-400, Augustus 1997.

Flores. E., Guerrero. M. G. and Losada. M. 1980. Short-term ammonium inhibition of nitrate utilization by *Anacystis nidulans* and other Cyanobacteria. Archives of Microbiology, 128, 137-144.

Flores. E., Ramos, J. L., Herrero. A. and Guerrero, M. G. 1983. Nitrate assimilation by Cyanobacteria. In G.C. Papageorgiou and L. Packer Editions. Photosynthetic Procaryotes: Cell differentiation and function, Elsevier North Holland, Amsterdam, 363-387.

Fogg G.E., Stewart W.D.P., Fay. P., Walsby. A.E. 1973. The blue-green algae. Academic Press, London-New York, 190-213.

Guerrero. M. and Lara. C. 1987. Assimilation of inorganic nitrogen in the Cyanobacteria. In Fay. Pand Van Baalen. C. Editions, 163-186, Elsevier Science Publisher, Amsterdam.

Herrero. A., Flores. E. and Guerrero. M . G. 1981. Regulation of nitrate reductase levels in the Cyanobacteria *Anacystis nidulans*, *Anabaena* sp. Strain 7119 and *Nostoc* sp. Strain 6719. Journal of Bacteriology, 145, 175-180.

Herrero, A. and Guerrero. M. G. 1986. Regulation of nitrite reductase cellular levels in the cyanobacterium *Anacystis nidulans*. Journal of Genetic Microbilogy, 132, 2463-2468.

Jourdan, JP. 1996. (Ed) 2001; Grown your own *Spirulina*. Notice: Manual of small scale *Spirulina* culture. Distributed by Antenna Technology.

Laws, E. A., Terry, K. L., Wickman, J. and Chalup, M. S. 1983. A single algal production system designed to utilize the flashing light effect. *Biotechnol. Bioeng*., 25, 2319.

Miller, A. G.and Colman B. 1980. Active transport and accumulation of bicarbonate by a unicellular cyanobacterium. *J. Bact*. 143: 1253-1259.

Morist, A., Montesinos, J.L., Cusido, J.A. and Godia, F. 2001. Recovery and treatment of *Spirulina* cells cultured in a continuous photobioreactor to be used as food. Process Biochemistry 37 (5): 535-547

Morist, A., Montesinos, J.L. and Godia, F. 1999. Harvesting operational mode and water purification. MELISSA Technical Note 43.21

Ohmori. M, Ohmori. K. and Strotmann. H. 1977. Inhibition of nitrate uptake by ammonia in a blue green algae, *Anabaena cylindrica*, Archives of Microbiology, 114, 225-229.

Pelizer L.H., Danesi G. E. D., de O Rangel C., Sassano C. E. N., Carvalho J. C. M., Sato S. and O. Moraes I. 2002. Influence of inoculum age and concentration in *Arthrospira platensis* cultivation. *Journal of Food Engineering* (20 May, in press)

Peschek. G. A. Czerny. T., Schmetterer. G. and Nitschmann. W. H. 1985. Transmembrane proton electrochemical gradients in dark aerobic and anaerobic cells of the Cyanobacterium (blue-green alga) *Anacystis nidulans*. Plant Physiology, 79, 278-284.

Pulz, O. 2001. Photobioreactors: Production systems for phototrophic microorganisms. Springer-report 2001. Link http://link.springer.de.

Richmond , A., Lichtenberg, E., Stahl, B. and Vonshak, A. 1990. Quantitative assessment of the major limitations on productivity of *Spirulina platensis* in open raceways. Journal of Applied Phycology, 2, 195.

Rippka, R. 1988. Isolation and purification of Cyanobacteria. Methods in Enzymology, 167, 3-27.

Vernerey, A., Montesinos, J.L., Godia, F. 1998. Biomass Harvester. MELISSA Technical Note 37.30

Vonshak, A., Abeliovich, A., Boussiba, S., Arad, S. and Richmond, A. 1982. Production of *Spirulina* biomass: effect of environmental factors and population density, Biomass, 2, 175.

Vonshak, A. 1997 (Ed). *Spirulina platensis* (Arthrospira): Physiology, Cell-biology and Biotechnology. Taylor and Francis. 256 pp.

Vonshak, A. 1987b. Strain selection of *Spirulina* suitable for mass production. Hydrobiologia, 151, 75.

Vonshak , A. and Richmond, A. 1988. Mass production of the blue-green alga *Spirulina*: An overview, Biomass, 2, 233.

Vonshak, A., Guy. R. and Guy. M. 1988. The response of the filamentous cyanobacterium *Spirulina platensis* to salt stress. Archives of Microbiology, 150: 417-420.

Zarrouk C. 1966. Contribution à l'étude d'une cyanophycée. Influence de divers facteurs physiques et chimiques sur la croissance et la photosynthèse de *Arthrospira maxima*. Geilter. PhD Thesis. France: Université de Paris.