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NTE SA

MELiSSA – Adaptation for Space

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TECHNICAL NOTE 72.7.3

TEST PROGRAM

Solid-Liquid Separation and Desalination Technologies

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1. Introduction

In the MELiSSA loop, liquid-solid separation is a crucial aspect because the different compartments in the loop contain their specific microorganisms. It aims to prevent the transfer of microorganism from one compartment to the next and at the same time to allow the transfer of fermentation products. Since each compartment contains a different type of organism with a different size and different physical characteristics, the requirements for a liquid-solid separation system will differ as well and it will probably not be possible to develop a single system for all compartments.

As discussed in Technical notes 72.7.1 and 72.7.2, the separation of *Arthrospira* from its culture medium is probably the most critical and challenging one in the MELiSSA loop. First of all, the algae need to be further processed for food. Therefore, cells need to be harvested and concentrated. Alteration and breakage of the cells should be minimised. *Arthrospira* can vary its density, surface and viscosity depending on its cultivation conditions. This variable physical appearance will evidently influence separation techniques based on gravity, and will contribute to clogging and fouling when filtration techniques are used. Because elevated salt levels will affect the taste of the final product, a washing step has to be included, either before or after cell concentration. Secondly, the medium is high in salinity and pH. After separation from the algae it will preferentially be recycled to the system to recover the salts. To this end, it should be devoid of dead cells, cell debris, etc. To reduce water input to the system, the washing solution for the algal cells could be produced from the culture medium after harvesting, by full or partial desalination.

In Technical note 72.7.2, several candidate harvesting and desalination systems were identified to be promising for compartment IV. For harvesting, the following techniques were selected for the test program:

- ultrasonic separation
- membrane filtration
- filtration
- centrifugation

For desalination, reverse osmosis, electrodialysis and diafiltration were evaluated.

The present technical note describes the test results for ultrasonic separation, centrifugation and filtration.

This report also contains the test results for the desalination of Zarrouk medium. It is however, important to notice that desalination technology was also investigated on Alpha Biotech culture medium for *Arthrospira* (see annex: culture), which contains much higher salt concentrations and which, was used for complementary tests on membrane filtration investigated by GEPEA-University of Nantes.

A trade-off of harvesting and desalination technologies for design of the breadboard will be made and the most promising technologies will be selected. The work dealing with the trade off will be included in technical note 72.7.4.

2. Solid-liquid separation technologies

The function of the solid-liquid separation system is to separate the algal cells from the culture medium with the objective to:

- produce a concentrated cell suspension with a high percentage of liquid elimination, and with a high nutritional quality
- provide a liquid stream free of dead cells and cell debris to be transferred to the next compartment

This was evaluated for ultrasonic separation , filtration (ultrafiltration) and centrifugation.

2.1 Ultrasonic separation

2.1.1 Principle

The separation process in ultrasonic separation devices is based on gentle acoustically induced aggregation followed by enhanced sedimentation.

An ultrasonic standing wave is the sum of two oppositely travelling waves, which originate from two independent transducers or from a transducer and a reflector. Over a distance of one wavelength the pressure amplitude reaches a maximum (antinodal) and zero (nodal) value twice (see Figure 1). Every discontinuity in the system acquires an acoustic potential energy and will tend to concentrate at positions of minimal potential energy. For example, cells will be driven by acoustic forces into the planes of the pressure nodes, which are separated by distances of half a wavelength. Secondary forces drive the cells to the local minima within the nodal planes. They then aggregate within these planes to form loose clumps which can be seen as parallel lines in the resonator chamber. These are held stationary by the acoustic forces against the fluid drag through the chamber. When the field is switched off, the aggregates settle rapidly due to gravitational forces.

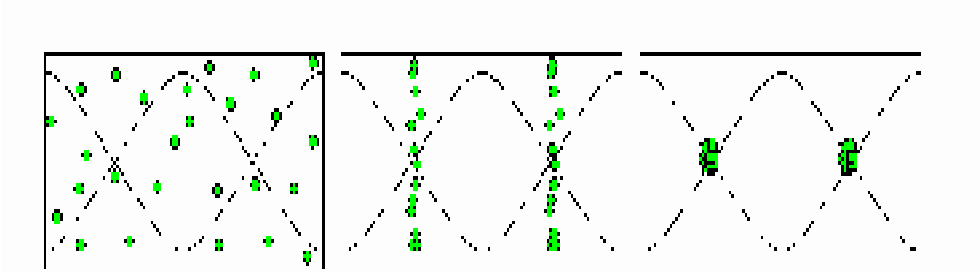


Figure 1. Principle of the acoustically induced aggregation (Bosma et al., 2002).

Ultrasonic separation hence creates an invisible barrier for cells which is not susceptible to fouling like e.g. membrane filters. Typically, acoustic filters have been used for long-term perfusion of mammalian and insect cells and for continuous separation of yeast. The system is then positioned on a fermentor from which cells are recirculated continuously. The clarified culture medium leaves the fermentor, the concentrated cells are returned into it. For cells smaller than 3 to 5 µm, the acoustic field is too weak to withhold them and no aggregation occurs. Therefore, bacterial cells are not efficiently retained. Viable cells are typically better retained than dead ones. Cell debris and dead cells will therefore be selectively removed and viable cells enriched in a fermentor.

The resonator typically consists of a housing with one inlet and two outlets and a resonator chamber in which the ultrasonic field is generated. One outlet will contain the concentrated cells, the other the purified harvest stream. Both inlet and harvest flow can be chosen and their ratio will determine the flow of concentrated cells in the recirculation line.

The separation efficiency that can be achieved will depend on several factors. The most important ones are discussed below:

- cell concentration at the input: High separation efficiencies can be reached at cell concentrations between 0.01 and 1% wet weight of cells per unit weight of suspension (Coakley, 1997). Within this range, efficiencies typically increase at increasing cell concentrations.

- field intensity or power applied to generate the field: When more power is supplied to the resonator chamber, the ultrasonic field becomes stronger. However, more heat will be dissipated in the medium. This causes temperature gradients and induces medium streaming due to thermal convection. Because the cells have to be held back against this background streaming, a higher power supply can interfere with cell aggregation and can reduce the separation efficiency.

- time during which the field is switched on and off: The time that the field is on or off can be chosen. When the field is switched off, the cells are no longer held by acoustic forces and will sediment into the recirculation flow. This should not occur too often, otherwise the cells will not form aggregates to sediment. At high cell concentrations, the field needs to be switched off more often to avoid that too many cells accumulate in the resonator chamber.

- harvest rate: The harvest rate for a particular type of acoustic filter is specified, e.g. 10 l/d at maximum. At higher harvest rates, separation efficiencies often drop substantially.

- ratio harvest to feed flow: When this ratio is too low, the resonator chamber will fill up with cells and biomass will be lost. At too high ratios, the dragging forces from the harvest stream will be too high compared to the acoustic forces and biomass will be lost as well. For the Biosep system of Applisens, ratios between 40 and 67% are recommended. Bosma (University of Wageningen) suggested ratios between 2 and 10% from his work with microalgae (oral communication).

2.1.2 Materials and methods

In the experimental work, the commercial apparatus Biosep ADI 1015 (10-50 l/d) from Applisens was used. The aim of the test programme was to find the optimal conditions for ultrasonic separation of *Arthrospira* and estimate the potential of the system as a harvesting technique.

2.1.2.1 Preparation of Zarrouk medium

The composition of the Zarrouk medium is given in Table 1. To avoid precipitation of salts which might interfere with optical density measurements, the medium was prepared as follows: carbonate, bicarbonate and phosphate salts were dissolved in 0.5 l of distilled water, the remaining salts in a separate volume of 0.5 l distilled water in quantities for 1 liter of final medium. Both solutions were autoclaved separately. When cooled down, they were mixed in aseptic conditions and separately sterilized solutions A5 and B6 were added in the appropriate amounts.

Table 1. Composition of Zarrouk medium used for *Arthrospira* cultivation

Composition	Amount (g/l)
$NaNO_3$	2.5
$NaHCO_3$	13.6
K_2SO_4	1
NaCl	1
$MgSO_4.7H_2O$	0.2
$CaCl_2.H_2O$	0.04
$FeSO_4.7H_2O$	0.01
EDTA-Na_2	0.08
K_2HPO_4	0.5
Na_2CO_3	7.6
Solution A5	4 ml
Solution B6	4 ml
pH	9.5-11

Solution A5	Amount (g/l)
H_3BO_3	2.86
$MnCl_2.4H_2O$	1.81
$ZnSO_4.7H_2O$	0.22
$CuSO_4.5H_2O$	0.08
MoO_3	0.015

Solution B6	Amount (g/l)
$KCr(SO_4)_2.12H_2O$	0.096
$NiSO_4.7H_2O$	0.048
$(NO_3)_2Co.6H_2O$	0.049
$Na_2MoO_4.2H_2O$	0.018

2.1.2.2 Growth of *Arthrospira platensis*

Strain PCC 8005 was ordered from the Pasteur Institute. As a result of several reasons mainly related to a lack of experience with *Arthrospira* cultivation, the strain did not grow well. Firstly, it was immediately transferred to Zarrouk medium, to which it was not yet adapted. Secondly, it was transferred at a fairly high dilution. Finally, it was aerated immediately and subjected to intensive illumination. Because the inoculum had turned yellowish and the cells clumped together instead of growing in a dispersed way, it was decided to subculture an algae inoculum from EPAS reactors. It was assumed that the bacterial contamination of this culture would not affect the ultrasonic separation results because bacteria are not expected to be retained by this technique, as explained in 2.1.1. Cell suspensions were maintained at room temperature in 2-liter Erlenmeyer flasks and illuminated with maximally four cool white OSRAM Dulux S lamps (type 11 W/21-840). Illumination was gradually increased until a dark green suspension was obtained with an optical density at 750 nm (OD_{750}) greater than 1. The suspensions were continuously aerated with air, passing through a 0.45 µm filter. Twice a week, 1 liter of cells was harvested in aseptic conditions and the same volume of fresh Zarrouk medium was added to the remaining culture. By this procedure, cell suspensions were never diluted more than twofold. The harvest was used in the ultrasonic separation tests as described below.

2.1.2.3 Description of ultrasonic separation system (Biosep)

For a period of one month, a Biosep filter ADI 1015 with a harvest capacity of 10 l/d was rented from Applisens/Applitek (Figure 2).

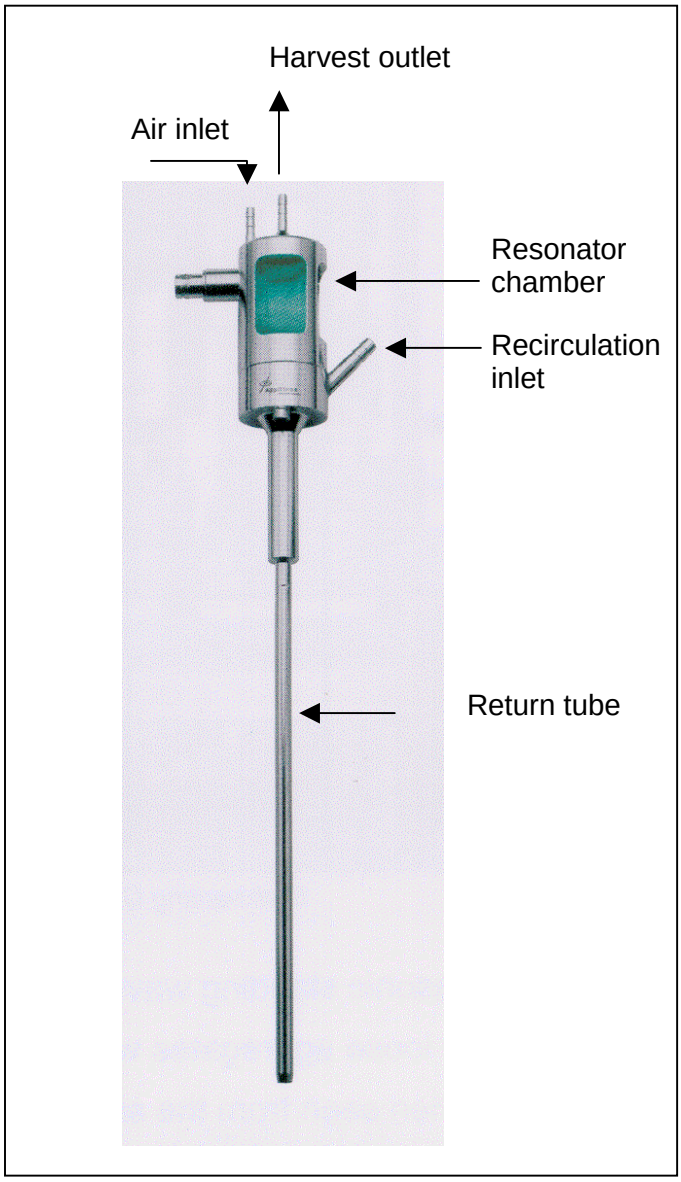


Figure 2. View of the 10 l Biosep (Applisens)

The system consists of a resonator chamber and an ADI 1015 controller. The latter contains a frequency generator, a power amplifier, an adjustable timer to specify run/stop cycle times and an automatic frequency controller (AFC) circuit that tracks the resonance frequency and provides the high-frequency voltage signal which generates the acoustic field in the resonator chamber. The controller exites a transducer which generates a resonant standing wave field at a frequency of around 2.1 MHz (User manual Applisens, 2000).

The typical configuration of the system is shown in Figure 2 and Figure 3. The resonator chamber is mounted on top of a bioreactor. The cell suspension is continuously recirculated through the resonator chamber by means of a recirculation pump. The system was first delivered with a recirculation pump of constant flow (0.96 l/h). Later, a pump with variable flow rate was obtained. The recirculation flow is split into a clarified harvest stream and a return flow. The ratio between these is determined by the harvest pump. The harvest rate should preferably not exceed the capacity of the system which is 10 l/d. Otherwise the separation efficiency may drop substantially. At regular time intervals the acoustic field and the harvest pump are stopped to recycle the aggregated cells to the bioreactor.

Operation of the ADI controller was performed according to the instructions of the manufacturer. The frequency control was left unaltered to operate in a 50 KHz range centered at 2.1 MHz. The AFC will then find the appropriate resonance frequency in this range. To obtain the desired power level (e.g. 3 W), the power scale knob was first set to 3 W and the output knob switched to zero. The output power was then slowly increased until a resonance peak was detected by the AFC, which then switches from scanning to fine tuning mode. When the output power level had stabilized, the output knob was adjusted to 100-120% of the scale. The power scale setting was then increased by 2 steps so that the power level became 65-75% of the scale (at a setting of 5 W).

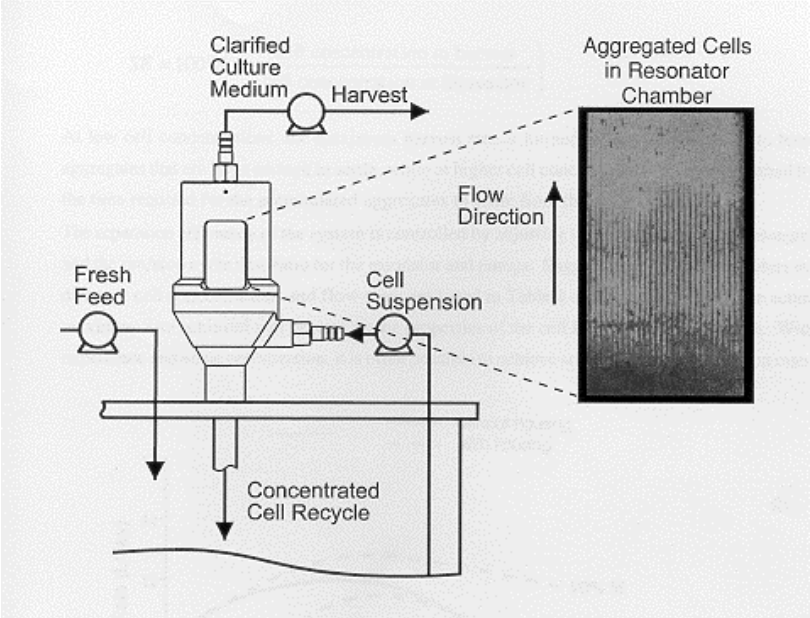


Figure 3. Typical configuration of the acoustic cell retention system (User manual Applisens, 2000).

The air inlet shown in Figure 2, is intended for cooling of the resonator chamber. According to the manufacturer however, it was not necessary to provide cooling as long as the applied voltages were not too high. Tests with yeast separation had indicated that cooling did not improve separation efficicencies.

2.1.2.4 Tests with Biosep

In our experiments, the Biosep was not used for perfusion purposes, but had to yield a concentrated algal suspension. Therefore, two different configurations could be envisaged. The first one was suggested by the manufacturer and is shown in Figure 4. In this case, the *Arthrospira* suspension is drawn from compartment IV by the recirculation pump to the resonator chamber. The clarified harvest stream and the concentrated algae are collected in different tanks for further treatment. To achieve a substantial degree of cell concentration, say 10-fold, the harvest flow must be of the same order of magnitude as the recirculation flow.

For these experiments, *Arthrospira* suspension harvested from the Erlenmeyer cultures was used as such, with OD_{750} varying between 1.0 and 1.4. The recirculation flow could initially not be varied and amounted to 0.96 l/h. At different harvest flow rates, increasing voltages were applied. For lower harvest flows, these varied between 3 and 6 W, for higher ones between 4 and 8 W. Once a recirculation pump with variable flow was available, the influence of increasing recirculation flow at constant harvest to recirculation ratio was investigated. The effect of timer adjustments was also studied.

Separation efficiency was evaluated by measurements of OD_{750} and suspended solids for feed, harvest and concentrate. In addition, several samples were analysed microscopically.

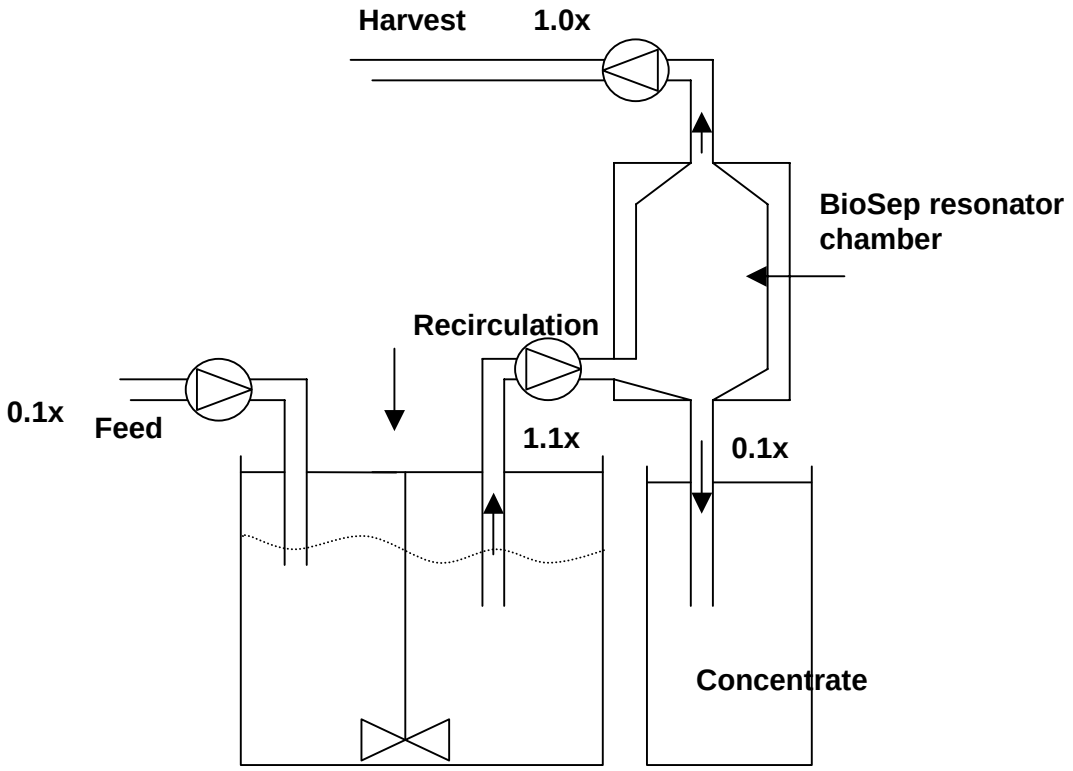


Figure 4. Concept 1: direct harvesting from compartment IV and collection of concentrate and harvest in separate tanks.

In a second configuration, the cell suspension is first removed from compartment IV and collected in a buffer tank. The Biosep filter is mounted on top of this tank and continuously removes clarified medium through the harvest stream to a desired level of concentration. The algae suspension is recirculated back to the buffer. Harvest and recirculation flows can now be varied much more independently because their ratio does not determine the final concentration level. Basically, this configuration is shown in Figure 3, except that the fermentor is now replaced by a buffer tank and that no fresh medium enters the system.

For the experiments performed according to this concept, the starting cell suspension was always diluted to an OD_{750} of between 1.0 and 1.15. A 400-ml volume was placed in a beaker and concentrated as far as possible (target 10-fold concentration). Again, variable recirculation and harvest flow rates were tested at varying ratios and at different voltages. In one experiment, a large volume of algal suspension was treated under optimal conditions (according to previous tests) to determine the maximum level of concentration without deterioration of the effluent. In one instance, a washing procedure was simulated by resuspending the concentrate in fourfold diluted Zarrouk medium to the original volume and by initiating a second cycle of ultrasonic separation.

In an additional experiment, three types of *Arthrospira* cultures were subjected to the same set of optimal conditions in order to verify whether the shape of the cells affects the separation efficiencies by ultrasonic treatment. The first culture was the one subcultured from EPAS and had long threedimensional spiral-shaped filaments. The second one was obtained from SCK and contained mostly two-dimensional curved filaments. The third one,

originating from the Pasteur Collection, had a morphology of straight filaments. At the onset of the tests, all cultures were diluted to the same OD_{750}.

The set-up used for the second configuration is shown in Figure 5.

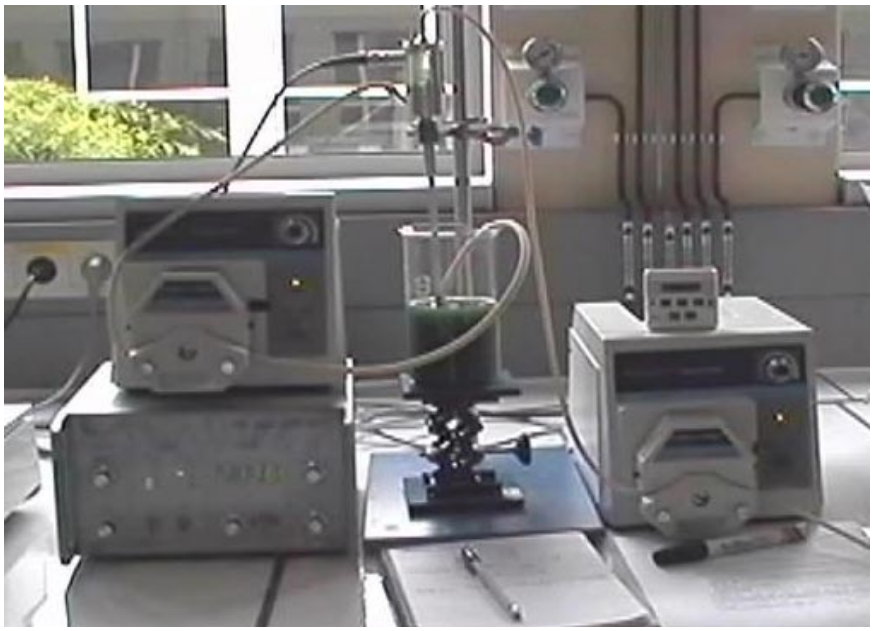


Figure 5. Picture of experimental set-up

2.1.2.5 Determination of OD_{750} and suspended solids

OD_{750} was measured against a blank of Zarrouk medium. Generally, five readings were taken and averaged to have a representative value.

Suspended solids were initially determined by filtering a known volume of suspension or sample over a predried 0.45 µm filter, and washing with an exces of distilled water. After drying at 105°C, the filter weight was determined again and suspended solids concentration calculated. This protocol was found to be highly variable in the first series of tests, probably because the removal of salts in the washing step was insufficient. When the washing step was standardised by applying 100 ml of distilled water, reproducible results were obtained. A calibration curve was determined for different dilutions of *Arthrospira* culture with Zarrouk medium by simultaneous and multiple measurements of OD_{750} and suspended solids concentration.

Particularly for the harvest stream of the ultrasonic separation tests, suspended solids (SS) concentrations were very low and calculation of the net amount of suspended solids as the difference between two similar weights would introduce a large error. Therefore, we also backcalculated the OD_{750} values to suspended solids concentrations by means of a calibration curve and used these values to determine the separation efficiencies.

2.1.2.6 Calculations

Separation efficiency = $(SS_{initial} - SS_{harvest})/SS_{initial}*100$

with SS = suspended solids

harvest = clarified outlet stream

Concentration factor = $V_{initial}/V_{final,\ recirculation}$

with V = volume

$V_{initial}$ = initial volume of suspension

$V_{final,\ recirculation}$ = final volume of concentrated cell suspension

2.1.3 Results and discussion

2.1.3.1 Relationship between OD_{750} and suspended solids concentration of *Arthrospira*

Because the growth of the *Arthrospira* cultures would be followed closely by optical density measurements, a correlation between OD_{750} and suspended solids needed to be established. In a MELiSSA report of 1992, Soyez stated that an optical density of one unit at 750 nm corresponds with a dry weight of *Arthrospira* of 1.093 g. For these determinations, samples of the algae were only filtered and not washed. We wanted to confirm these results but decided to wash the filtered cells in order to remove the excess of salts.

Only after standardisation of the washing procedure, a satisfying correlation between OD_{750} and SS measurements was obtained. The equation is given below:

$SS\ (mg/l) = 771.26\ OD_{750} - 12.653 \quad (r^2 = 0.999)$

In this case, an optical density of 1 corresponds to a SS concentration of 760 mg/l.

For the test programme the target initial cell concentration was 1 g/l. Since our cultures never reached these concentrations, it was decided to focus on OD_{750} values and keep these around 1.

2.1.3.2 CONCEPT 1: continuous ultrasonic harvesting directly from compartment IV

In the Applisens user manual operating parameters for different cell concentrations and harvest rates are suggested. Because these are not derived from tests with algal cells, these parameters settings were complemented with other suggestions to choose the starting point for optimisation of algal harvesting. Because Keyser (Applisens, oral communication) and Bosma (Agricultural University of Wageningen, oral communication) tested the same system with yeast and microalgae respectively, their experience was taken into consideration as well. Bosma suggested to keep the ratio between harvest and recirculation between 2 and 10%, to keep the intensity of the field between 2 and 6 W and to keep the ‘on’ time of the timer between 30 and 120 seconds. Data from Keyser indicate that for cell densities between 0.5 and 1 g/l, harvest rates as high as the nominal capacity

of the Biosep filter give separation efficiencies well above 95%, at field intensities between 3 and 5 W and variable on/off timer regimes.

- Effect of applied voltage on separation efficiencies

For the first experiments, the recirculation rate was set at 0.96 l/h or 23 l/d because of the type of pump delivered (fixed flow). Taking into account that the ratio between harvest and recirculation should be around 0.5, the recirculation rate was set at 0.41 l/h or around 10 l/d. This is the nominal capacity of the Biosep filter used, so this should not result in too low separation efficiencies. Initial field intensity was chosen to be between 3 W and 6 W with a timer setting of 600 s on and 3 s off.

The results of this first experiment are shown in Figure 6.

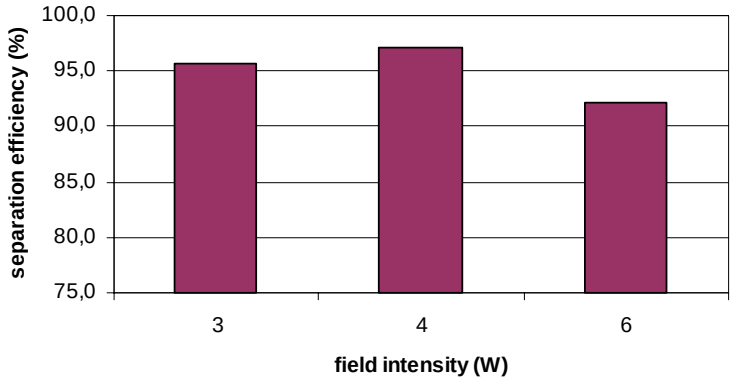


Figure 6. Effect of increasing field intensity on separation efficiency. The recirculation flow was set at 0.96 l/h and the harvest flow at 0.41 l/h.

Separation efficiencies well above 95% were obtained in this first experimental run. An increase in field intensity only improved the results to a certain level. At 6 W, the separation efficiencies dropped again. This observation may be associated with heat dissipation in the medium at higher field intensities. Thermal convection can interfere with cell agglomeration or the drag forces from convective flow may become more important than acoustic forces and will reduce the separation efficiency.

Based on the suggestion of Bosma (Agricultural University of Wageningen), the same experiment was also performed at a lower ratio of harvest to recirculation flow. At a fixed recirculation rate of 0.96 l/h, the harvest rate was set at 0.15 l/h. The same field intensity and on/off timer regime were applied. As shown in Figure 7, the evolution in separation efficiency with applied field intensity differs from the one in Figure 6. Based on SS measurements, the efficiency decreases when the field intensity is increased. Due to a high variability on the SS determinations at this stage (washing not yet standardized) and the large error introduced by subtracting two nearly identical filter paper weights for the determination of the SS in the harvest flow, we also calculated the separation efficiency with SS numbers backcalculated from OD_{750} measurements. These results do not give the same trend as the SS based values. The separation efficiency remains fairly constant at increasing field intensity and finally drops at 6 W, probably due to thermal convection phenomena, as mentioned before.

Visual observation showed that the resonator chamber was almost free of algae at the harvest rate of 0.15 l/h. This probably indicates that the field was strong enough to retain the cells before they actually entered the resonator chamber. It is logical then that a further increase in field intensity did not further improve the separation efficiency.

Decreasing the on/off sequence for application of the field, also did not much affect the separation efficiencies. At on/off regimes of 300s/3s and 180s/3s and a field intensity of 4 W, removal was between 93 and 95%, which is similar to the result presented in Figure 7.

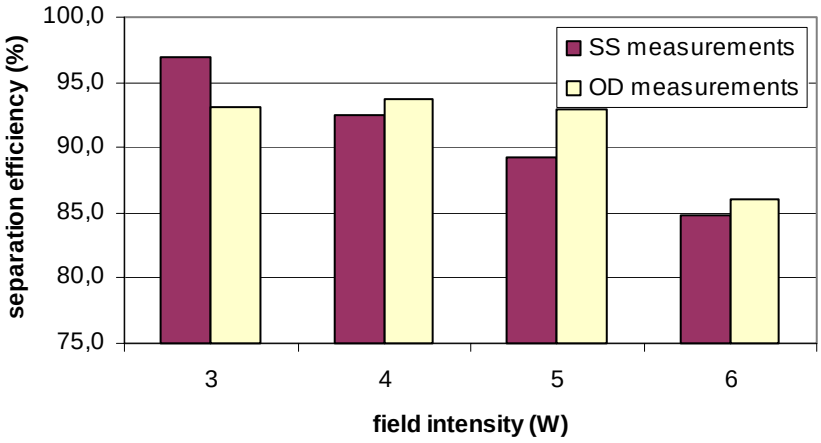


Figure 7. Effect of increasing field intensity on separation efficiency. The recirculation flow was set at 0.96 l/h and the harvest flow at 0.15 l/h. Separation efficiencies were calculated directly from SS measurements and from OD_{750} measurements backcalculated to SS values using the calibration curve.

The tested harvest to recirculation ratio of 0.16 was close to the range suggested by Bosma. The resulting concentration factor for the recirculation stream then equals 1.19. In other words, starting from an initial *Arthrospira* concentration of 0.76 g/l, a concentrate of 0.90 g/l would be obtained. From the perspective of algae harvesting, this level of concentration is inadequate. In fact, in the present concept, sufficient thickening of cells can only be achieved if the harvest to recirculation ratio approaches 1. We therefore decided not to try to optimize the ultrasonic separation in the lower range of ratios and to focus on the higher range.

For the same recirculation flow of 0.96 l/h, the harvest was now set to 0.75 l/h to achieve a concentration factor of 4.6. At higher harvest rate, power generally needs to be increased to achieve the same separation or the field has to be switched off more often to empty the resonator chamber from cells. Power was therefore varied between 3 and 8 W. The timer 'on' time was reduced at higher field intensities and 'off' time increased to give the cell aggregates the time to settle out.

In spite of the adjustments, the overall results were a lot worse than the previous ones (see Figure 8). Increasing the power did improve the achieved results, but even at a field intensity as high as 8 W, separation efficiencies did not exceed 90%. We could visually observe that at 3 W aggregation was bad and that the drag from the harvest flow was stronger than the acoustic forces which should keep the algae in the resonator chamber. At a field intensity of 6 W, aggregation proceeded satisfactorily, but when the field was switched off every 60 s to empty the resonator chamber from cells, part of the cells was rising instead of sedimenting. This of course substantially increased the suspended solids concentration in the harvest stream. At 8 W, aggregation further improved, but the same rising of cells took place when the field was switched off. This unexpected movement of cells is probably due to thermal effects, but may also be related to the variable density of *Arthrospira* that is also problematic for centrifugation and gravity-based separation techniques. Figure 9 shows cell aggregation in the resonance chamber. Cells present in the top part of the chamber have high probability to appear in the clarified' harvest stream.

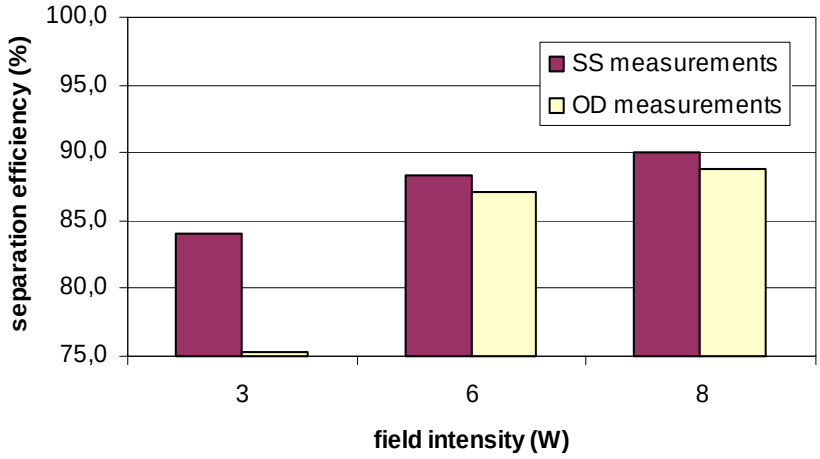


Figure 8. Effect of increasing field intensity on separation efficiency. The recirculation flow was set at 0.96 l/h and the harvest flow at 0.75 l/h. Separation efficiencies were calculated directly from SS measurements and from OD_{750} measurements backcalculated to SS values using the calibration curve.

Figure 9. Aggregation of algae cells in the resonance chamber.

Samples from the initial cell suspension and from the harvest were checked microscopically to find out whether the ultrasonic treatment affected the filament morphology and cell viability. As shown in Figure 10, the structure of the cells in both streams was found to be quite different. It was first assumed that the cells had lost their gas vacuoles or other cellular material during passage through the resonator chamber and that this might explain the absence of a 'granular' appearance in the cells from the harvest stream. However, we believe that this is not the case. The Biosep filter is known to be more effective in trapping viable cells than dead ones. During yeast fermentations, this property is advantageous in the sense that dead cells are selectively removed from the fermentors. At the same acoustic field power, the escape rate of non-viable cells can be about 5% higher than the one for viable cells. Considering the fact that the acoustic energy is low, that shear stress is low and that the system has been applied successfully for cell perfusion, it is unlikely that passage of *Arthrospira* through the resonator results in cell death or lysis. Rather, non-viable cells will be enriched in the harvest due to a higher escape rate.

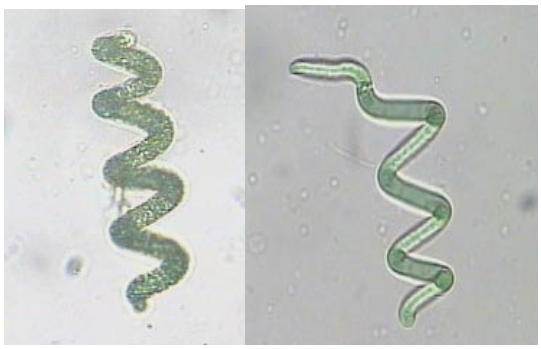


Figure 10. Microscopic view (400x) of *Arthrospira* in suspension before ultrasonic treatment (left) and in the harvest (right).

From the present results, it seems that the effect of increasing field intensity on the separation efficiency is highly dependent on the ratio of the harvest to the recirculation flow. Therefore, the recirculation pump with fixed flow was replaced with another one with variable flow. This allowed us to vary the ratio at different absolute recirculation flows.

- Effect of harvest flow on separation efficiencies

When the best results obtained in the previous experiments for a given harvest rate were plotted against the harvest flow (see Figure 11), it is clear that separation efficiencies start to decrease drastically at the higher range. The increase in field intensity applied can probably not compensate for the increased fluid drag of cells through the resonator chamber. To find out whether this is due to the absolute harvest flow or rather the ratio of harvest to recirculation flow, additional experiments were performed at lower recirculation flows of 0.33 and 0.45 l/h. Different harvest to recirculation ratios were tested by varying the harvest flow.

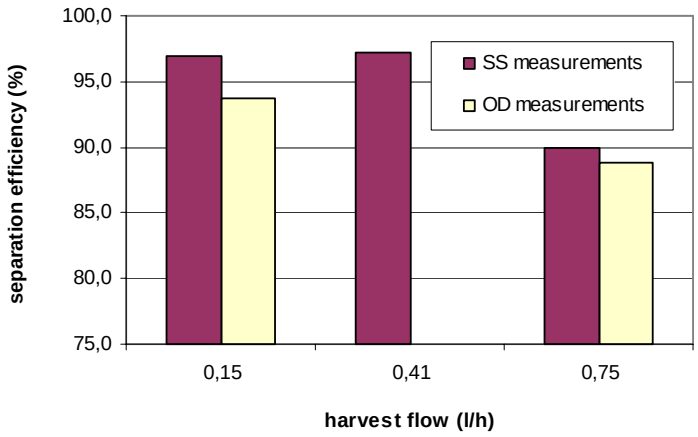


Figure 11. Variation of separation efficiency with harvest flow at a fixed recirculation rate of 0.96 l/h. Values are the best results obtained at a given harvest flow over different field intensities.

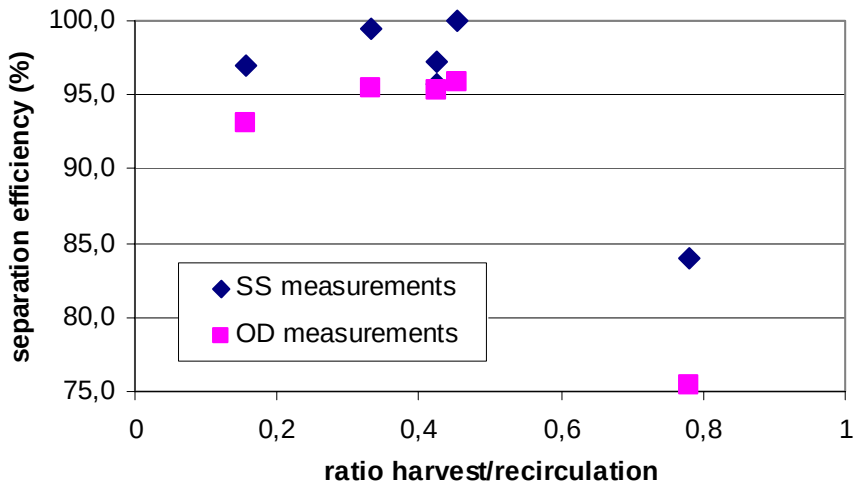


Figure 12. Effect of increasing harvest/recirculation ratio on separation efficiency. All tests were performed at a field intensity of 3 W and an on/off timer regime of 600 s/3 s.

In Figure 12, all the results obtained at a field intensity of 3 W were plotted against the harvest to recirculation ratio. An optimum seems to exist around a ratio of 0.4, which is close to the range suggested by the manufacturer of between 0.3 and 0.5. In addition, similar separation efficiencies were obtained at different setpoints of harvest and recirculation flow. It therefore seems that particularly the ratio of flows is important with regard to the separation results, at least in a range of flows below the nominal capacity of the Biosep filter.

Because the degree of cell concentration in the present configuration was insufficient for the application envisaged, further tests at still other flow rates were not performed.

- Effect of on/off timer settings on separation efficiencies

It is suggested that the on/off timer regime should be adjusted when higher cell concentrations have to be trapped in the resonator chamber. To avoid accumulataion of cells the 'on' time should be decreased and the 'off' time increased to allow the aggregates to settle. From the information we have, it seems that it is rather a trial and error process to try to optimize these settings. From the experience we have it seems that a decrease in 'on' time does not much affect the results when separation efficiencies are high. On the contrary, each time the field is switched off, part of the aggregates starts to float in the resonator chamber instead of settling and leaves the system with the harvest flow. It is therefore advisable to keep the 'on' time as long as possible. We also believe that further optimisation of these settings should be part of the test programme with the breadboard, provided that ultrasonic separation will be the selected harvesting step.

Because the present concept does not allow for a high degree of cell concentration, we decided to switch to a second concept, in which *Arthrospira* cells would be harvested from Compartment IV and stored in a buffer tank. When the Biosep filter is mounted on the buffer tank, cell concentration can take place by continuous extraction of culture medium and recirculation of the trapped cells through the return tube to the same tank.

2.1.3.3 CONCEPT 2: batch ultrasonic *Arthrospira* thickening from a buffer tank

- Effect of field intensity on separation efficiency

As for concept 1, separation efficiencies were measured for different harvest and recirculation flows at increasing field intensities. In all experiments, a tenfold thickening of the algal suspension was aimed for. At flow rates of 0.15 and 0.33 l/h respectively, high efficiencies well above 95% were obtained (see Figure 13). At a field intensity of 4 W these were close to 100%. Therefore, higher power inputs were not tested.

At higher harvest and recirculation flows (see Figure 14), separation efficiencies of close to 100% were obtained when field intensity was increased to 5 W. As expected, a stronger field has to be applied to achieve the same degree of cell separation at higher harvest rates. The high efficiencies correspond well with the optimal results mentioned in literature for yeast and mammalian cell applications, which indicates that the system is also well suited for the separation of algae. Based on a model to predict efficiencies, Bosma *et al*. (2002) stated that after optimisation, microalgae can be harvested with an efficiency of 93 ± 14% and a concentration factor of 11. Although the results might depend on the type of algae used, our data are definitely better than the ones predicted by these authors.

The fact that equally high separation efficiencies can be achieved at much higher harvest and recirculation rates is interesting from the point of view that thickening of a batch of algae can be achieved in a much shorter period of time.

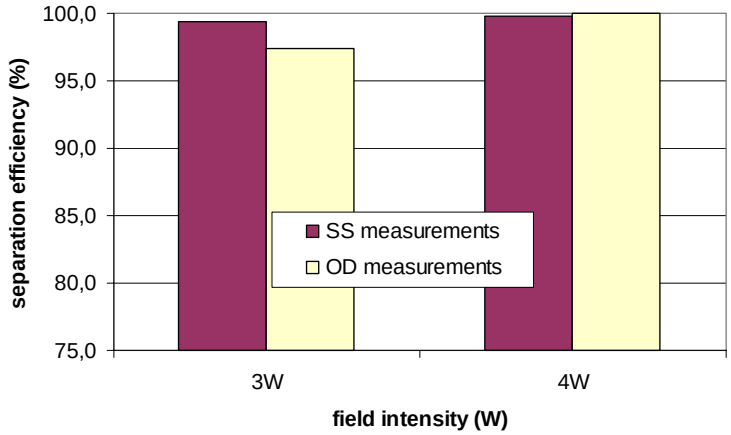


Figure 13. Effect of increasing field intensity on separation efficiency. Cells were concentrated 10-fold. Harvest and recirculation flows were 0.15 and 0.33 l/h respectively

Visually, conditions for optimal cell separation were characterized by the absence of cells in the resonator chamber and consequently the absence of cell aggregate lines. The field was then apparently strong enough to prevent the cells from entering the resonator chamber. To our experience, breakthrough of cells in the harvest line would occur soon after the appearance of cells in the resonator chamber. This generally happened quite suddenly as if large numbers of cells had accumulated at the bottom of the chamber and clogged the entrance. Presumably, numerous aggregates could at some point no longer be retained by the acoustic forces.

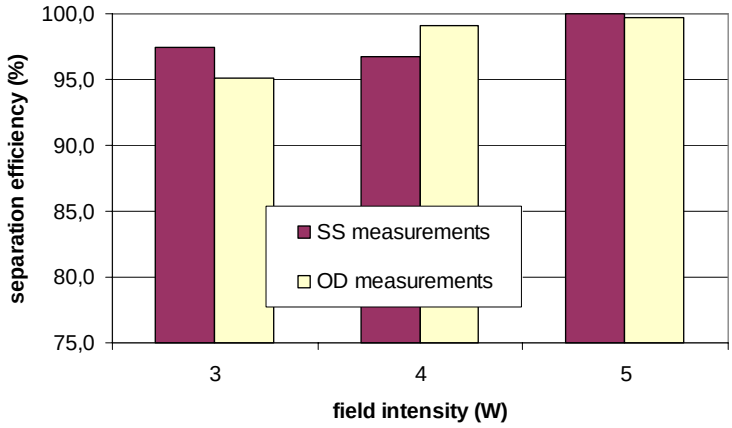


Figure 14. Effect of increasing field intensity on separation efficiency. Cells were concentrated 10-fold. Harvest and recirculation flows were 0.41 and 0.96 l/h respectively.

- Effect of flow rate on separation efficiency

  Tests were performed at different harvest and recirculation rates, as mentioned above. Results were very good as long as the harvest rates remained in the order of magnitude of the nominal capacity of the Biosep filter (see Figure 13 and Figure 14). When the flow rates were further increased at the same harvest to recirculation ratio, the separation efficiencies were no longer satisfying at a reasonable field power input. The remaining experiments were therefore performed at the highest tested flow rates still giving good results, i.e. a harvest flow of 0.41 l/h and a recirculation rate of 0.96 l/h.

- Concentration factor

  To be able to see the potential change in separation efficiency in the course of the experiment, i.e. at increasing cell concentrations, visual observations were complemented with OD_{750} measurements at frequent time intervals. As an example, the evolution in OD_{750} is shown for two experimental runs in Figure 15. Apparently, a constant separation efficiency could be achieved when a field intensity of 5 W was applied from the onset of the experiment (test B). This was however not true for test A with a lower initial power input of 3 W. As cell thickening proceeded, a gradual deterioration of the water quality in the harvest line was observed. This could be counteracted temporarily by an increase in field intensity, which was applied after 20 min or at a concentration factor of about 2, and again after 40 min or at a concentration factor of about 4. The strategy to gradually increase power inputs however proved to be less effective then starting at a higher but constant power input. According to our experience, it seems difficult to obtain a clear harvest once cells have appeared in large numbers in the chamber. Indeed, each time the ultrasonic field is switched off, some of them will rise and appear in the harvest line. Therefore, it is better to apply a stronger though not excessively high field and prevent the cells from entering the resonator chamber in the first place.

  Experience in fermentation industry where Biosep is applied, indicates that a continuous adjustment in operational settings is necessary to achieve optimal separation efficiencies and control cell bleeding because cell concentrations in the fermentors vary in time. For our experiments, more detailed experiments on this matter will be included in the test plan of the breadboard in case the trade-off is in favor of the Biosep filter.

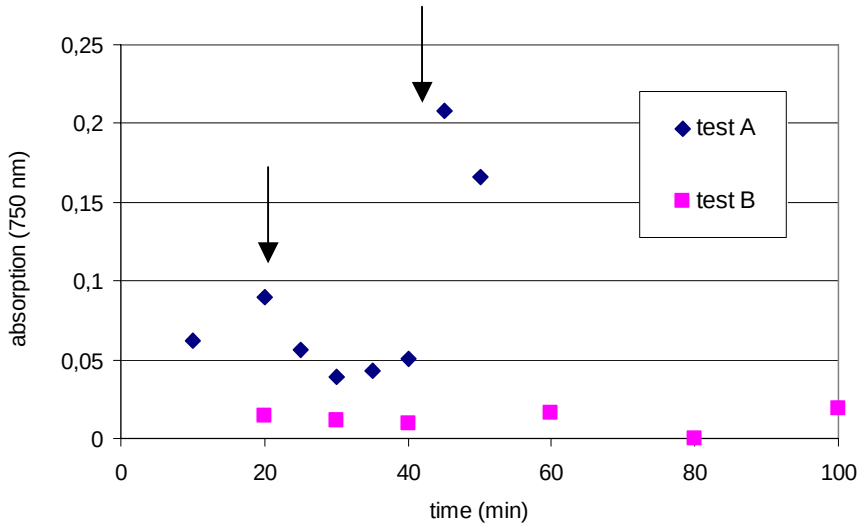


Figure 15. Change of OD_{750} in time for the harvest line during ultrasonic separation of *Arthrospira*. Harvest flow was 0.41 l/h and recirculation flow 0.96 l/h

The initial on/off timer regime 600 s on/4.5 s off, and the OD_{750} of the start solution between 1.0 and 1.1. In test A, field intensity was gradually increased from 3 W to 4 W after 20 min (first arrow) and to 5 W after 40 min (second arrow) with a simultaneous decrease in duration of 'on' time to 60 s. In test B, field intensity was kept constant at 5 W.

In a next experiment we wanted to concentrate the algae suspension to biomass levels of between 10 and 20 g/l. Again harvest rate was set at 0.41 l/h, recirculation rate at 0.96 l/h, timer regime was 300 s 'on' and 4.5 s 'off' and field intensity was 5 W. An initial cell suspension volume of 1200 ml was concentrated to 54 ml, corresponding to a concentration factor of 22. Because the recirculation from the Biosep filter has to be submerged in the cell suspension to avoid contact with air (risk of cavitation), it was not possible to reduce the final volume of the suspension more. Starting with a cell concentration of 0.7 g/l, a concentrate of 16.6 g/l was obtained, which corresponds fairly well with the volumetric concentration factor. The overall separation efficiency was higher than 99%. As the test was stopped after 3 hours of treatment, breakthrough of algae was observed. We therefore believe that at the chosen conditions, thickening to higher concentrations is not advisable. In this respect, it is interesting to note that no results were found on tests with yeast cells at concentrations higher than 20 g/l.

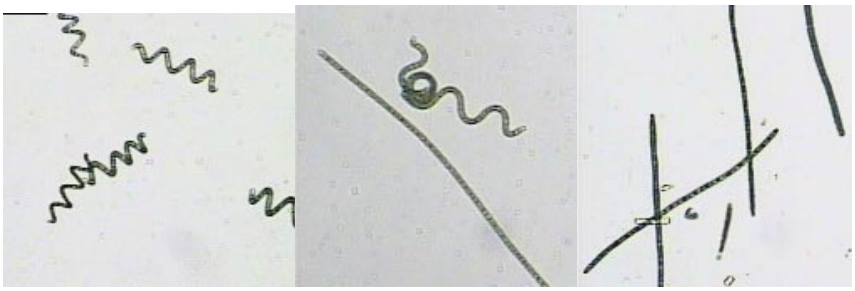
As opposed to the observations in 2.1.3.2, neither the harvest or the concentrated cell suspension seemed to contain dead or injured cells. This is another indication that the ultrasonic treatment does not affect the viability of the algae and that the earlier presence of empty cells in the harvest was due to a preferential trapping of viable cells in the acoustic field and preferential leakage of dead cells.

As a final remark, we want to mention that gas bubbles appeared in the resonator chamber during several tests. As long as these remain at fixed positions in the chamber, they will not cause turbulence or interfere with the separation process (Keyzer, personal communication). They probably originate from dissolved gas in the medium which might be liberated due to temperature changes.

- Effect of cell shape

Since more or less optimal separation conditions had been identified, we wanted to obtain further information on the effect of different cell shapes on the separation efficiency. Three different cell samples were subjected to a field intensity of 5 W, a harvest rate of 0.41 l/h and a recirculation rate of 0.96 l/h. The first one originated from EPAS and had been used in all the previous experiments. Cells in this culture generally had a nice spiral shape. The second culture was obtained from SCK and was found to contain straight curved cells. The third culture was also provided by EPAS and was subcultured from a PCC inoculum. The cells were mostly straight and not curved. Our own PCC culture which had recovered from the initial light shock (mentioned in 2.1.2.2), also contained spiral cells and was therefore not included.

When the shape of the cells was checked microscopically at the onset of the tests, some changes had occurred in that the culture from SCK, now contained straight cells and a fraction of spiral-shaped filaments, but no more curved cells. Typical pictures are shown in Figure 16.



(a) (b) (c)

Figure 16. Microscopical observation of three *Arthrospira* cell suspensions at the start of a comparative experiment. (a) spiral filaments in a culture from EPAS, (b) mixed spiral and straight filaments in a culture from SCK, (c) straight cells in a PCC-subculture from EPAS.

Separation efficiencies were very different for the three different cultures, as indicated in Table 2. Because breakthrough of cells was observed after 10 min already for the SCK culture, the test was stopped at that point. Separation efficiency was as low as 70%. For the PCC-derived culture consisting of straight filaments only, the results were better but not satisfying. The spirally shaped filaments clearly gave the best separation results.

Table 2. Separation efficiencies for three differently shaped *Arthrospira* cultures at identical test conditions.

Shape/origin	**Separation efficiency (%)**
Spiral, EPAS	98%
Spiral and straight filaments, SCK	Due to breakthrough of cells, test was stopped at an early stage
Straight filaments, PCC	90%

The test demonstrates that cell shape or culture condition does have an impact on the efficiency of the technique. Whether this is due to a difference in 'working' diameter of the filaments in the ultrasonic field or a different growth condition of the cells or rather a different level of aggregation of the different cell shapes, is not clear at this point.

- Cell integrity

In different tests, the integrity of the algal cells was checked by microscopical observation. No indications were found that the ultrasonic forces induced cell lysis or loss of cell viability or the release of cell constituents.

To confirm these results, protein determinations according to the Lowry protocol were made on filtered samples from the initial *Arthrospira* suspension, the harvest and the recirculation or concentrate line. According to our measurements none of the samples contained any proteins. If we can assume that the high salt concentration of the Zarrouk medium did not interfere with the determinations, this would indicate that no cell lysis occurred and no cell constituents were released during tenfold or higher concentration of the cells. In fact, this is not surprising because the ultrasonic separation is typically used to retain viable cells in fermentors. It does not interfere with the viability of the yeast or mammalian cells in the perfusion cultures for which it is applied.

- Washing procedure

  In one instance, a washing procedure was simulated as follows. A cell suspension was thickened 10 times, then 10 times diluted to the original cell concentration with 4-fold diluted Zarrouk medium and finally subjected to a second concentration test under the same conditions. Due to samplings from the concentrated suspension after the first test, the suspension could not be diluted to the original volume and hence, thickening in the second run was less than in the first one. However, over similar periods of time, similar separation efficiencies were obtained. In addition, cell desintegration due to the osmotic shock was not observed. These results indicate that the tested concept can be used for thickening and washing and that the separation efficiency is not affected in a second cycle of ultrasonic treatment. Whether washing with demineralized water results in cell disrupture was not investigated in this test.

- Reproducibility

  Ultrasonic treatment of the same *Arthrospira* culture harvested at different time points but subjected to the same operational conditions, showed a fairly good reproducibility. Repeated experiments at optimal conditions showed a variation in separation efficiency between 97 and 99%, which is considered to be satisfactorily taking into account the variability in shape and growth conditions of the cells with time.

- energy consumption

  Energy consumption was calculated for the following conditions: harvest of 100 l *Arthrospira*/d and concentrating from 1 tot between 10 and 20 g/l. Because the cells have to be harvested and washed several times over a period of one day, we assumed that the volume of 100 l had to be concentrated 10- to 20-fold in about 2 hours. This means that the clarified harvest stream from the resonance chamber needs to be around 50 l/h and the recirculation rate around 100 l/h.

  The following information on power consumption was obtained from the manufacturer:

  - Biosep controller: 150 W at maximum (assumed, not available from manufacturer). Taking into account that the maximal output power is 15 W and that optimal separation conditions were achieved at 5 W, we assume that power consumption will amount to approximately 50 W.

  - Acoustic resonator assembly: estimated to be between 100 and 400 W. As for the controller, we assume that the actual consumption amounted to about one third or say 100 W.

- 2 pumps with a capacity of 200 l/h: each 250 W at maximum. One of the pumps will operate at approximately one third of its capacity (harvest line), the other one at two third (recirculation line). Conservatively we therefore assume an energy consumption of 250 W.

This means that the total power consumption amounts to 400 W to harvest 100 l/d. Because the cells will be harvested over a period of 2h, the energy consumption amounts to 8 kWh/m³.

- Total mass of the system

  Mass was calculated for the same assumptions as for the energy calculations. The Biosep controller for a 40 l/h system weighs 50 kg, the resonator assembly 120 kg and the two pumps 20 kg each. Tubing weight is negligible. Total mass of the system would be around 210 kg. Taking into account a harvest of 100 l/d this amounts to 2100 kg/m³.

2.1.4 Conclusions

From the present results ultrasonic separation seems to be a promising technique for the batchwise harvesting of *Arthrospira*. Ten- to twentyfold thickening to concentrations between 10 and 20 g/l of dry matter can be achieved and washing cycles can be performed in the same apparatus. Separation efficiencies can be optimized to 99% and dead cells are preferentially removed from the concentrate which is to be further processed as food. The technique is non-destructive, not susceptible for fouling and can be performed in axenic conditions. Disadvantages are however that

- probably continuous adjustment of operational parameters to varying cell concentrations is required
- the separation efficiency depends on the cell shape or the growth condition of the cells and never attains 100%
- a risk of breakthrough always exists
- it is partially based on gravitational forces, which might be problematic in view of the varying physical characteristics of *Arthrospira*
- the clarified water is not completely free of cells, even at optimal separation efficiencies

This implies that the technique must be coupled with an additional membrane filtration step before the clarified water can be subjected to desalination by reverse osmosis or electrodialysis.

In addition, energy consumption is high.

2.2 Membrane technology: Filtration

2.2.1 Principle

Filtration (dead-end or tangential, with screens or membranes) consists in the separation of two or more components from a fluid stream, using generally, pressure as driving force. In conventional usage, it usually refers to the separation of solid, immiscible, particles from a liquid. Membrane filtration extends this application further to include the separation of dissolved solutes in liquid feeds. The primary role of a membrane is to act as a selective barrier (Figure 17). It should allow the passage of certain components and retain other ones of a mixture. As a consequence, either the permeating stream or the retained phase should be concentrated in one or more components. Among membrane separation processes themselves, the distinction between the various processes is based on the nature of the active force (which controls the transport process) and the size of the retained particles or molecules (Table 3).

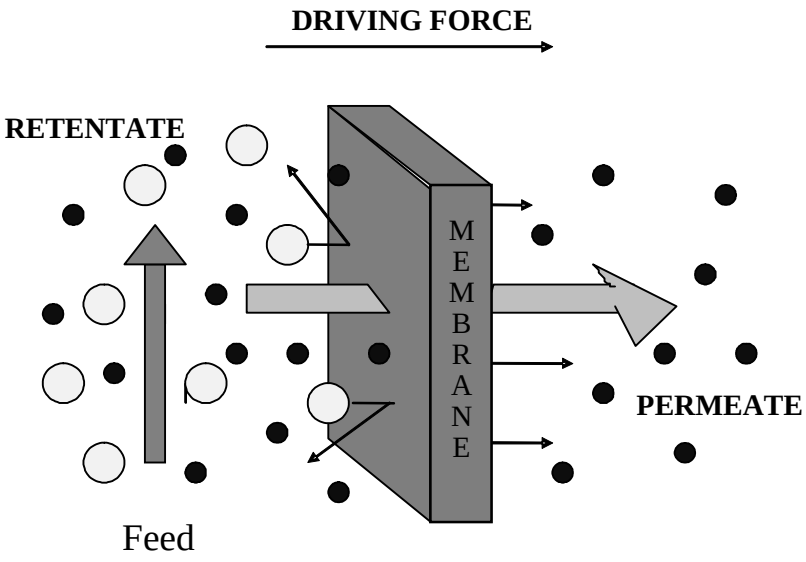


Figure 17. Principle of membrane separation process.

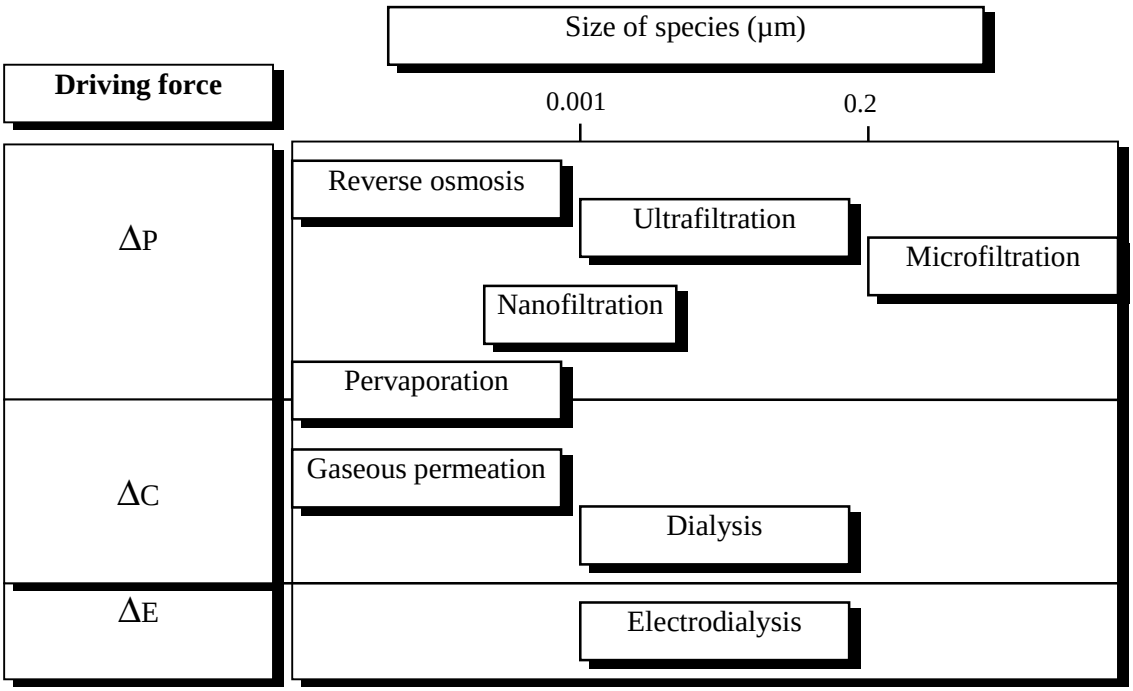


Table 3. Classification of membrane separation processes.

During pressure filtration, the pressure will be gradually increased to avoid damage to the filters. When a specified pressure is reached, operation of the system will proceed at a fixed overpressure. As a result of cake build-up, the filtrate flow will decrease in time. It can be

theoretically calculated that a linear relationship exists between the ratio time/filtrate volume and the cumulative filtrate volume. The slope itself is proportional to the specific resistance to filtration of the cake.

The choice of the membrane and the type of filtration (microfiltration, ultrafiltration, nanofiltration, diafiltration, …) is not innocuous. Tests have been carried out to compare performances for *Arthrospira platensis* harvesting with microfiltration and ultrafiltration membranes. The following operating parameters have to be taken into account in the choice of a membrane: organic or inorganic membranes, influence of membrane molecular weight cut-off, influence of membrane materials on fouling, adsorption, selectivity, … on top of which comes the membrane regeneration stage. The complementary experiments for the choice of the type of membranes (organic or inorganic) for A*rthrospira platensis* concentration are not reported in this note. The study was already conducted by GEPEA-Nantes and is actually under investigations. The detailed experimental data will be reported in TN 72.7.5.

The direct concentration of the culture by nanofiltration presents the advantage of simultaneous concentration of cells, cell-fragments, dissolved proteins and amino-acids. Moreover, the use of the same pilot-plant for the concentration step and the final desalting procedure is possible. Nanofiltration would allow to obtain a liquid concentrate directly usable for diet preparation. However, the quality of the final product can be affected, indeed cells are probably partly disrupted due to circulation in high pressure pumps and numerous passages in throttling valves.

In this section, we aimed to compare the filterability or the resistance to filtration of differently shaped cultures of *Arthrospira.* It was assumed that this might give an indication of the fouling the cultures may cause during membrane filtration. Complementary experiments dealing with the study of the fouling in terms of hydraulic resistance, influence of the operating conditions (resistance, velocity and cell concentration) will be widely reported in TN 72.7.5.

2.2.2 Materials and methods

In a first test, *Arthrospira* suspensions were filtered in conditions which are similar to real pressure filtration, in the sense that pressure was gradually increased. At the onset of the experiment 500 ml of suspension was brought in a closed mini pressure filter with a diameter of 90 mm and filtered over 0.45 µm. The chamber was closed and the first filtrate was collected. Pressure was then increased every 20 s with steps of 1 bar and the amount of collected filtrate determined. At a final pressure of 6 bar, filtrate volumes were determined at regular time intervals until the vacuum breaks.

The second test was performed to determine the actual resistance to filtration. A 1 l volume of suspension was dewatered over a 0.45 µm filter at a constant overpressure of 1 bar. The filtrate was collected and at regular time intervals the volume was determined.

The ratio time/filtrate volume was plotted against the cumulative filtered volume. The specific resistance to filtration can then be determined from the slope of the initial linear part of the curve according to the following formula:

$$R = 2 P A^2 b/\mu W$$

with R = specific resistance
P = overpressure
A = filter surface

b = slope of plot time/volume versus volume
µ = viscosity of the filtrate
W = weight of cake/filtrate volume

2.2.3 Results and discussion

Results of the first test are presented in Figure 18. Apparently, the filterability of differently *Arthrospira* cultures does depend on their shape, although slightly different curves were also obtained for two cultures with the same spiral shaped filaments (EPAS and PCC). More significantly, the time required to filter the same volume of suspension increased substantially when the fraction of straight filaments increased. This seems to indicate that the spiral filaments are easier to filter under the chosen conditions. However, it cannot be excluded that differences in cell growth condition and extracellular polymers content also affected the filterability. Overall, the filterability of all solutions is good, probably as a result of the low cell concentration in the suspension.

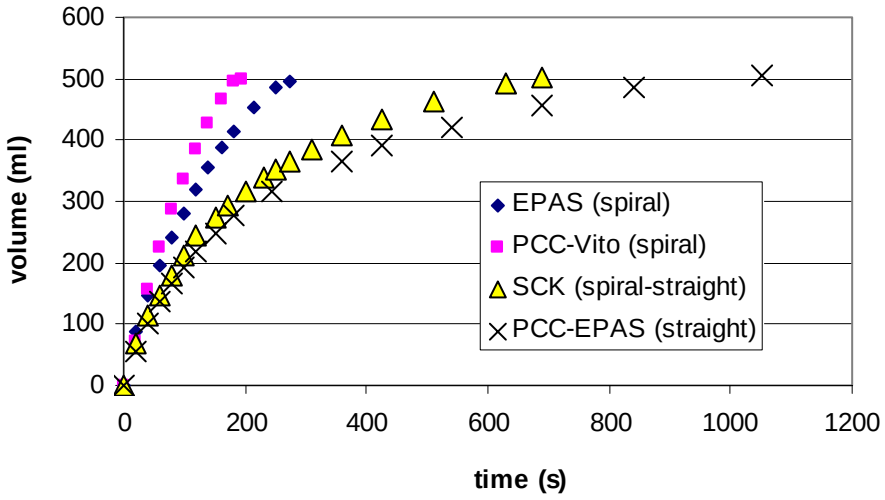


Figure 18. Pressure filtration curve for differently shaped *Arthrospira* suspensions at increasing pressures from 1 to 6 bar.

To be able to determine the exact specific resistance to filtration, an additional test at fixed overpressure was conducted. The ratio time/filtrate volume was plotted versus the cumulative filtered volume (see Figure 19) to allow the calculation of the specific resistance to filtration. It has to be mentioned that this type of calculation is normally used for the filtration of concentrated sludge suspensions. A real sludge cake is formed during the filtration procedure, which presents a certain resistance to filtration. In the present tests, the concentration of *Arthrospira* was too low for a cake to form. This probably explains the atypical form of the plots. Except for the spiral PCC culture from Vito, none of the curves seems to contain a linear part from which the specific resistance to filtration can be calculated. Still, an attempt was made to calculate the specific resistance to filtration. Values were always above 2.10^{12} m/kg which would imply a rather poor filterability.

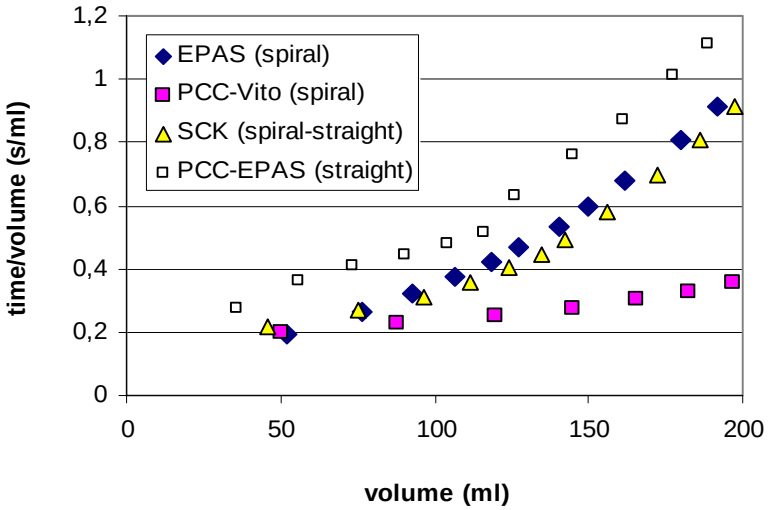


Figure 19. Pressure filtration curve for differently shaped *Arthrospira* suspensions at a constant overpressure of 1 bar.

The order in filterability which had been observed in the first test and seemed to be related to the morphology of the cells, is no longer apparent. In addition, the behaviour of two cultures with the same morphology was not similar at all.

Remark

Suspended solids concentrations were much lower than could be expected from the OD_{750} measurements.

2.2.4 Conclusions

Arthrospira cultures with different morphology were compared in pressure filtration tests. Testing at increasing pressure indicated that straight filaments were less easily dewatered than spiral shaped ones. Attempts to calculate the specific resistance to filtration at constant pressure were unsuccessful. An atypical filtration curve was obtained probably because the cell concentration was too low or because the physiology of the cultures was different. The tested cultures showed a different filtration behaviour, but this no longer seemed to be related to their morphology.

From the present results, it is not possible to evaluate the potential for fouling of the different morphologies for membrane filtration applications. It is evident however that filterability will vary largely depending on culture conditions of the algae.

2.3 Centrifugation

The separation technique proposed in this study is the centrifugation. Two common speed intensities were used: Low speed centrifugation at speed below 10.000 rpm and Super Speed Ultracentrifugation at speeds between 10.000 and 20.000 rpm. Because of difficulties, related to find a laboratory having the appropriate material to perform the Ultracentrifugation at speed higher than 20.000 rpm, the latter was not investigated in this study.

In the present work, the centrifuge is envisaged to recover the cells, producing a paste with a high percentage of liquid elimination (up to 99%) and conserving the integrity of the harvested cells.

2.3.1 Principle

The system, which was selected to be tested for the harvesting of Arthrospira platensis, consists of a centrifuge. This is a device for separating particles from a solution according to their size, shape, density, viscosity of the medium and rotor speed. In biology, the particles are usually cells, sub cellular organelles, viruses, large molecules such as proteins and nucleic acids. For filamentous cells, Low-Speed, Super Speed Ultracentrifugation and Ultracentrifugation are mainly used.

2.3.2 Material and methods

2.3.2.1 Organism and culture conservation

Two strains of *Arthrospira platensis*, type PCC8005 were used for the centrifugation tests. One was ordered at Pasteur Institute (Institut Pasteur, France, web-site: www.pasteur.fr and e-mail: ntmarsac@pasteur.fr) and the second was kindly provided by GEPEA-Nantes-University. Despite the fact that both originated from the same strain and cultivated in the same Zarrouk medium in EPAS for long time, they were remarkably different in shape. *A. platensis* of Pasteur Institute was coiled type as shown in Figure 20 and the one of GEPEA-Nantes was 100% of straight form (Figure 21)

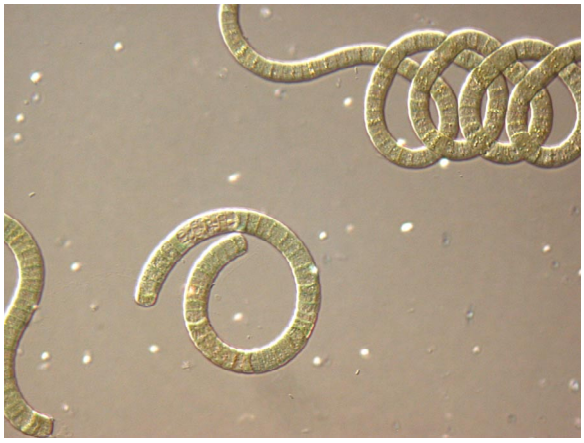


Figure 20. Coiled and S-shaped *Arthrospira platensis* PCC 8005, viewed with phase contrast microscopy (400 x enlargement). From *Pasteur Institute* -France

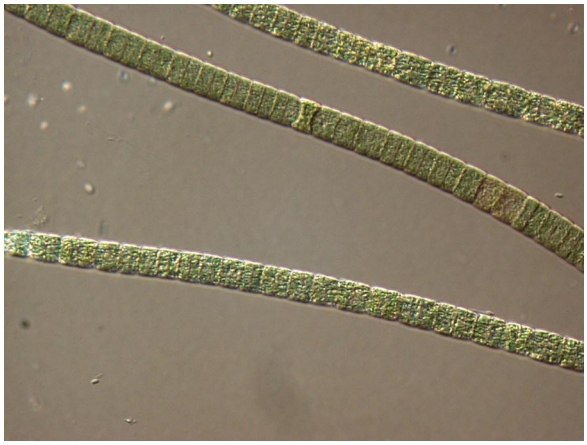


Figure 21. Straight *Arthrospira platensis* PCC 8005, viewed with phase contrast microscopy (400 x enlargement). From GEPEA-Nantes, France

2.3.2.2 Growth of Arthrospira platensis

As mentioned above, two different forms of *Arthrospira platensis* type PCC8005 were tested. The strain ordered from Pasteur Institute was difficult to cultivate because of the differences in salt concentrations of the initial feed medium (see TN 72.7.1, BG-II + ASN-III be; Pasteur Institute -France, Rippka, 1988) and Zarrouk medium. The culture could, effectively, be used for the tests when concentrations of 1 g/L were obtained after 3 weeks, in sufficient volumes in Zarrouk medium

The culture obtained from GEPEA-France was easy to cultivate in Zarrouk medium despite the fact that the initial culture medium contained high salt concentrations (ALPHA BIOTECH-France) with 20% seawater (see annex: culture).

In EPAS laboratory, permanent cultures of both *Arthrospira platensis* forms were kept in Erlenmeyers-flasks with Zarrouk medium at low light intensities originated from 6 cool white fluorescent lamps OSRAM Dulux S 11W/21-840 each. The measurements of the light intensity were done with a luxmeter probe type Testo 545. Illumination was gradually increased until a dark green suspension was obtained. The optical density (OD), measured at 750 nm was measured and the suspensions diluted, if necessary, to $OD_{750\ nm}$ slightly greater than 1, corresponding to a concentration of 1-1.2 g/L. The cultures were continuously aerated with air-pumps (120L/h), passing through a 0.45µm filters. The harvested suspensions were used for the centrifugation tests as described below.

2.3.2.3 Description of the centrifugation system

The system was based on the rate or speed at which the centrifuge was turning. As mentioned above, two speed centrifugations were tested for the liquid-solid separation of *Arthrospira platensis*: Low Speed centrifugation at speeds of 6000 rpm and 7000 rpm and Super Speed Ultracentrifugation at speeds of 10.000 rpm, 12.000 rpm and 13.000 rpm. The centrifugation time was set at 15 minutes in all experiments and the temperature during centrifugation was 4°C. For each test, the centrifugation tubes were autoclaved (120°C, 20 mn) to avoid contamination of the culture during harvesting. The centrifuged volumes were 40 ml with 4 replicated for each tested speed. The results were representing the mean value of the four readings to have an accurate value.

2.3.2.4 Operation of the centrifuge

- Clear digital display and easy-to-use knobs
- Automatic “soft-touch” locking
- Setting in rpm or in x g
- Extremely quiet operation
- Separate short spin button
- Speed can be set from 1000 x g to 18,000 x g
- Timer can be set up to 99 minutes or continuous run

Model	**Sigma 2K15 (Van der Heyden)**
Capacity (ml)	4 x 40
Maximum Speed (x g)	18,000
Speed Control	Variable, microprocessor controlled
Energy max	750 W
Timer	30 sec. to 99 min + hold

2.3.2.5 Determination of $OD_{750\ nm}$, Suspended Solids and microscopic analyses

Separation efficiency was evaluated by measurements of OD_{750nm} and suspended solids and completed by microscopic observations of the filtrate and concentrate to investigate the state of the cells after treatment.

2.3.2.6 Biomass measurements

The biomass concentration is directly related to the optical density when the latter is below 1 (Beer-Lambert law):

$$\text{Biomass (g/L)} = \varepsilon \times \text{OD}$$

The biomass is then determined by measuring the optical density at a wavelength of 750 nm. At this wavelength, neither the Cyanobacteria nor the Exopolysaccharides absorb, it is only the diffusion of the light through the cells, which is being measured. This measurement is an indirect method for biomass determination and the best approach is the correlation of dry weight (DW) to optical density (OD).

To allow effective suspended solids measurements, known volume of harvested samples were first filtrated through a 0.45 µm pre-dried MILLIPORE® filter, washed up to three times (3 x 20 ml) with distilled water. The suspended solids (SS) concentration was determined after drying the sample at 105°C until the sample weight remained constant. A calibration curve was determined with different dilutions of *Arthropira* culture where both SS and OD_{750nm} were simultaneously determined.

2.3.2.7 Optical density measurements

At an optical density of 750 nm neither the Cyanobacteria nor the exopolysaccharides absorb. It is only the light through the cells, which is being measured. Also chlorophyll a, phycocyanins and Beta-carotene do not contribute (Cornet, 1992). The optical density measurement is an indirect method of biomass growth determination. In most cases, the best approach is the correlation of dry weight (DW), replaced in our experiments by suspended solids determination (SS) to optical density (OD) determined at 750 nm.

2.3.2.8 Microscopic observations

The aim of the microscopical analyses of the algal suspensions after harvesting was to verify if the cells conserve or not their integrity after centrifugation. The filtrates as well as the concentrate were directly observed with phase contrast and light microscopy (type Microscope Olympus). The images where loaded on PC with the image Pro Express program. All images were observation at 400 x enlargement.

2.3.2.9 Mass balance

The establishment of the mass balance was based on the determination of the suspended solids of the initial suspension, the concentrate and the filtrate after centrifugation. The calculations were performed as follows:

$$\textbf{Separation efficiency} = (SS_{\text{initial}} - SS_{\text{harvested}}) / SS_{\text{initial}} \times 100$$

With:
SS = suspended solids (g/L)
Harvested = clarified outlet stream
Initial = concentration before treatment

Concentration factor = $V_{initial} / V_{final, recirculation}$

With:
V = volume (ml)

$V_{initial}$ = initial volume before treatment
$V_{final, recirculation}$ = final volume of concentrated cells

2.3.3 Results and discussion

Because of difficulties to maintain the same suspended solids concentration in both right and coiled *Arthrospira platensis* during the whole test experiment, a range suspended solids concentration was defined for the tests, between 1and 1.45 g/L of suspended solids.

The results presented in Table 4 show the data obtained with the straight and coiled *Arthrospira platensis* with the different centrifugation speeds.

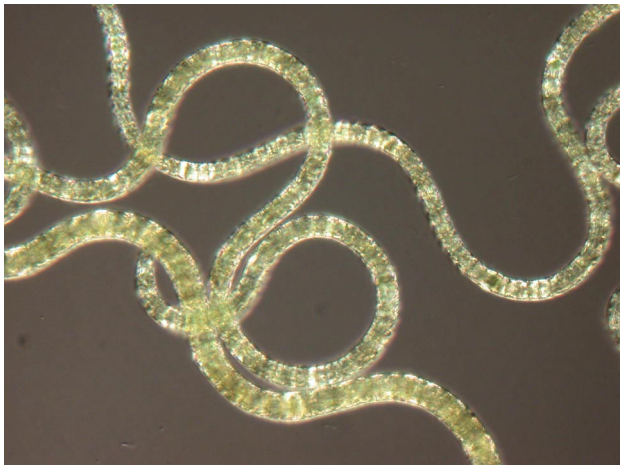
Table 4. Separation efficiency and concentration of the right and coiled *Arthrospira platensis* with centrifugation

	Centrifugation speed (x g)	Centrifugation speed (rpm)	Concentration factor (Fold)	Separation efficiency (%)
Right Arthrospira				
	6000	8403	8.00	94.17
	7000	9077	8.00	94.78
	10 000	10849	8.00	94.86
	12 000	11884	12.00	97.93
	13 000	12369	13.33	100
Coiled Arthrospira				
	6000	8403	5.71	52.25
	7000	9077	6.67	53.09
	10 000	10849	8.00	59.14
	12 000	11884	8.00	59.52
	13 000	12369	10.00	74.77

A 100% separation could be reached at high centrifugation speed of 13.000 rpm with the right *Arthrospira platensis*. From an initial suspension concentration of 1.42 g/L, one could concentrate the alga up to 18.9 g/L, giving a slurry with a nice dark greenish colour.

The obtained results from the coiled cells were not as promising as these of the right shaped cells. At high centrifugation speed, the maximum obtained concentration in the pellet was 11.1 g/L. Moreover, the supernatant was not clear as expected. A yellowish colour was present in all the replicates on which the SS were determined. It was, indeed very difficult to filtrate the samples and the filters were most of the time clogged.

All samples were observed with the microscope to verify the integrity of the cells. The results of the investigations performed on the coiled fragments are reported in Figure 22.



(a)

(b)

(c)

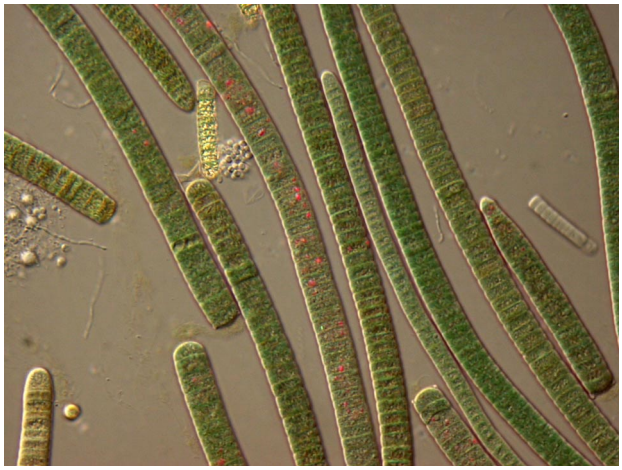
Figure 22. Microscopic observations of coiled type *Arthrospira platensis* at 400 x enlargement; (a) initial suspension, (b) concentrate and (c) filtrate of 13.000 rpm centrifugation speed

The images show clearly the fragmented cells in the filtrate (22c), which may explain the yellowish color of the samples after centrifugation. The fragmentation of the cells induced probably diffusion of the inclusions of the cells into the medium and difficulties during the filtration of the samples may be because of the stickiness of these inclusions and constituents (e.g. EPS and phycocyanin…).

The concentrate showed more consistent cells and much less fragmented filaments. One could propose that the use of centrifugation at high speeds of the order of 13.000 rpm may separate the entire coiled fragments from the fragmented ones, even if the separation efficiency is not satisfactory. The latter seems to be related to the density of the cells since the coiled filaments, when observed by the microscope contained a lot of vacuoles inside the cells, viewed as empty zones in the filaments (Figure 22a). This could probably hinder the centrifugation efficiency of this type of filaments. At this state of the study, we could not verify the impact of the presence of the vacuoles on the centrifugation efficiency and on which circumstances they are formed during the growth conditions.

The microscopic observations of the right shaped cells are shown in Figure 23.

(a)



(b)

(c)

Figure 23. Microscopic observations of straight type *Arthrospira platensis* at 400 x enlargement; (a) initial suspension, (b) concentrate and (c) filtrate of 12.000 rpm centrifugation speed

The separation efficiency of the right shaped cells was much higher than of the coiled cells. A 100% separation efficiency was reached with super speed centrifugation of 13.000 rpm. In the filtrate neither fragments nor cells were found. At a speed of 12.000 rpm an almost 98% separation efficiency was reached. As shown in Figure 23b, the pellet was constituted of intact fragments with dense inclusions. However, the filtrate contained some fragmented cells and empty filaments. This may indicate, ones more, that the centrifugation could be used as LSSS for *Arthrospira platensis* separation from the feed medium, first because of the high separation efficiency, mainly obtained with the straight shaped fragments and secondly because of its ability to separate the debris, fragments from the intact fragments. The latter could be further processed in the chain of *Arthrospira platensis* for consumption and the recuperated filtrate could be discharged to be further re-processed as wash medium for instance.

2.3.3.1 Energy consumption

Energy consumption was calculated for the following conditions: harvest of 100 l *Arthrospira*/d and concentrating from around 1 tot between 10 and 20 g/l. Because the cells have to be harvested and washed several times over a period of one day, we assumed that the volume of 100 l had to be concentrated 10- to 20-fold in about 2 hours. This means that the clarified harvest stream from the centrifuge needs to be around 50 l/h and the recirculation rate around 100 l/h.

For industrial centrifuges, the total power consumption amounts 750 to 1200 W to harvest 100 l/d. Because the cells will be harvested over a period of 2h, the energy consumption amounts 15 to 24 kWh/m^3.

2.3.4 Conclusions

From the present study on the centrifugation technique as possible LSSS for *Arthrospira platensis* harvesting, it was shown that thickening of the alga suspension up to 13.3 folds could be obtained with the straight shaped *Arthrospira*, giving concentrations between 15 and 18.9 g/L. Microscopic observations allowed to verify the integrity of the filaments in the concentrate after centrifugation and the withdrawal of the debris, dead cells from the suspension in the filtrate.

Coiled filaments seemed to be more sensitive to centrifugation than the right type filaments of *Arthrospira platensis*. After centrifugation, the filtrate was first predominantly full with debris and empty filaments, probably resulting from the centrifugation speed and second the centrifugation efficiency was lower than with the right shaped cells.

In general, and more specifically, in the case of right shaped filaments of *Arthrospira platensis*, the centrifugation technique is not destructive and can be performed in axenic conditions. However, the main disadvantages are:

- The separation efficiency seems to depend on the cell shape.
- The culture conditions of the cells and their physical characteristics, which still have to be optimised.
- The clarified water is not completely free of cells and debris, even at optimal separation efficiencies approaching the 100%.
- The technology may not be adequate to be used in microgravity conditions.

3. Desalination technologies

The objective of the desalination of the washing water is twofold. The first objective is to obtain a diluate which contains only a small amount of salts and which can be used to wash the algal cells, after they have been separated from the culture medium. The second objective is to obtain a concentrate, containing a high concentration of salts, which can be reused as culture medium for the algal cells.
Both reverse osmosis (RO) and electrodialyis (ED) were evaluated for their desalination performance. The aim was to obtain a permeate (in the case of RO) or a diluate (in the case of ED) with a dry matter content below 300 mg/l.

3.1 Reverse osmosis

3.1.1 Principle

In reverse osmosis a solute, salts in this case, is separated from a solution by forcing the solvent, water in this case, to flow through a membrane by applying a pressure greater than the osmotic pressure. The total flux is the sum of the solvent flux and the solute flux. The higher the selectivity of the membrane, the lower the solute flux compared to the solvent flux. Figure 24 shows the principle of reverse osmosis.

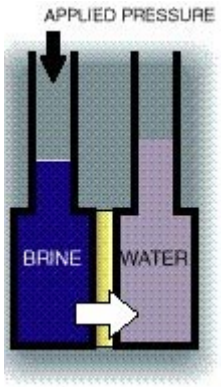


Figure 24. Principle of reverse osmosis

3.1.2 Materials and methods

3.1.2.1 Preparation of the Zarrouck medium

The composition and preparation of the Zarrouck medium is described in 2.1.2.1.

3.1.2.2 Reverse osmosis tests

The aim of the reverse osmosis process is to produce desalinated permeate that can be used to wash the algal cells. Previous tests showed that completely desalinated water leads to cellysis so a certain amount of salts should be present in the washing water. Therefore the separation performance of three reverse osmosis membranes with a different salt recovery was investigated. All reverse osmosis tests were performed on the Zarrouck medium (conductivity 24000 µS/cm). Table 5 gives an overview of the different membranes that were tested and their separation characteristics as given by the membrane suppliers.

Table 5. Description of the tested reverse osmosis membranes

Membrane type	*Average NaCl rejection*	*Permeability (l/hm^2bar)*
Trisep TS80*	90%	6,23
Nitto LES 90**	95%	5
Dow SW 30***	99.4%	0,48

* : Trisep: tested with model 8040-TS80-TSA (8 inch) at 100 psi net pressure, 25°C, 15% recovery, pH8 and 30 minutes operation, permeability is pure water permeability; NaCl rejection for solution containing 500 ppm NaCl

** : Nitto : tested with model D2 (2.5 inch) at 10 bar, 25°C, 20-35 % recovery, permeability is the pure water permeability, for Na Cl rejection for solution containing 0.2 wt% NaCl

*** : Dow : tested with model SW30-2514 (2.5 inch) at 55 bar, 25°C, 32000 ppm NaCl, pH 8 and 2% recovery

All experiments were performed in a dead end filtration mode. Circular, flat sheet membranes with a membrane area of 0.0044 m^2 were used. Before the start of every test, a preconditioning of the membranes was performed with RO water to remove preservatives that might be present and to compact the membrane as the membrane performance might change due to the pressure applied to it. Therefore the membranes were operated with pure RO water for approximately two hours

and until a stable flux was obtained, at the test conditions that were used in the actual reverse osmosis test with the Zarrouck medium. During this period, the pure water flux was measured.

3.1.2.3 Sample analysis

The conductivity of the feed mixture was determined before the start of every test to be able to determine the selectivity of the membranes after completion of the test. A permeate and concentrate sample was taken at the end of every test. These samples were analysed for their Cl^-, NO_3^-, CO_3^-, Na - and HCO_3^--content. Also the pH, conductivity, DOC (dissolved organic carbon), DIC (dissolved inorganic carbon), DC (dissolved carbon) and dry matter content were determined.

3.1.3 Results and discussion

3.1.3.1 Reverse Osmosis experiments

The three membranes described in the previous section were tested for their separation performance at a feed pressure of 20 bar and a feed flow of approximately 270 l/h (2 m/s).

Figure 25 shows the results for the preconditioning experiments with the three different membranes.

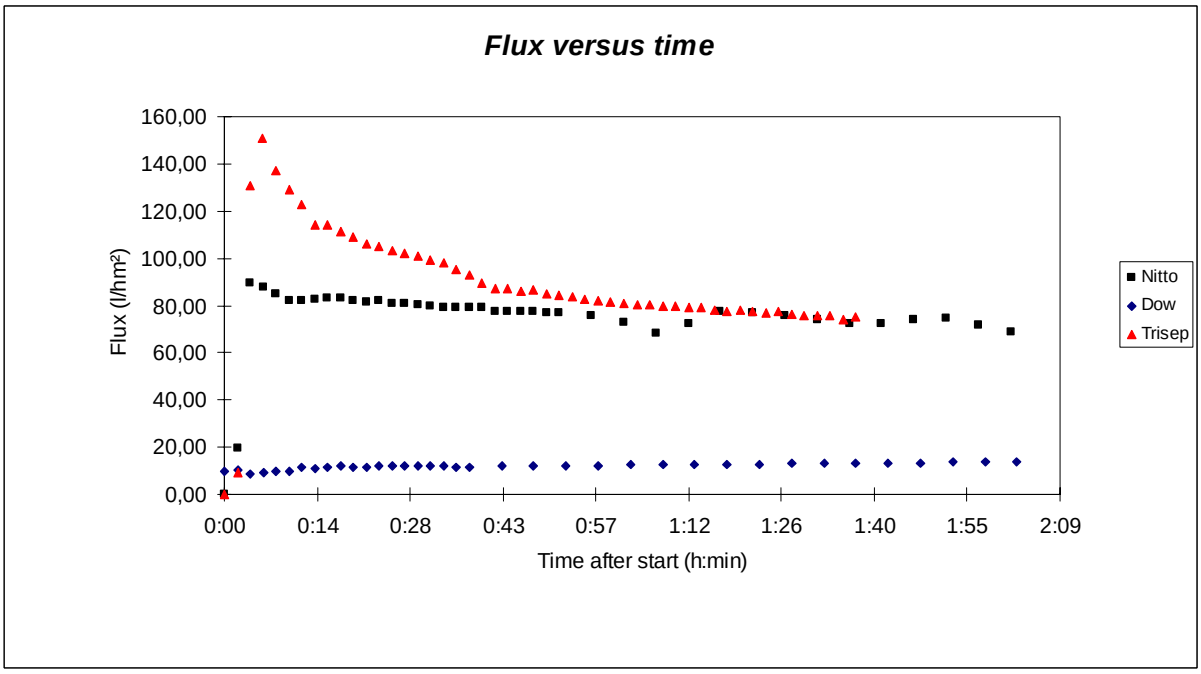


Figure 25. Results for the compacting experiment with the three different membranes.

Figure 26 shows the results for the RO experiments on the Zarrouck medium with the three different membranes.

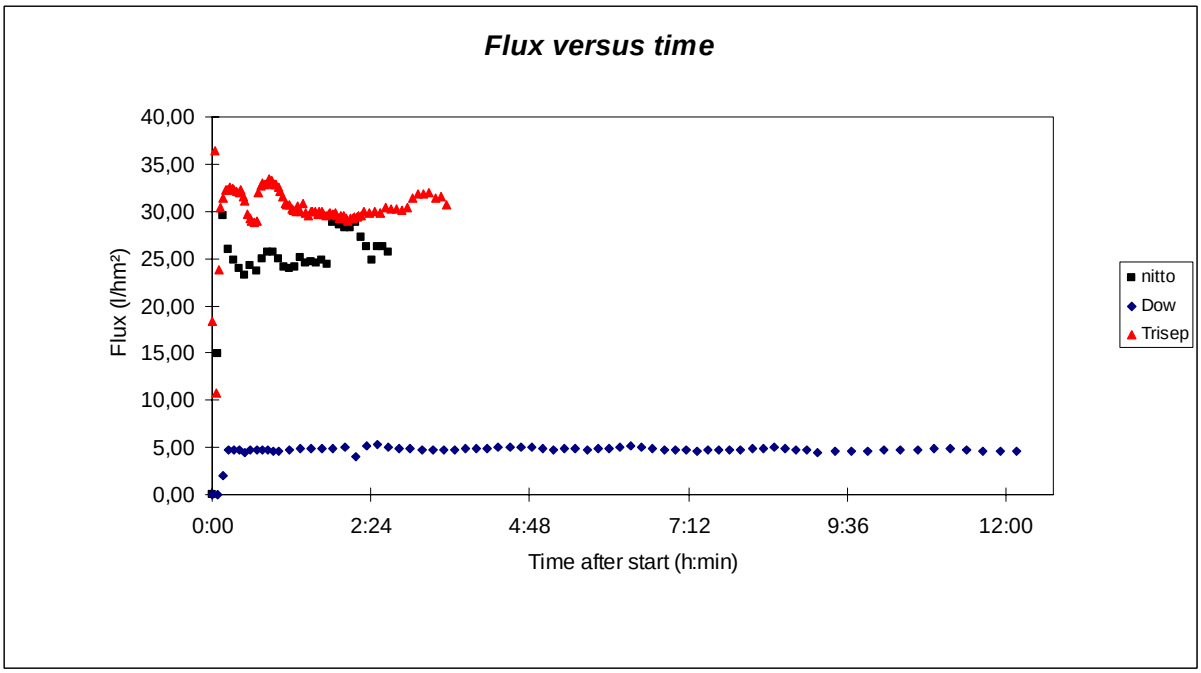


Figure 26. Results for the reverse osmosis experiment with the three different membranes for the Zarrouk medium

Table 6 shows the analysis results for the retentate (R) and permeate (P) mixtures of the reverse osmosis experiments with the Zarrouk medium for the three different membranes investigated. Table 7 gives an overview of the fluxes and the ion rejections that were obtained with the different membranes. The calculation of the ion rejection was based on the conductivity.

Table 6. Analysis results

Parameter	***Trisep R***	***Trisep P***	***Retention Trisep (%)****	***Nitto R***	***Nitto P***	***Retention Nitto (%)****	***Dow R***	***Dow P***	***Retention Dow (%)****
pH	9,6	9,4		9,6	9,7		9,6	9,5	
conductivity (µS/cm)	25800	2540	90,16	24300	4460	81,65	25500	1132	95,56
NO_3 (mg/l)	1790	660	63,13	1680	510	69,64	1850	120	93,51
Na (mg/l)	6860	461	93,28	6390	1060	83,41	6840	207	96,97
Cl (mg/l)	602	180	70,10	560	130	76,79	606	34	94,39
CO_3 (mg/l)	1740	48,4	97,22	1660	277	83,31	1720	45,6	97,35
HCO_3 (mg/l)	10440	461	95,58	9950	1320	86,73	10330	344	96,67
DOC (mg/l)	190	10	94,74	<50	16		300	<2,5	>99,1
DIC (mg/l)	2750	<10	>99,6	2620	371	85,84	2720	86	96,84
DC (mg/l)	2940	108	96,33	2630	387	85,29	3020	85	97,19
Dry matter (mg/l)	25800	1700	93,41	19600	3340	82,96	31500	710	97,75

* : retention based on retentate concentration

Table 7. Overview of experimental results: an overview of the fluxes and the ion rejections that were obtained with the different membranes

Membrane type	*Pure water flux (l/hm^2)*	*Mean flux for zarrouck medium (l/hm^2)*	*Ion rejection (%)*	*Dry matter in permeate (mg/l)*
Trisep TS 80	75	30,7	89,80	1700
Nitto LS 90	72	25,5	81,53	3340
Dow SW 30	13	4,8	95,42	710

Table 8 gives an overview of the permeabilities according to the membranes suppliers and the permeabilties obtained in the pure water- and Zarrouck tests. The difference in permeability between the pure water- and Zarrouck- tests is due to the osmotic pressure of the Zarrouck medium.

Table 8. Overview of experimental results: overview of the permeabilities according to the membranes suppliers and the permeabilties obtained in the pure water- and Zarrouck tests

Membrane type	*Permeability (l/hm^2bar) according to membrane supplier*	*Permeability (l/hm^2bar) in pure water test*	*Permeability (l/hm^2bar) in Zarrouck test*
Trisep TS80	6,23*	3,75	1,54
Nitto LES 90	5,00*	3,60	1,28
Dow SW 30	0,48	0,65	0,24

* : for the nitto and trisep membrane, the permeability is the pure water permeablilty

Among the three membranes tested, only the Trisep membrane reached the salt rejection that was given by the the membrane supplier. For the Nitto and the Trisep membrane the permeability obtained in the experiments was lower that the permeability according to the membrane suppliers. As expected, the Dow SW30 membrane shows the highest ion rejection but the lowest flux.
From the results it is clear that the dry matter content of the permeate in the reverse osmosis experiment using the Trisep and Nitto membrane is far above the goal of 300 mg/l so a single stage RO process with these membranes can not be used. With the Dow membrane, the amount of dry matter in the permeate is 710 mg/l which is better than the permeate with the two other membranes but still is not below the desired 300 mg/l.
As the selectivity that was given by the provider of the Dow membrane was higher than the selectivity found with this membrane, an additional experiment was performed using this membrane in a larger (0,6 m^2 membrane area) spiral wound lab-scale module, Type SW30-2514 as shown in Figure 27 (Dow FILMTEC). The objective was to investigate whether higher selectivities could be obtained when using higher feed pressures and a spiral wound module in a pressure tube, which is the configuration that is actually used in practical applications. Therefore a Zarrouck feed mixture with a conductivity of approximately 24000 µS/cm was desalinated at 5 different feed pressure : 20, 30, 40, 50 and 55 bar at isoconcentration. The feed flow rate was approximately 750 l/h. The selectivity and flux were determined for the different pressures. Table 9 and Figure 28 show the results obtained at the different feed pressures.

Figure 27. SW30-2514 module

Table 9. Overview of results obtained with SW30-2514 module at different feed pressures

Feed pressure (bar)	*Flux for zarrouck medium (l/hm^2)*	*Permeability (l/hm^2 bar)*	*Permeate conductivity (µS/cm)*	*Selectivity (%)*
20	4,5	0,38	147	99,39
30	13,3	0,74	71	99,70
40	21,5	0,90	53	99,78
50	31	1,03	43	99,82
55	36,5	1,11	40	99,83

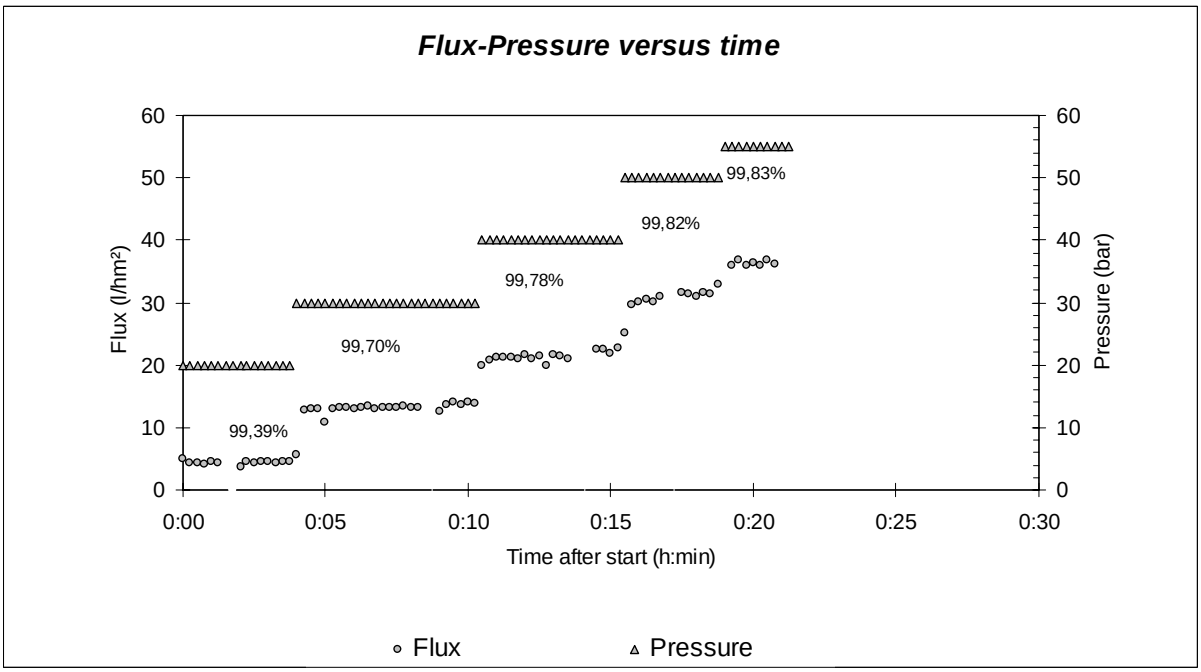


Figure 28. Flux and selectivity at different feed pressures

From the results it is clear that the selectivity is higher than the selectivity found with the Dow membrane in the previous test and the selectivity is even higher than the NaCl selectivity given by the membrane provider. As expected, the selectivity increases with increasing pressure due to the increase in pure water flux.

The flux at 20 bar is approximately the same as the flux found with the same membrane in the previous test. Figure 29 shows that the flux increases linearly with increasing pressure as expected. Based on Figure 29, the osmotic pressure is calculated to be 15.37 bar.

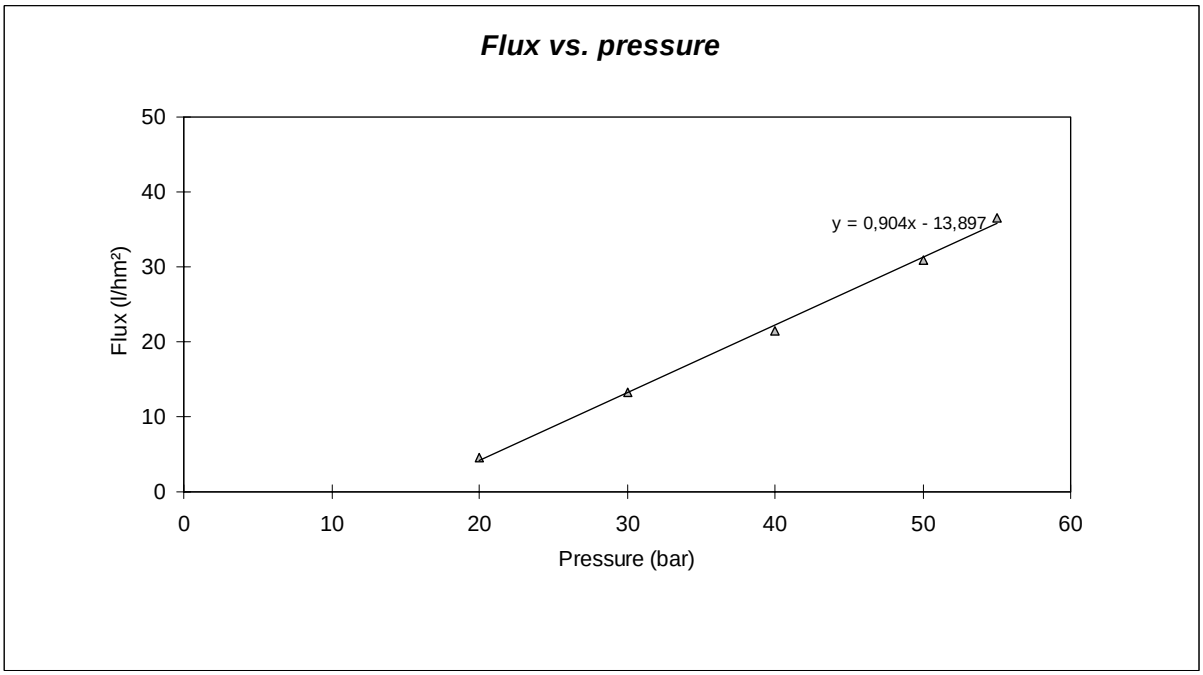


Figure 29. Flux versus pressure

A second experiment was performed with the SW30-2514 membrane module at 55 bar in which permeate was continuously collected to obtain a doubling in the conductivity of the feed mixture. In case of 100 % ion rejection this would mean a volumetric recovery of 50 %. The flux was measured at regular time points and the experiment was stopped when the retentate conductivity was approximately double of the initial feed conductivity. A feed- and permeate- sample were taken at the start of the experiment and a retentate- and permeate- sample were taken at the end of the experiment. The analysis results of the samples are shown in Table 10. Figure 30 shows the fluxes as a function of time.

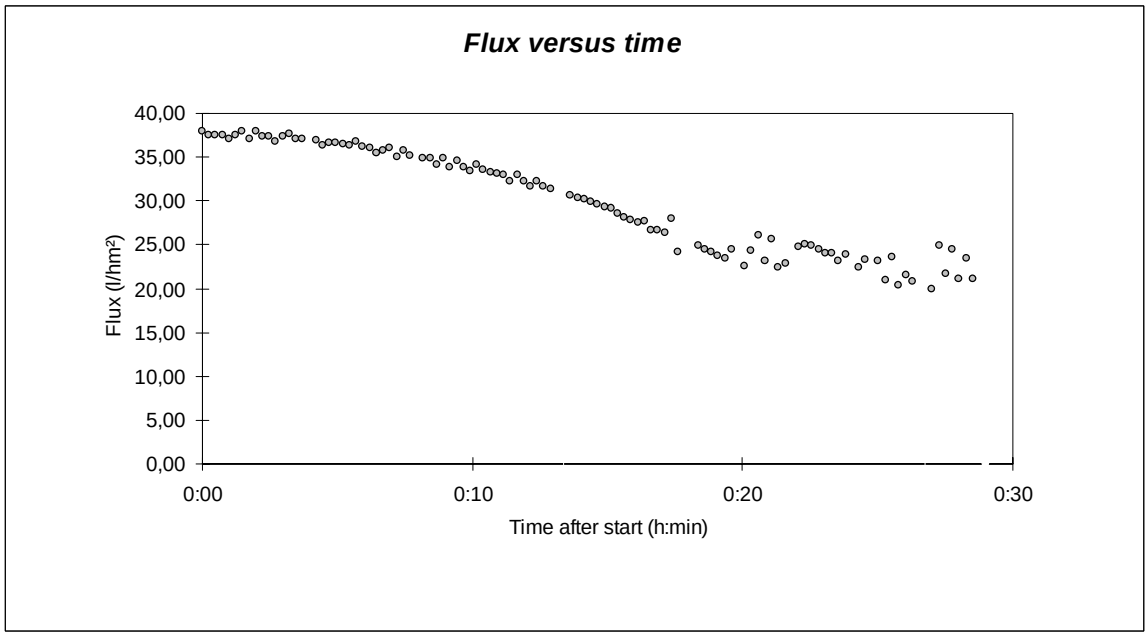


Figure 30. Flux versus time

Table 10. Analysis results

Parameter	***Feed***	***Permeate 1 (start)***	***% retention (start)***	***Permeate 2 (end)***	***% retention (end)***	***Retentate***
pH	9	7,3		6,7		8,9
conductivity (µS/cm)	24600	41	99,83	187	99,60	47200
NO_3 (mg/l)	1940	13,9	99,28	76,7	98,13	4106
Na (mg/l)	7130	10,3	99,86	33,3	99,80	16300
Cl (mg/l)	740	1,03	99,86	4,75	99,68	1470
CO_3 (mg/l)	580	*	99,99	*	99,99	1090
HCO_3 (mg/l)	13800	7,8	99,94	16	99,95	32700
DOC (mg/l)	220	<1	>99,55	<1		<50
DIC (mg/l)	2950	1,7	99,94	4,5	99,93	6880
DC (mg/l)	3170	1,4	99,96	4,1	99,94	6880
Dry matter (mg/l)	22900	88	99,62	180	99,63	48400

* : negligible

Based on these results the selectivity at the start and at the end of the experiment were calculated. The selectivity was 99.83 % at the start and 99.60 % at the end. As expected, the flux decreases as a function of time because the salt concentration in the feed increases, leading to an increase in osmotic pressure.

3.1.3.2 Energy requirements

Based on the results obtained with a 2.5 inch Dow membrane module which has a membrane surface of 0.6 m^2 (experiment performed at different feed pressures), calculations were performed to obtain the amount of energy that is required to desalinate the Zarrouck medium. For reverse osmosis the only energy required is the pumping energy. The pumping energy can be calculated by the following formula:

$$E_p = \frac{\Delta P * q_f}{\eta * 1000}$$

in which E_p is the energy requirement of the pump (kW), q_f is the feed flow rate (m^3/s), ΔP is the pressure drop across the membrane (Pa) and η is the pump efficiency. The energy required per m^3 of permeate (kWh/m^3) was calculated by dividing the pumping energy by the permeate flow rate.
The energy calculations were performed for a reverse osmosis process in which only one module type 2514 was used. Therefore the recovery, which is the permeate flow divided by the feed flow, is very low as shown in Table 11. From the results it is clear that recovery is the highest at a pressure of 55 bar due to the higher flux at this pressure.

Table 11. Energy requirements and recovery for the RO process with Dow membranes

Pressure (bar)	recovery (%)	Energy (kWh/m^3) for process with 1 module
20	0,36	205,76
30	1,064	104,43
40	1,72	86,13
50	2,48	74,67
55	2,92	69,76

In practical applications, more modules are connected and larger membrane modules are used, so the recovery becomes higher resulting in a lower energy requirement. Table 12 shows the energy requirement for a reverse osmosis process with one or more Dow SW30-2514 modules connected in series at a pressure of 55 bar.

Table 12. Energy requirements and recovery for the RO process with Dow membranes

Number of modules	recovery (%)	Energy (kWh/m^3)
1	2.92	69,76
2	5.84	34,88
3	8.76	23,25
4	11.68	17,44

In the experiments a 2.5 inch module was used. In practical applications however larger modules (e;g; 4.5 inch)are used leading to a higher recovery per module and consequently a lower energy requirement (in kWh/m^3) for the same number of modules.

3.1.4 Conclusions

Based on the experiments it can be concluded that the Dow SW30 membrane is the only membrane, among the membranes tested, that gives a permeate with a dry matter content below the required amount of 300 mg/l . The energy calculations, performed based on the tests with one SW30-2514 module clearly show that the energy requirement decreases when a higher feed pressure is used which is due to the increase in permeate flux and an recovery. The energy calculations are based on a reverse osmosis process with only one SW30-2514 module, resulting in a low recovery value and consequently a high energy requirement. When a larger module (e.g. a 4.5 inch instead of a 2.5 inch module) would be used or when more modules would be

connected, the pumping energy could be used more efficiëntly and the energy requirement would decrease. So the more modules are linked together, the less the energy requirement. On the other hand, more modules require more space and represent more mass. A major disadvantage of reverse osmosis, bearing in mind the application in space, is the large and heavy high pressure pump that is needed for this process causing also vibration when it is in operation.

3.2 Electrodialysis

3.2.1 Principle

The principle of the electrodialysis process is depicted in Figure 31 for desalination of a NaCl solution.

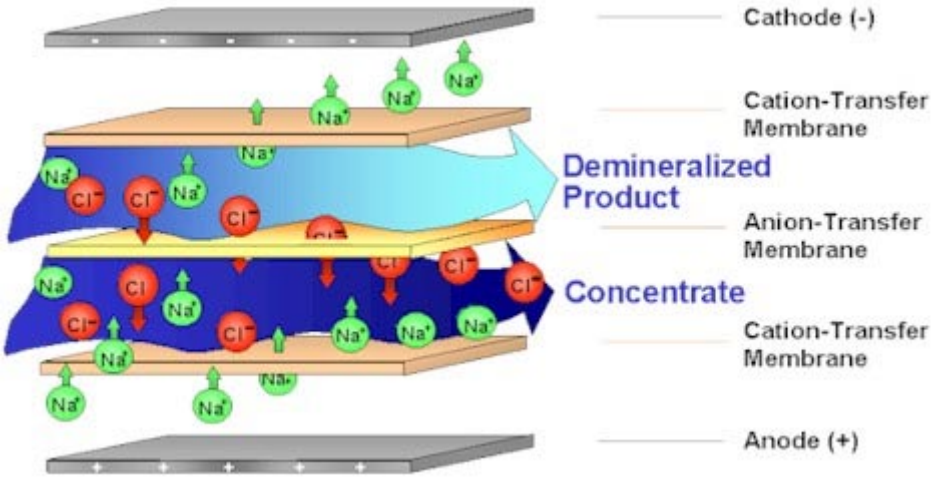


Figure 31. Principle of electrodialysis for the desalination of a NaCl solution

In this process electrically charged membranes are used to remove ions from an aqueous solution. A number of cation- and anion-exchange membranes are placed in an alternating pattern between a cathode and an anode. When an ionic feed solution (Zarrouck medium in this case) is pumped through the cell pairs, nothing will happen as long as no current is applied. However, when a direct current is applied, the positively charged ions migrate to the cathode and the negatively charged ions migrate to the anode. The negatively charged anions can not pass the negatively charged membrane and the positively charged cations can not pass the positively charged membranes. This means that the overall effect is that the ionic concentration increases in alternating compartments accompanied by a simultaneous decrease in ionic concentration in the other compartments. Consequently alternate dilute and concentrate solutions are formed. Electrolysis occurs at the electrodes, with hydrogen formation at the cathode and oxygen formation at the anode.

3.2.2 Materials and methods

3.2.2.1 Preparation of the Zarrouck medium

The composition and preparation of the Zarrouk medium is described above.

3.2.2.2 Electrodialysis tests

For the ED experiments a stack of 9 cation and 8 anion membranes was used, resulting in 8 concentrate- and 8 diluate-chambers. The cation- and anion membranes used (type FKS and FAS respectively) were standard ion-exchange membranes that can be used in a pH-range from 1 to 14 (FUMA-TECH GmbH, Germany). The effective size of the membranes was 0.1 m x 0.1 m so the effective membrane area of the stack was 0.08 m^2. All experiments were performed in batch mode. During the experiments, the voltage was kept constant. A 'standard' experiment with a NaCl solution was regularly performed to check whether the membrane performance did not change due to membrane fouling. Therefore desalination of a 0.011 M NaCl solution was performed at 14.87 V.
Figure 32 shows the conductivity versus time for the standard NaCl experiments. The results show that the membrane performance remained approximately the same.

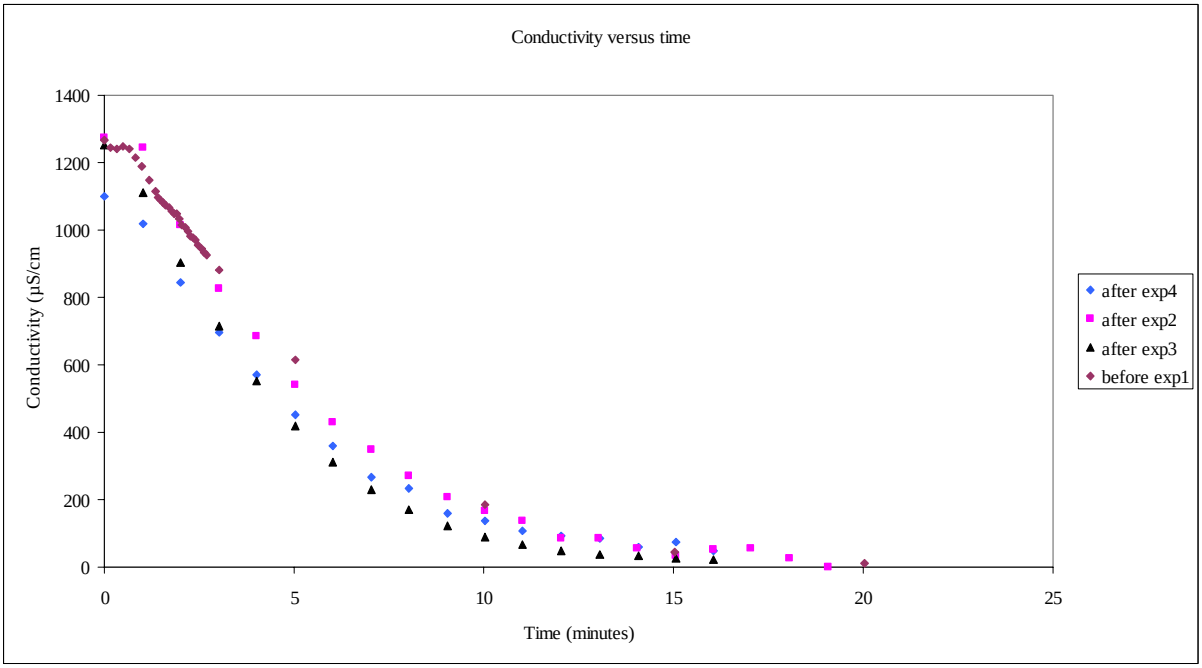


Figure 32. Conductivity versus time for NaCl experiments

A number of experiments were performed to investigate the effect of the current density and the diluate/concentrate ratio. The current density is an important operation parameter as it influences the energy and time required for the desalination. The diluate/concentrate ratio is important as a low ratio (e.g. 2/8) means that a low diluate-volume (1 l) is produced compared to a large concentrate-volume (4 l). However the ratio can not be too high as this might lead to scaling.
To investigate the effect of the current density the first experiment was performed at a voltage of 6.93 V to obtain a starting current density of approximately 100 A/m^2 and a second experiment was performed at a voltage of 21.92 V to obtain a starting current density between 500 A/m^2 and 600 A/m^2 . Both experiments were performed at a diluate/concentrate ratio of 2/8.
To investigate the effect of the diluate/concentrate ratio on the separation process two additional experiments (experiment 3 and experiment 4) were performed at approximately the same voltage as used in experiment 2 but with a different diluate/concentrate ratio. In experiment 3 the diluate/concentrate ratio was 2/4 while in experiment 4 the ratio was 2/2.

An overview of the exact operating conditions in all experiments is given in Table 13.

Table 13. Overview of operating conditions for the different experiments

	experiment 1	**experiment 2**	**experiment 3**	**experiment 4**
voltage (V)	6,93	21,92	21,84	21,84
current density at start (A/m^2)	110	530	590	570
flow diluate (l/h)	103	105	112	101
flow concentrate (l/h)	108	116	116	112
flow rinsing solution (l/h)	184	172	185	188

3.2.2.3 Sample analysis

For experiment 1, a feed sample was taken at the start of the experiment and a diluate sample was taken at the end of the experiment. These samples were analysed for their Cl -, NO_3 -, CO_3 -, Na - and HCO_3 -content. Also the pH, conductivity, DOC, DIC, DC and dry matter content (dry matter only for experiment 1) was determined. For experiments 2 to 4, no samples were analysed as the the same membranes were used in every experiment so the analysis are expected to be approximately the same for all experiments. Based on experiment 1, the amount of dry matter in the diluate can be calculated based on the rest conductivity.

3.2.3 Results and discussion

3.2.3.1 Electrodialysis experiments

Figure 33 shows the conductivity versus time after start of the experiment for all experiments. From this figure it is clear that a increase in current density with a factor 5 leads to a decrease in the time required for the desalination process with approximately the same factor. This will influence the energy requirement as will be described in more detail in section 3.2.3.2.

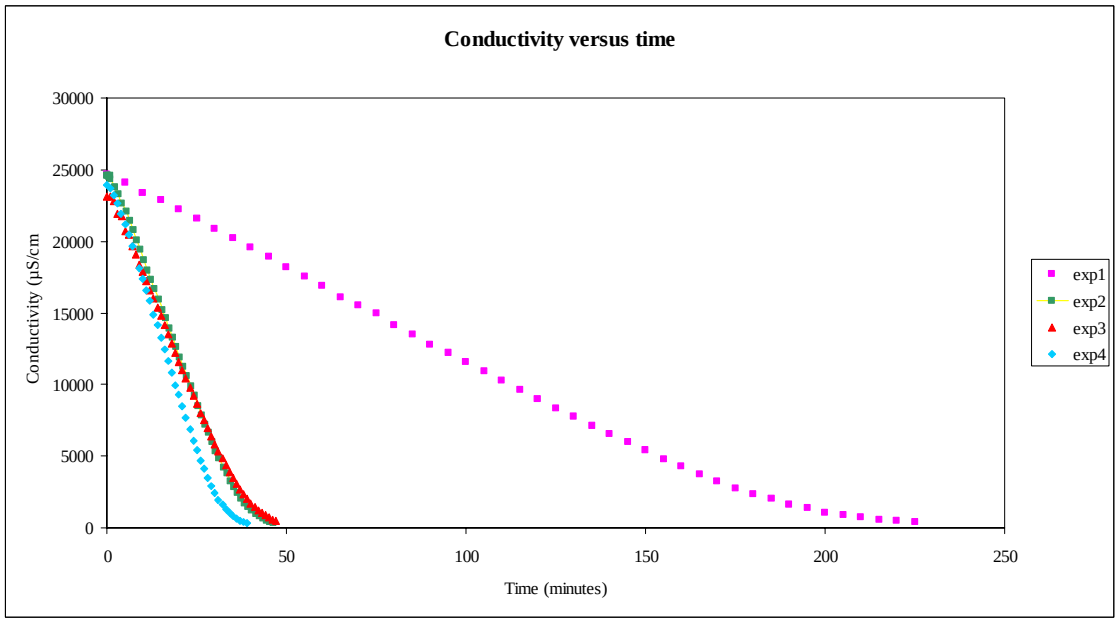


Figure 33. Conductivity versus time

Table 14. Analysis results for feed (F) and diluate (D) stream of experiment 1

Parameter	***Exp1 F***	***Exp1 D***	***% removal***
pH	9,6	8,8	
conductivity (µS/cm)	24629	371	98,49
NO_3 (mg/l)	1700	11,9	99,30
Na (mg/l)	6310	61	99,03
Cl (mg/l)	580	3,04	99,48
CO_3 (mg/l)	1650	2	99,88
HCO_3 (mg/l)	9910	112	98,87
DOC (mg/l)	<50	11	
DIC (mg/l)	2610	23	99,12
DC (mg/l)	2590	34	98,69
dry matter (mg/l)	20300	270	98,67

Table 15 gives an overview of the results obtained in the different experiments.

Table 15. Results overview

	exp1	**exp2**	**exp3**	**exp4**
experiment duration (min)	225	46	47	39
final diluate conductivity (µS/cm)	371	321	499	351

Based on the conductivity and dry matter content in the first experiment, the conductivity correlating with a dry matter content of 300 mg/l was deduced, at which the experiments were stopped.
When experiment 1 is compared with experiment 2 it is clear that the desalination process is much faster for experiment 2 in which a higher voltage (and accordingly a higher current density) is used. When experiments 2, 3 and 4 are compared, it can be concluded that the diluate/concentrate ratio does not influence the desalination process, in the range tested. The small difference in the time required for desalination between experiments 2, 3 and 4 is probably not due to the difference in diluate/concentrate ratio but rather to fluctuations in the system performance (small flow rate variations, small variations in membrane performance).

3.2.3.2 Energy requirements

The energy required for the ED process can be split up in the energy required for the transport of ions and the pumping energy required for the circulation of diluate, concentrate and rinsing solution (three pumps are needed).
The energy requirement of the pumps can be calculated by the following equation :

$$E_p = \frac{q_f * \Delta P}{\eta * 1000}$$

in which E_p is the energy requirement of the pump (kW), q_f is the feed flow rate (m^3/s), ΔP is the pressure drop (Pa) and η is the pump efficiency. The energy required per m^3 of permeate (kWh/m^3) was calculated by dividing the pumping energy by the permeate flow rate.

The energy required for the transport of ions can be calculated from the experimental results by the following formula :

$$E = V * I$$

In which E (kW) is the energy required for the transport of ions, V is the charge (volts) and I is the current (A).

Table 16 gives an overview of the energy requirements for the different experiments. In all experiments, 2 l of Zarrouck medium was desalinated. Based on the energy requirements to desalinate 2 l of Zarrouck medium, the energy requirement was calculated to desalinate 1 m^3 of the Zarrouck solution.

Table 16. Energy requirements for different experiments

	Exp1	**Exp2**	**Exp3**	**Exp4**
energy for ion transport (kWh/m^3)	11,17	31,54	37,58	28,55
pumpenergy (kWh/m^3)	13,72	2,82	3,01	2,42
total energy (kWh/m^3)	24,89	34,36	40,59	30,97

When comparing the energy requirements in experiment 1 and 2, it is clear that the energy required for the ion transport is higher when a higher voltage is applied while the pumping energy is lower when a higher voltage is applied. The total energy is higher when a higher voltage is applied.

3.2.4 Conclusions

Based on the ED desalination experiments it can be concluded that more energy but less time is required when a higher voltage is applied. Consequently the desalination of a given flow rate will require less membrane area but more energy when a higher voltage is used.

3.3 Susceptibility of *Arthrospira* to osmotic shocks

3.3.1 Introduction

Arthrospira cells nedd to be washed to achieve the final salinity level required for consumption as food. Because alteration of the cell structure has to be avoided as much as possible in view of the nutritional quality, it may be advisable to wash the cells sequentially with solutions which are desalinated to different final conductivity levels.

3.3.2 Materials and methods

To assess the impact of the conductivity of the washing solution on cell integrity, the following series of tests was set up. Thirty ml of an *Arthrospira* culture was centrifuged (15 min at 10000 rpm, 4°C), and resuspended in 30 ml of different solutions to similar OD_{750} values. The solutions were: demineralized water, Zarrouk medium and three solutions of intermediate salinity, generated by electrodialysis of Zarrouk medium to different end conductivities, namely 550 µS/cm, 1200 µS/cm and 5.6 mS/cm.

Non-centrifuged samples in Zarrouk medium were sed as a reference.

The suspensions were evaluated over a period of several hours by visual observation (color) and microscopical observation. After about 5 h, cells were again centrifuged, stored at 4°C and analysed for their protein content.

3.3.3 Results and discussion

After centrifugation, cell shape had slightly changed in the sense that parts of the *Arthrospira* filaments were flattened. Incubation at different salinity levels did not lead to any color change or to microscopically visible morphological changes.

Attempts to determine the protein content of the centrifuged cells were hampered as follows. Centrifugation of the cells prior to the determination did not concentrate all the cells in a pellet. Part of them was present as a floating layer in the centrifuge tubes and could not be recovered. In addition, it was not possible to remove all the supernatant without any additional loss of biomass. As a result, protein contents did not show any relation to the salinity of the suspension tested.

It is probably not surprising that the influence of salinity on cell integrity is limited. *Arthrospira* cells are expected to be very resistant to osmotic shock because they have a rigid cell wall such as bacteria. Therefore, washing with water does probably not result in cell lysis or loss of macromolecules, but may cause some physiological changes at the level of ions (Dubertret, oral communication).

3.3.4 Conclusions

From our experiments, no influence of salinity on cell integrity could be observed. The issue was not considered important enough at this stage of the work to require further investigations.

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ANNEX 1:

CULTURE MEDIUM
(ALPHA BIOTECH)

ALPHA BIOTECH	PROCEDURE N° 4.1.98	1/1

Rédigée le : 07/03/02 Validée par : B Lépine Le : Visa :	PROD / ENTRETIEN DE LA SPIRULINE EN PETIT VOLUME

1. OBJECTIF

La spiruline doit être entretenue en petit volume dans un mélange de solutions S1, S2, S3, S4 et de préparations A5, B6.
Quelques unes d'entre elles sont aussi utilisées pour la préparation du milieu de culture en grand volume de la Spiruline.

2. PREPARATION DES SOLUTIONS

2.1. Les macro-éléments

Utiliser une eau de mer à 2 %

S1 Mélanger 100 ml eau de mer + 700 ml eau osmosée
Peser 0,2 Mg SO_4 + 1 g K_2 SO_4 + 2,5 g Na NO_3 + 10 g Na HCO_3
Dissoudre dans le 1er mélange
Autoclaver 20 mn à 120 °C

S2 Peser 2,5 g K_2 HPO_4, qsp 1 L eau osmosée
Autoclaver 20 mn à 120 °C

Penser à autoclaver une éprouvette de 200 ml.

S3 Préparer une solution de Ca Cl_2 à 40 g/l
Autoclaver 20 mn à 120 °C

S4 Peser 0,2 g Fe SO_4 + 1,6 g EDTA, qsp 100 ml eau osmosée ou 43 g. de Fe SO_4 + 100 g. EDTA qsp 20 l d'eau osmosée. Autoclaver 20 mn à 120 °C

2.2. Les micro-éléments

A5 Peser 2,86 g H_3 BO_3
1,81 g Mn Cl_2, 4 H_2O
95 mg Zn Cl_2
79 mg Cu SO_4, 5 H_2O
15 mg Mo O_3
qsp 1 L eau milliro

Autoclaver 20 mn à 120 °C

B6 Peser 36 mg Co Cl_2, 6 H_2O
qsp 1 L eau milliro

3. PREPARATION DE 1 L DE MILIEU

Près d'une flamme, mélanger
- S1 + 200 ml S_2
- 1 ml S3
- 5 ml S4
- 1 ml A5
- 1 ml B6

Ensemencer le milieu avec 1/5 de Spiruline, placer le flacon devant la lumière à 35 °C

De : ALPHA BIOTECH Tél : 02 40 01 79 11
: M. Bertrand LEPINE Fax : 02 40 01 73 22

CONFIDENTIEL

à : CRTT Tél :
: Pascal JAOUEN Fax : 02 40 17 26 18

Date : 11/04/2002 Heure 12:03

Cher Pascal,

Voici les renseignements sur la Spiruline et le milieu de culture :

Souche : Arthrospira platensis

Origine : PCC 8005 de l'Institut Pasteur

Milieu utilisé en grand volume (pour 1 m^3) :

Eau de mer : 200 l. (filtré et stérilisé)
Eau douce : 800 l. (filtré + charbon actif)

Na HCO_3 / 9 KG	S4 : 1,5 l
Na NO_3 : 1,5 Kg	A5 : 1l
K_2HPO_4 : 50 g.	B6 : 1l
$MgSO_4$: 200 g.	S3 : 1 l
K_2SO_4 : 50 g.	

Macro-éléments

S3 Préparer une solution de Ca Cl_2 à 40 g/l
Autoclaver 20 mn à 120 °C

S4 Peser 0,2 g Fe SO_4 + 1,6 g EDTA, qsp 100 ml eau osmosée ou 42 g. de Fe SO_4 + 100 g. EDTA qsp 20 l d'eau osmosée . Autoclaver 20 mn à 120 °C

Micro-éléments

A5 Peser 2,86 g H_3 BO_3
1,81 g Mn Cl_2, 4 H_2O
95 mg Zn Cl_2
79 mg Cu SO_4, 5 H_2O
1,5 mg Mo O_3
qsp L eau milliro

Autoclaver 20 mn à 120 °C

B6 Peser 36 mg Co Cl_2, 6 H_2O
qsp 1 L eau milliro → osmosed water