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MELiSSA – Adaptation for Space

ESA contract 15671/01/NL/ND

TECHNICAL NOTE 72.9.2

Functional test results

Version : 1
Issue : 1

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16/12/03

H.R. GENT 165.321 - BTW BE 447.422.101 - KBC 441-0619121-21

DOCUMENT CHANGE LOG

Version	Issue	Date	Observation
1	0	03/11/03	Draft
1	1	16/12/03	Final

DISTRIBUTION LIST

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1. Introduction

A breadboard was constructed for the harvest and desalination of *Arthrospira* algae. In technical note TN72.9.1 a plan for functional tests in the hardware and operation phase has been described. In the present technical note the results and evaluation of the breadboard are presented.

2. Test principles

Table 1. Test principles for hardware and operation phase

Nber	Failures	Test principle
Hardware phase		
1	Insufficient mixing force or non homogeneous mixing	Visual check of homogeneity and power sufficiency of the mixing
2	Incorrect cooling of the reactors	Control on long period of stability and precision of temperature with a portable temperature sensor
3	Liquid/gas leakage	Visual check of absence of leakage
4	Erroneous measurement/control	Check with portable measuring device
5	Unstable measurement/control	Check with portable measuring device
6	Reactor/tank break	Visual check
Operation phase		
7	Clogging	Visual check + check right flow through pipes
8	Corrosion	Visual check
9	Deterioration of measurement/control	Check with calibration+status of instrument
10	Instrument break	Visual check
11	Reactor/tank break	Visual check

3. Test results for hardware phase

3.1 Part 1: Photoreactor and buffer tank

Table 2. Test results for photoreactor and buffer tank in hardware phase

Test objective	Instrument ref	Instrument description	Test perfomed (Y/N)	Test result	Comments
Check sufficient/homogeneous mixing	R1	Mixer for photoreactor	Y	Pass	
	R2	Mixer for buffer tank	Y	Pass	
Check correct cooling of the reactor/tank	K1	Cooler	Y	Fail	1. Distribution of cooling liquid to both the growth reactor T1 and the buffer tank T2 did not function well, leading to insufficient cooling capacity for T1. Therefore, a second cooler was installed. K1 is connected to T1 with a temperature-controlled valve, cooler K2 is connected to T2. 2. When water is used as cooling liquid, the danger exists that it freezes when pumping rates through the cooler are low. Therefore, a mixture of glycol and water is used.
Check absence of liquid/gas leakage	T1	Photoreactor	Y	No liquid leakage	
	T2	Buffer tank	Y	No liquid leakage	
	T_T1	Temperature sensor	Y	No liquid leakage	
	P1	Pump to feed reactor	Y	No liquid leakage	
	V1	Temperature controlled valve between reactor and cooler	Y	No liquid leakage	
	V2	Manually controlled valve between buffer tank and ultrasound system	Y	No liquid leakage	

	V13	Manually controlled valve for air supply to photoreactor	Y	No liquid leakage	
	V16	Manually controlled valve between cooler and buffer tank	Y	No liquid leakage	
		Connections	Y	No liquid leakage	
Check accuracy and stability of measurement	T_T1	Temperature sensor	Y	Pass	
	KT1	Cooler temperature control	Y	Pass	Problem of insufficient distribution of cooling capacity was solved by providing a second cooler (see above)
		Lamps photoreactor	Y	Pass	1. Light intensity could not be set at fixed values. Therefore, extra connections were provided for coupling to volt meter or indicator. 2. Calibration performed with luminometer.
Check absence of reactor/tank break	T1	Photoreactor	Y	Pass	
	T2	Buffer tank	Y	Pass	

3.2 Part 2: Ultrasound unit

Table 3. The test results for the ultrasound unit in the hardware phase.

Test objective	Instrument ref	Instrument description	Test perfomed (Y/N)	Test result	Comments
Check sufficient/homogeneous mixing	R3	Mixer for the concentration tank	Y	Pass	
Check absence of liquid/gas leakage	T3	Concentration tank	Y	Pass	
	P2	Recycle pump	Y	Pass	
	P3	Harvest pump ultrasound unit	Y	Pass	
	C2	Resonance cell	Y	Pass	
	L_T1	Level sensor	Y	Pass	
	V11	Manually controlled valve to remove concentrated cells from the concentration tank	Y	Pass	
	V12	Manually controlled valve for air cooling of resonance cell	Y	Pass	
	V15	Manually controlled valve after ultrasound unit for sampling	Y	Pass	
		connections	Y	Pass	
Check accuracy and stability of measurement	C1	Ultrasound controller	Y	Pass	
	L_T1	Level sensor	Y	Pass	Alarm lit when low level reached
Check absence of reactor/tank break	T3	Concentration tank	Y	Pass	
	C2	Resonance cell	Y	Pass	

3.3 Part 3: Filtration unit

Table 4. Test results for filtration unit in hardware phase

Test objective	Instrument ref	Instrument description	Test perfomed (Y/N)	Test result	Comments
Check absence of liquid/gas leakage	UF1	Membrane	Y	No liquid leakage	
	UF2	Membrane	Y	No liquid leakage	
	T4	Demineralised water tank	Y	No liquid leakage	
	P4	Pump in recirculation loop	Y	No liquid leakage	
	Ti1	Temperature sensor	Y	No liquid leakage	
	Pi1	Pressure sensor before membranes	Y	No liquid leakage	
	Pi2	Pressure sensor after membranes	Y	No liquid leakage	
	Pi3	Pressure sensor on permeate flow	Y	No liquid leakage	
	Fi1	Flow meter	Y	No liquid leakage	
	V3	Manually controlled valve in retentate recycle to ultrasound unit	Y	No liquid leakage	
	V4	Manually controlled valve between ultrasound and filtration unit	Y	No liquid leakage	

	V5	manually controlled valve between demineralised water tank and membranes	Y	No liquid leakage	
	V6	Manually controlled valve in water supply pipe to demineralised water tank	Y	No liquid leakage	
	V7	Manually controlled valve for deaeration of demineralised water tank	Y	No liquid leakage	
	V8	Manually controlled valve for air supply to demineralised water tank	Y	No liquid leakage	
	V9	Manually controlled valve for permeate drain	Y	No liquid leakage	
	V10	Manually controlled valve for retentate sampling	Y	No liquid leakage	
	V14	Manually controlled valve in filtration recycling loop	Y	No liquid leakage	
	PR1	Pressure reducer	Y	No gas leakage	
	PR2	Pressure reducer	Y	No gas leakage	
		Connections	Y	No liquid/gas leakage	

Check accuracy and stability of measurement	Ti1	Temperature sensor	Y	Pass	
	Pi1	Pressure sensor before membranes	Y	Pass	
	Pi2	Pressure sensor after membranes	Y	Pass	
	Pi3	Pressure sensor on permeate flow	Y	Pass	
	Fi1	Flow meter	Y	Pass	
Check absence of reactor/tank break	T4	Demineralised water tank	Y	Pass	

4. Updated scheme of the breadboard

As a result of corrective measures taken in the functional tests of the hardware phase, the design of the breadboard was corrected. The only change in Figure 1 compared to previous schemes is the addition of an extra cooler.

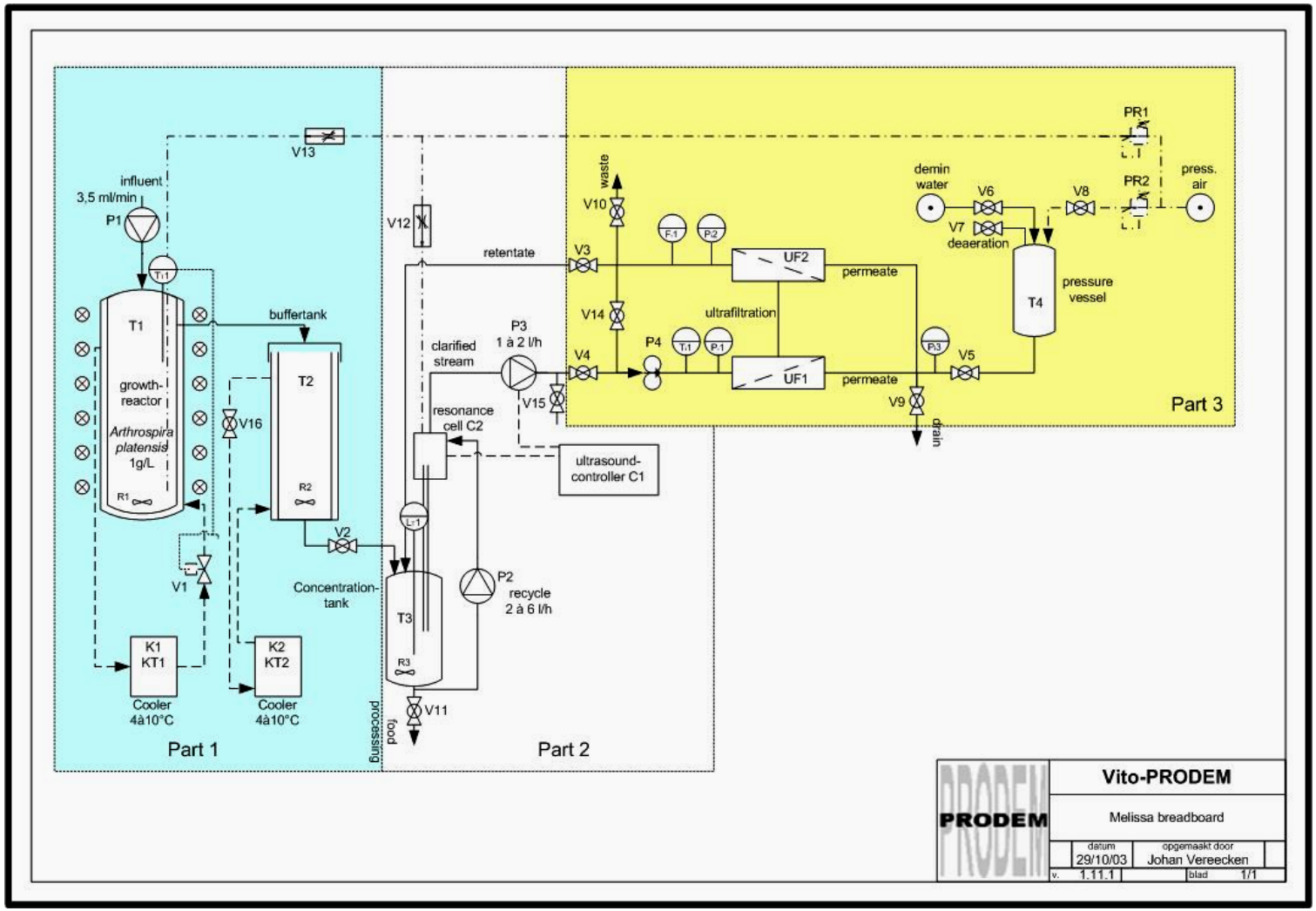
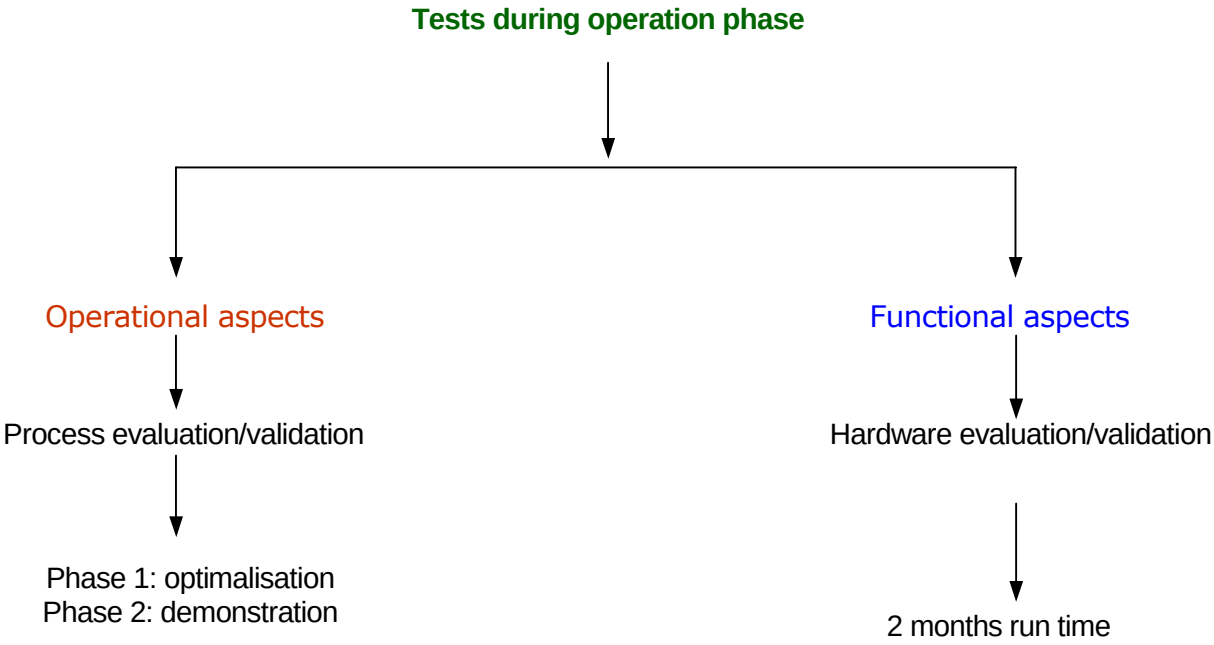


Figure 1. Overview scheme of the breadboard for *Arthrospira* harvesting.

5. Test results for operation phase

According the test plan procedure, the tests during the operation phase were performed taking into account two major aspects: the operation aspects and the functional aspects as follows:



As already mentioned in TN72.9.1, the components of the breadboard need to fulfil some requirements during the operation phase. the major requirements are listed in Table 5.

Table 5. Requirements of the breadboard components for the operation phase

Requirements	Related instrumentation
Functional aspects	
1. Is there clogging of components?	Reactors, pumps, valves, connections, membranes
2. Is there corrosion of components?	Reactors, cooler, pumps, connections
3. Is there deterioration of measurement/control?	Sensors, controller
4. Do instruments/units break?	Cooler, pumps, mixers, valves, sensors, lamps, connections, controller, membranes
Operational aspects	
1. Is algae growth rate as expected at the chosen environmental conditions?	Photoreactor
2. Does biomass quality deteriorate during storage?	Buffer tank
3. Are the conditions for ultrasonic concentration of the harvested algae optimised?	Ultrasonic system
4. Is the cell concentration of the retentate of the membrane filtration unit acceptable?	Filtration unit
5. Is the biomass recovery in the membrane filtration unit acceptable?	Filtration unit
6. What is the optimal membrane cleaning procedure?	Filtration unit
7. Is the number of washing steps optimised to obtain the desired final salinity of the algae concentrate?	Tanks, membranes, pumps, valves

5.1 Test results for functional aspects

5.1.1 Part 1: Photobioreactor and buffer tank

Table 6. Test results for photobioreactor and buffer tank during operation phase (functional aspects)

Test objective	Instrument ref	Instrument description	Test perfomed (Y/N)	Test result	Comments
Check sufficient/homogeneous mixing	R1	Mixer for photoreactor	Y	Pass	
	R2	Mixer for buffer tank	Y	Pass	
Check correct cooling of the reactor/tank	K1	Cooler	Y	Pass	
Check correct cooling of the buffer tank	K2	Cooler	Y	Pass	
Check absence of liquid/gas leakage	T1	Photoreactor	Y	No liquid leakage	
	T2	Buffer tank	Y	No liquid leakage	
	T_T1	Temperature sensor	Y	No liquid leakage	
	P1	Pump to feed reactor	Y	No liquid leakage	
	V1	Temperature controlled valve between reactor and cooler	Y	No liquid leakage	
	V2	Manually controlled valve between buffer tank and ultrasound system	Y	No liquid leakage	
	V13	Manually controlled valve for air supply to photoreactor	Y	No liquid leakage	

	V16	Manually controlled valve between cooler and buffer tank	Y	No liquid leakage	
		Connections	Y	No liquid leakage	
Check accuracy and stability of measurement	T_T1	Temperature sensor	Y	Pass	
	KT1	Cooler temperature control for reactor	Y	Pass	
	T_T2	Temperature sensor	Y	Pass	
	KT2	Cooler temperature control for buffer tank	Y	Pass	
		Lamps photoreactor	Y	Pass	Calibration performed with light sensor “photometer type LI-COR (Quantum/Radiometer/Photometer) model LI-189 provided for use by UBP-France”. .
Check absence of reactor/tank break	T1	Photoreactor	Y	Pass	
	T2	Buffer tank	Y	Pass	

5.1.2 Part 2: Ultrasound unit

Table 7. The test results for the ultrasound unit during operation phase (functional aspects).

Test objective	Instrument ref	Instrument description	Test perfomed (Y/N)	Test result	Comments
Check sufficient/homogeneous mixing	R3	Mixer for the concentration tank	Y	Pass	
Check absence of liquid/gas leakage	T3	Concentration tank	Y	Pass	
	P2	Recycle pump	Y	Pass	
	P3	Harvest pump ultrasound unit	Y	Failed	Over-dimensioning of P3 caused decrease in power delivery of the pump at low flow rates (< 100 ml/min)
	C2	Resonance cell	Y	Pass	
	L_T1	Level sensor	Y	Pass	
	V11	Manually controlled valve to remove concentrated cells from the concentration tank	Y	Pass	
	V12	Manually controlled valve for air cooling of resonance cell	Y	Pass	
	V15	Manually controlled valve after ultrasound unit for sampling	Y	Pass	
		connections	Y	Pass	
Check accuracy and stability of measurement	C1	Ultrasound controller	Y	Pass	
	L_T1	Level sensor	Y	Pass	Alarm lit when low level reached
Check absence of reactor/tank break	T3	Concentration tank	Y	Pass	
	C2	Resonance cell	Y	Pass	

5.1.3 Part 3: Filtration unit

Table 8. Test results for filtration unit during operation phase (functional aspects)

Test objective	Instrument ref	Instrument description	Test perfomed (Y/N)	Test result	Comments
Check absence of liquid/gas leakage	UF1	Membrane	Y	No liquid leakage	
	UF2	Membrane	Y	No liquid leakage	
	T4	Demineralised water tank	Y	No liquid leakage	
	P4	Pump in recirculation loop	Y	No liquid leakage	
	Ti1	Temperature sensor	Y	No liquid leakage	
	Pi1	Pressure sensor before membranes	Y	No liquid leakage	
	Pi2	Pressure sensor after membranes	Y	No liquid leakage	
	Pi3	Pressure sensor on permeate flow	Y	No liquid leakage	
	Fi1	Flow meter	Y	No liquid leakage	
	V3	Manually controlled valve in retentate recycle to ultrasound unit	Y	No liquid leakage	
	V4	Manually controlled valve between ultrasound and filtration unit	Y	No liquid leakage	
	V5	manually controlled valve between demineralised water tank and membranes	Y	No liquid leakage	

	V6	Manually controlled valve in water supply pipe to demineralised water tank	Y	No liquid leakage	
	V7	Manually controlled valve for deaeration of demineralised water tank	Y	No liquid leakage	
	V8	Manually controlled valve for air supply to demineralised water tank	Y	No liquid leakage	
	V9	Manually controlled valve for permeate drain	Y	No liquid leakage	
	V10	Manually controlled valve for retentate sampling	Y	No liquid leakage	
	V14	Manually controlled valve in filtration recycling loop	Y	No liquid leakage	
	PR1	Pressure reducer	Y	No gas leakage	
	PR2	Pressure reducer	Y	No gas leakage	
		Connections	Y	No liquid/gas leakage	
Check accuracy and stability of measurement	Ti1	Temperature sensor	Y	Pass	
	Pi1	Pressure sensor before membranes	Y	Pass	
	Pi2	Pressure sensor after membranes	Y	pass	
	Pi3	Pressure sensor on permeate flow	Y	Pass	
	Fi1	Flow meter	Y	Pass	
Check absence of reactor/tank break	T4	Demineralised water tank	Y	Pass	

5.2 Test results for operational aspects

5.2.1 Part 1: Photobioreactor and buffer tank

Table 9. Test results for photoreactor and buffer tank during operation phase (operational aspects)

Test objective	Instrument ref	Instrument description	Test perfomed (Y/N)	Test result	Comments
Check optimal growth rate (1 g biomass /L.d) .	R1	Lamps light intensity (300 W/m^2)	Y	Pass	The corresponding voltage to 300 W/m^2 = 3.42 V (calibration of the photoreactor performed with light sensor type LI-COR (Quantum/Radiometer/Photometer) model LI-189 provided for use by UBP-France". .
	T_{T1}	Temperature sensor	Y	Pass	
Check the quality of the biomass after storage	R2	Buffer tank	Y	Pass	No deterioration in the structure of the biomass was observed in the buffer tank after 24 h storage compared to the biomass in the photobioreactor

5.2.2 Part 2: Ultrasound unit

Table 10. Test results for ultrasound unit during operation phase (operational aspects)

Test objective	Instrument ref	Instrument description	Test perfomed (Y/N)	Test result	Comments
Optimization of the conditions for ultrasonic concentration of the harvested algae	R3	Concentration tank	Y	Pass	
	C1	Ultrasound controller	Y	Pass	
	P2	Recycle pump	Y	Pass	
	P3	Harvest pump ultrasound unit	Y	Failed	The function of the pump which normally was controlled by ultrasound controller was over taken by pump (P4)
	C2	Resonance cell	Y	Pass	

5.2.3 Part 3: Filtration unit

Table 11. Test results for filtration unit during operation phase (operational aspects)

Test objective	Instrument ref	Instrument description	Test perfomed (Y/N)	Test result	Comments
Acceptability of cells concentration of the retentate	UF1	Membrane	Y	Pass	
	UF2	Membrane	Y	Pass	
	P4	Pump in recirculation loop	Y	Pass	Biomass retention of 100% was achieved at membranes level
Recovery of biomass in the membrane filtration	UF1	Membrane	Y	Failed	Due to the shear stress, microscopic observations of the retentate from the filtration unit showed drastic changes in the shape of the biomass.
	UF2	Membrane	Y	Failed	Due to the shear stress, microscopic observations of the retentate from the filtration unit showed drastic changes in the shape of the biomass.
	Fi1	Flow meter	Y	Pass	

6. Hardware performances

6.1 Photobioreactor

The photobioreactor was operated in a continuous mode. The growth conditions for *Arthrospira platensis* are described in Technical notes 72.6 and 72.7.1. The wet reactor volume was 5 litres and the harvested volume per day to be processed was 5 litres with an algal concentration of around 1 g/L.

6.1.1 Calibration

The photobioreactor was calibrated with a light sensor type LI-COR (Quantum/Radiometer/Photometer) model LI-189 provided for use by UBP-France". For an algal optimal growth rate of 1 g biomass/L.d., light intensity of 300 W/m^2 was necessary. The light sensor was used to measure the incident flux in the centre of the reactor expressed as Eb (µmol/m^2.s). The measurements were performed at 6 different levels, from the bottom of the photoreactor to the top. Table 12, shows the average data collected at the 6 different levels.

Table 12. Average light intensity calculation over the whole reactor

Volts	Eb (µmol/m2.s)	F0 (µmol/m2.s)	W/m2
1,8	93,5	80,1	20,9
2,4	316,9	271,5	70,8
3,1	658,3	564,0	147,1
4,1	1394,5	1194,8	311,7
5,0	2312,3	1981,2	516,8
6,0	3597,4	3082,2	804,1
6,9	4745,7	4066,1	1060,7
7,7	5934,3	5084,5	1326,4
8,9	7729,5	6622,6	1727,6
9,3	8569,8	7342,6	1915,5

Conversion from Eb to W/m^2 (PAR)

Conversion from Eb into incident flux F0 at the outer side of the photobioreactor

F0 = Eb x rb/ 3,1415926 x R (µmol/m2.s)

F0= Flux at the outer side of the reactor

Eb= Flux measured by the light sensor central in the reactor

rb= Radius of the sensor = 3 cm

R= Interne radius of the reactor = 11 cm

π = 3,1415926

Conversion from µmol/m2.s to W/m2:

F0 /4,6 x 1,2

4.6 = approximate estimation of the emission spectrum of the lamps

1.2 = correction factor depending on emission spectrum

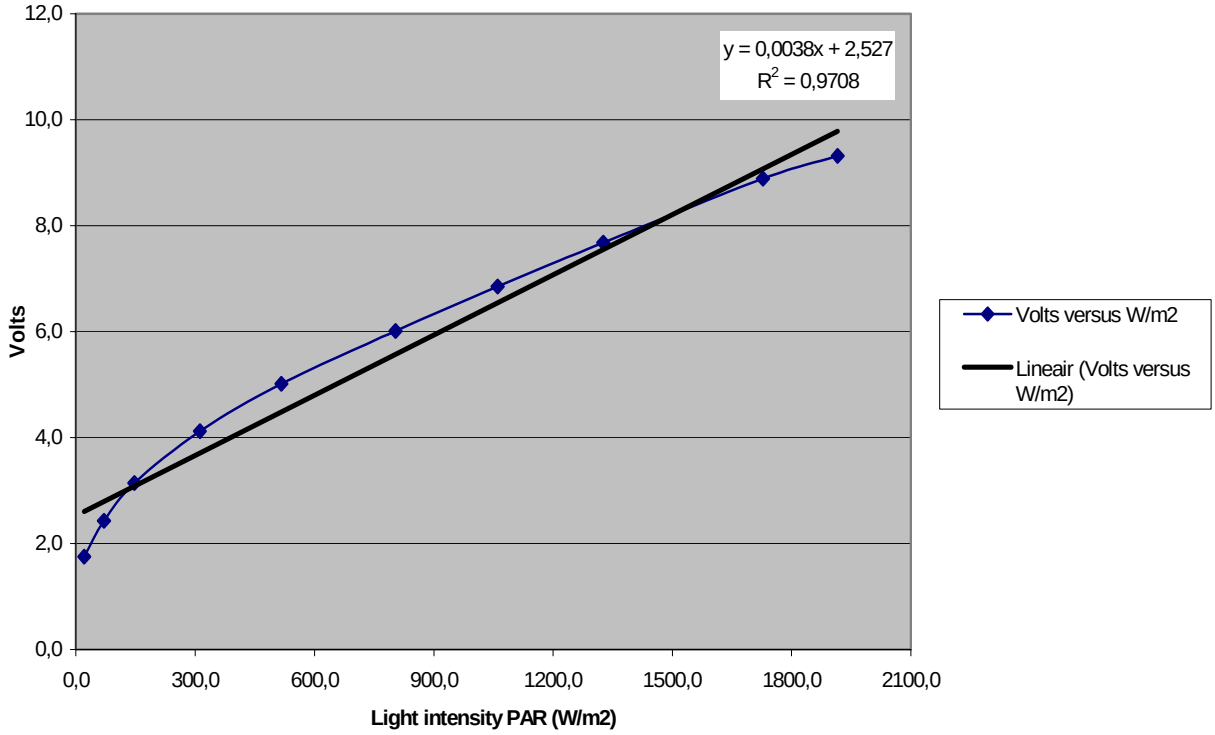


Figure 2. Results of light calibration inside the photobioreactor

For a corresponding light intensity of 300 W/m^2, 3.67 Volts were needed.

For 300 W/m2	
Voltage (volts) =	3,67

A volt meter was continuously connected to the lighting system of the photoreactor. A continuous follow-up of this system could be performed by switching on the volt meter and by adjusting the light intensity to 3.67 Volts when necessary.

For the start-up of the photoreactor, 5 L of the *Arthrospira platensis* at concentration of 0.6 g /L was introduced into the glass vessel, the magnetic stirrer R1 switched on and started aeration. The growth medium consisted of Zarrouk medium fed at a flow of 5 L/d. Over the whole period of running of the hardware, the biomass was almost stable in the reactor, fluctuating between 0.8 and 1 g/L with an almost stable production of 5 L algal suspension per day.

6.1.2 Origin of the algal culture

The algal suspension , *Arthrospira platensis* PCC-8005 was kindly provided by SCK-Mol in Belgium and cultivated in Zarrouk medium at EPAS laboratory before being inoculated in the photobioreactor. Microscopic observations showed that the culture had a nice spiral

shape, the same as the one used by VITO (provided by EPAS) during the test program for the ultrasound system.

6.2 Performances of the Ultrasound Unit

The principle of ultrasound separation in fully described in TN 72.7.3 of ESA contact 1567/01/NL/ND.

Based on preliminary experiments performed by VITO with the Applisens® ultrasound separation system and on the operating parameter mentioned on the user manual of the unit mounted on the breadboard, the begin settings of the ultrasound system were defined for the test procedure.

Several paramaters needed to be optimised:

Harvest flow rate: in TN 72.8 the ultrasound system was chosen to process 5 l of algae in about 2 h. Because optimal separation efficiencies can only be achieved below the maximum capacity of the ultrasound system (50 l/d), the harvest flow has to be limited to 2 l/h.

Ratio harvest to recirculation flow: Preliminary experiments performed by VITO indicated that the ratio has to be between 1:2 and 1:3. For testing of the breadboard a 1:2 ratio was chosen. A 1:3 ratio was not selected because part of the aggregates started to float in the resonator chamber due to turbulences given by the recirculation flow at this ratio.

Power input or field intensity: based on tests reported in TN 72.7.3, a field intensity of 5 W should give optimal results. At this field intensity, separation efficiencies were quite low around 12-15% (results not shown). Therefore, it was decided to test the system for field intensities from 6 W to 10 W (maximum capacity). Separation efficiencies below 90% were not selected. High field intensities were to be used to achieve more than 90% separation efficiencies.

On/off time: To avoid accumulation of cells in the resonance chamber, the 'on' time should be decreased and the 'off' time increased to allow the aggregates to settle. It was a trial and error process to try to optimize these settings. From the data collected from the work of VITO in this field, 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 was switched off, part of the aggregates started to float in the resonator chamber instead of settling and leaved the system with the harvest flow. It was therefore advisable to keep the 'on' time as long as possible. Settings used for testing the hardware were: 300 s "on" and 10 s "off" times

Separation efficiency was calculated as follows based on a mass balance:

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

With: SS = suspended solids

Harvest = Clarified outlet stream from ultrasound system

V = volume

6.2.1 Effect of applied voltage on separation efficiencies

For the experiments, the harvest rate was set at 2 L/h or 48 L/d since the type of the ultrasound unit is made to harvest continuously up to 50 L/d. Taking into account that the

ratio between harvest and recirculation should be around 0.5, the recirculation rate was set at 4 L/h or around 96 L/d. Initial field was chosen to be between 6 W and 10 W with a timer setting of 300 s “on” and 10 s “off”. The results of the experiments are shown in Figure 3.

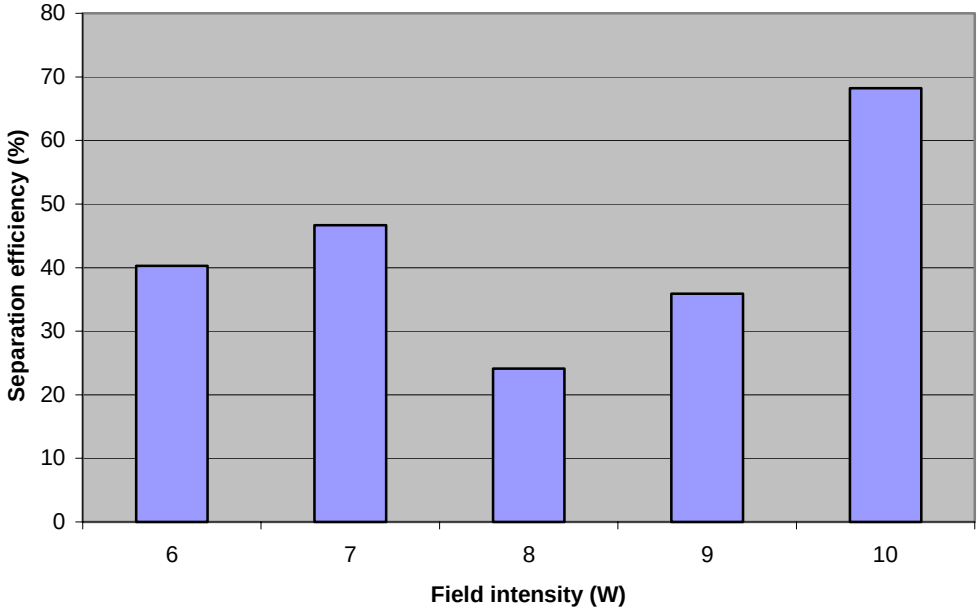


Figure 3. Effect of increasing field intensity on separation efficiency. The recirculation flow was 4 L/h and the harvest flow 2 L/h.

Separation efficiencies lower than 90% were obtained in the first set experiments. An increase in field intensity only improved the results to a certain level. A separation efficiency of 69% was obtained at 10 W (maximum intensity of the system). These results are associated with one major failure reported on the breadboard during the operation phase (Ref: section 5.1: test results for functional aspects and section 5.2: test results for operation aspects) and which is summarised here down:

- Low performances of the micro gear pump (P3) during operation of the recirculation gear pump (P4) of the ultrafiltration unit. P4 was applying a suction force on the filtrated stream from the ultrasound unit also during the “off” time of the ultrasound controller leading to a wash out of the agglomerates from the resonance chamber instead of settling back to the concentration tank.

Visual observation showed that the resonance chamber was almost full of alga at the upper part at a harvest flow of 2L/h and even at a lower harvest flow of 1L/h (Figure 4). This indicate that despite the fact that the field was strong enough to retain the cells, the suction capacity of the P4 livered a higher flow, which lead to turbulences in the resonance chamber and therefore a wash out of the aggregates.

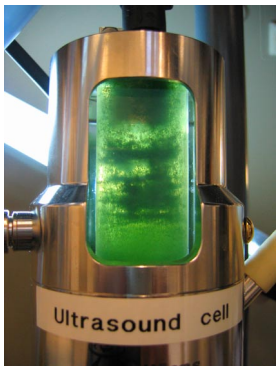


Figure 4: Aggregation of alga cells in the resonance chamber

6.2.2 Effect of on/off timer settings on separation efficiencies

Decreasing the on/off sequence for application of the field did not much improve the separation efficiencies since the "off" time of the ultrasound controller did not definitely stop the stream to pass through the resonance chamber.

In spite of the adjustments, the overall results were a lot worse than the ones presented in Figure 12. Decreasing the power, varying the on/off time of the ultrasound system, decreasing the ratio harvest to recirculation flow and decreasing the harvest flow to less than 2L/h did not improve the achieved results.

6.2.3 Concentration factor

After different trials with different field intensities, a constant separation efficiency could be achieved when a field intensity of 10 W was applied from the beginning of the experiment. For lower field intensities from 6 W to 9 W, drastic oscillations in OD_{750} were observed on the clarified stream samples taken at regular times during the tests (Figure 5).

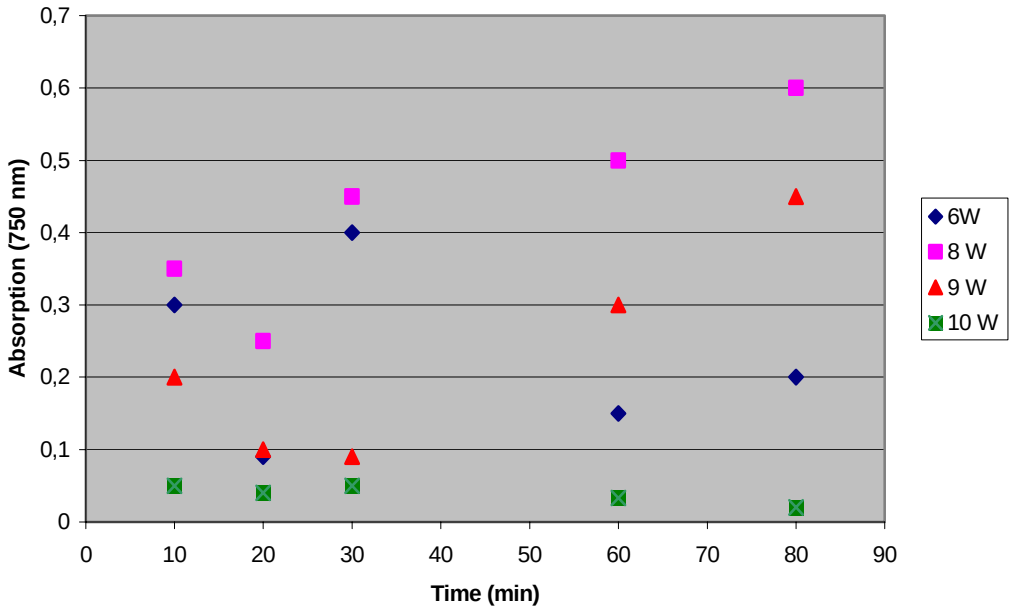


Figure 5. Changes in OD_{750} in function of time at different field intensities. The recirculation flow was 4 L/h and the harvest flow 2 L/h.

The strategy to gradually increase power inputs was not successful. The most efficient way to achieve a satisfactory separation was to put a high power input from the beginning

of the test. It seemed to be difficult to obtain a clear harvest stream once the biomass has concentrated the resonance chamber. Each time the ultrasound controller switched off, the cells were released in the clarified stream.

The breadboard was operated with an initial volume in the concentration tank of 5 litres at time 0 and was stopped when the volume in the concentration tank was around 0.7 to 0.5 Litres at time 2h 15 min, independently of the concentration of solids.

The biomass concentration factor was calculated as follows:

Concentration factor = $SS_{\text{final concentrated suspension}} / SS_{\text{initial}}$

With:

$SS_{\text{final concentrated suspension}}$ = Suspended solids concentration in the concentrated cell suspension

SS_{initial} = Suspended solids concentration initial

The volumetric concentration factor was calculated as follows:

Volumetric concentration factor = $V_{\text{initial}} / V_{\text{final concentrated suspension}}$

With:

V_{initial} = initial volume of suspension

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

The results of the experiments with the different field intensities are reported in Figure 6.

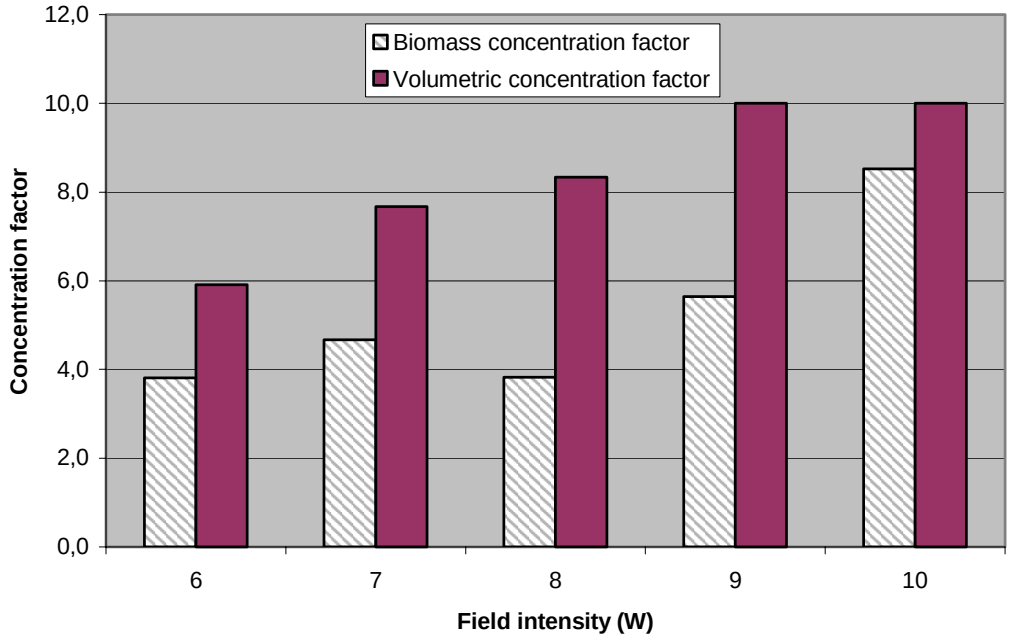
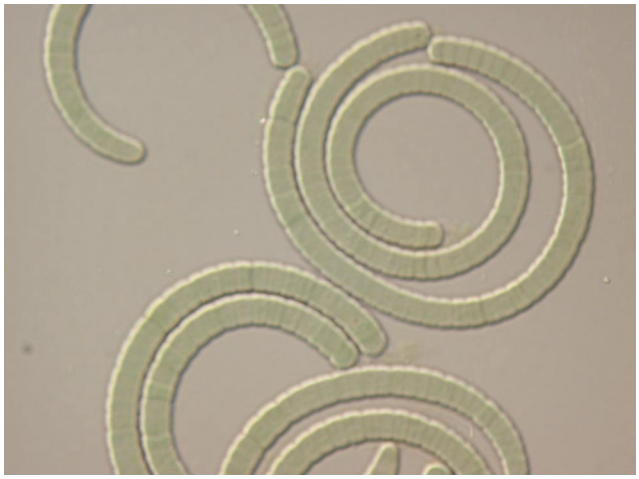


Figure 6. Effect of increasing field intensities on biomass concentration factor. The recirculation flow was 4 L/h and the harvest flow 2 L/h.

As shown in Figure 6, the biomass concentration factor does not correspond fairly well with the volumetric concentration factor for most of the field intensities applied to the system. This gives an additional confirmation to the wash out of the biomass from the ultrasound system. Indeed, a biomass concentration factor of 10 could not be achieved and the maximum obtained value at 10 W field intensity was a biomass concentration factor of 8.5. This is different from the results obtained by VITO during the test program with the ultrasound unit where a biomass concentration factor of 23 was obtained. These differences are associated with the failures in the breadboard reported in paragraph 6.2.1.

6.2.4 Effect on cell shape

The shape of the cells was checked for each set of experiment with the different field intensities (from 6 W to 10 W). In all the experiments, the shape of the algal cells seemed not to be altered during processing as shown in Figure 7.



(A)

(B)

Figure 7. Microscopic view (400 X) of *Arthrospira platensis* before ultrasonic treatment **(A)** and in the harvest **(B)** at field intensity of 10 W.

Neither the harvest or the concentrated cell suspension seemed to contain injured or fragments of dead cells. This is an indication that the ultrasound treatment does not affect the viability of the algae.

Contrarily to what was observed on the results of VITO during the test program with the ultrasound unit, no gas bubbles appeared in the resonance chamber during all the experiments. Therefore, the relative low separation efficiency obtained is not related to turbulences in the resonance chamber but as mentioned previously to breadboard specific failures.

6.2.5 Effect on cell integrity

In all the tests, the integrity of the cells was checked by micrscopical observations. No indications were found to confirm that the ultrasound forces induced cell lysis or loss of cell viability or even release of cell constituents.

6.2.5.1 Proteins determination

Additional to microscopic observations, proteins determination were performed according to Lowry method, using the kit: P5656 (protein assay kit, Sigma diagnostics®). The analysis were made on cell suspensions and on filtrated samples to presence of absence of proteins in the filtrated samples. According to the obtained results, none of the samples contained any proteins. This would indicate that no cell constituents were released during the concentration step of the algae. As already mentioned in TN 72.7.3, these results are not surprising since the ultrasound separation technology is typically used to retain viable cells in fermentors. It does not interfere with the viability of yeast or mammalian cells in the perfusion cultures for which it is applied. The proteins concentration in the biomass itself was rather high representing around 60% to 80% of the weight of the total biomass (expressed as dry weight). This percentage is quite acceptable since literature data report a 50 % to 70% of proteins of the total biomass (DW) in normal conditions and even more during high lighting periods (Falquet, 1996). Depending on the medium where it is cultivated, proteins content in *A.platensis* fluctuate significantly (Table 13).

Table 13. Changes in phycocyanin and protein contents in *Arthrospira platensis* cultivated in different ionic strengths (according to Cornet, 1992).

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

6.2.5.2 Exoplysaccharides (EPS)

To simulate the exoplysacchrides production in the filtrate stream, the “Photosim” program (J.F. Cornet version 3.0 – 2002) was used. Based on the reactor geometry, the value of the optical thickness for the reactor, the value of the incident radiant energy flux (W/m^2) and the value of the illuminated working volume in the photobioreactor (%) as well as some analysis results like: the biomass concentration in the incoming and outgoing flows, nitrates and sulphate concentrations, the calculations have been made from the “Photosim” program (Figure 8). The amount of EPS in the photobioreactor seem to approach 40 to 60% of the total biomass (DW). In optimal growth conditions, the normal production of EPS at low pH and under high light intensities is around 30% but high EPS are formed at high pH values. At an insufficient nitrogen supply, photosynthesis may

produce EPS, exclusively (Cornet, 1992) . Even if nitrates are sufficiently present in the culture medium, limitations in ammonium seem to favorise EPS production. This is more pronounced if temperature and lighting conditions are not respected.

EPS are more or less biodegradable, depending on the circumstances, which limits the quantity found back in the concentrated alga. An *A. platensis* with 60% proteins content, seems to contain around 30% EPS (Melissa Report 1996, pp 90). In our suspension, a 40% EPS was balanced with a 60% proteins content which indicate that EPS were produced in rather high amounts in the photobioreactor.

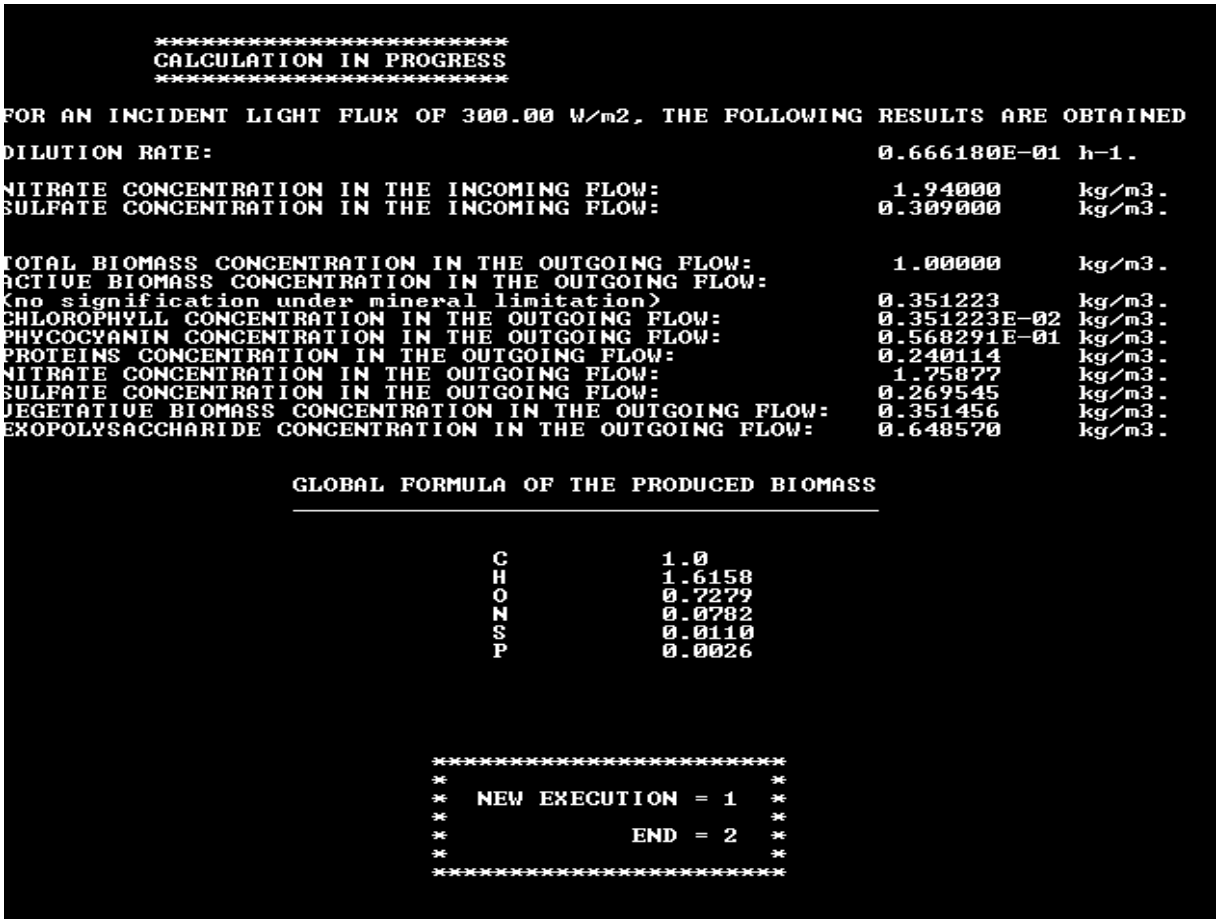


Figure 8. Results of data simulation for the photobioreactor using "Photosim" program.

6.2.5.3 Sugars

Sugars represent globally 15% to 25% of the total dry weight of *A.platensis* biomass. Simple sugars (glucose, fructose and saccharose) represent only a very low fraction of the total sugars like glucosans, glycogen and glycerol.

In our investigations, total sugars were determined using inversion method. The total percentage was quite low not exceeding 5% of the total dry weight. For effective determination of total sugars and more precisely the amount of glucosamines and meso-inositol phosphate which represent the major sugars in *A. platensis* another method of analysis should be used like the GC/MS.

The composition of the most important elements in collected *A. platensis* is as follows:

Proteins = 65 % in weight (norm : >50)
Sugars = 15 % in weight
Minerals = 7 % in weight (Total ash : <10)
Lipids = 6 % in weight
Fibres = 2 % in weight
Water = 5 % in weight (norm : <10)

Energetic Content = 5000 calories or 20,9 kJ/ g dry.

6.2.6 Reproducibility

The most effective results were obtained with the highest field intensity of 10 W. To check the reproducibility of the obtained results, four repeated tests were performed at different days. Repeated experiments showed a variation in separation efficiency between 69% and 75%.

6.2.7 Energy consumption

The energy consumption of the system for specific conditions: harvest of 100 litres *Arthrospira platensis* per day and concentration from 1 g/L to at least 10 g/L is reported in TN 72.7.3. To summarize, the total energy consumption of the ultrasound system should be in the neighbouring of 8 to 10 kWh/$m^{3.}$

6.3 Performances of the filtration unit

The clarified stream coming from the ultrasonic system is sent to a UF unit. This stream is supposed to contain approximately 50 mg/l cell suspension at the best separation efficiencies as reported in TN 72.3.3. The concentrate of the UF unit is sent back to the ultrasonic separation. As described earlier, each cycle should be finished in about 2 h 15 minutes. During each cycle, approximately 4.5 l has to be processed by the UF unit.

The following components were used in the UF unit :

- Two UF membranes ordered from TAMI Filtration. Type Céram inside, ATZ, 50 kD, 120 cm length, 3 channels, 10 mm outer diameter, 0.045 m^2 membrane area. Housing in stainless steel.
- Centrifugal pump (Verder, type V-MD 30C)
- Thermometer (temperature range 0-100°C)
- Three manometers in stainless steel (pressure range 0-4 bar)
- Flow meter polysulfon/PVC (flow range 50-500 l/h)
- PVC tank for demineralized water for back washing to concentrated stream in the UF membranes
- 9 valves

The operation of the ultrafiltration unit is described according to Figure 1.

Pump P4 was operated during the entire filtration process. Because of the pressure built up in the filtration loop, water was permeated through the membranes as long as clarified stream was transported from the ultrasound unit to the ultrafiltration loop. When the cell suspension in the concentration tank had reached the desired final volume of around 0.7-0.5 L, pump P3 was switched off, and valves V4 and V9 were closed. The demineralised water tank was filled with 4.5 l demineralised water which was used to back flush the biomass concentrate accumulated in the membranes. Pressure on the demineralised water tank was increased with pressurized air and with pump P4 still running, the demineralised water diluted the cell suspension in the ultrasound system (washing of the cells). The concentrate that was still in the ultrafiltration loop was also transported to the concentration tank while at the same time the ultrafiltration loop was cleaned.

The consumption of chemicals is mainly an item for the operation of the membrane filtration unit. Hydrodynamic conditions are chosen to reduce membrane fouling to the highest extent. Furthermore, a backwash with clean water was provided after each run in the present concept of the harvesting system with the double aim to wash the cell suspension and to reduce membrane fouling. Since the membranes did not clog at any moment (no pressure drop was observed) during the whole experimental period of 2 months, membranes cleaning was not necessary. It was experimentally observed that the degree of irreversible algae binding to ceramic membranes was rather low not exceeding 10% to 16% (minimum cell recovery from the ultrafiltration unit is of around 84%) compared with the tests performed by GEPEA-Nantes during the test program (TN72.7.5 not delivered yet) where around 20% cell binding on the membranes was observed.

6.4 Separation efficiencies of the breadboard

Despite the fact that the ultrasound unit did not succeed to reach separation efficiencies of 90% to 95%, the cell suspension leaving the ultrasound unit was completely retained by the membranes. The filtrated stream was completely empty of biomass for all the tests (Figure 9) except for the test performed at 8 W field intensity. During this test, the ultrafiltration membranes were overloaded with biomass that has not been retained by the ultrasound unit. At 8 W field intensity, the separation efficiency of the ultrasound unit was 24%, which means that the ultrafiltration membranes had to retain 76% of the remained cell suspension. During this test, the colour of the collected filtrate from the breadboard turned into bluewish and the one of the retentate into yellowish colour. To complete the visual observations, microscopic evaluation of the shape and viability of the cells was performed (Figure 10). It was evident that filaments fragmentation was occurring due to the shear stress applied on the membranes which explains the discharge of the cell constituents in the filtrate. There is an evidence that the membranes could ensure a 100% separation efficiency of the whole system, but till a certain extend. According to our test results, the membrane filtration unit could ensure a 100% separation efficiency of a maximal initial algal suspension of 0.5 g/L. For biomass concentration exceeding 0.5 g/L the separation efficiency decreases significantly and cells are seriously damaged.

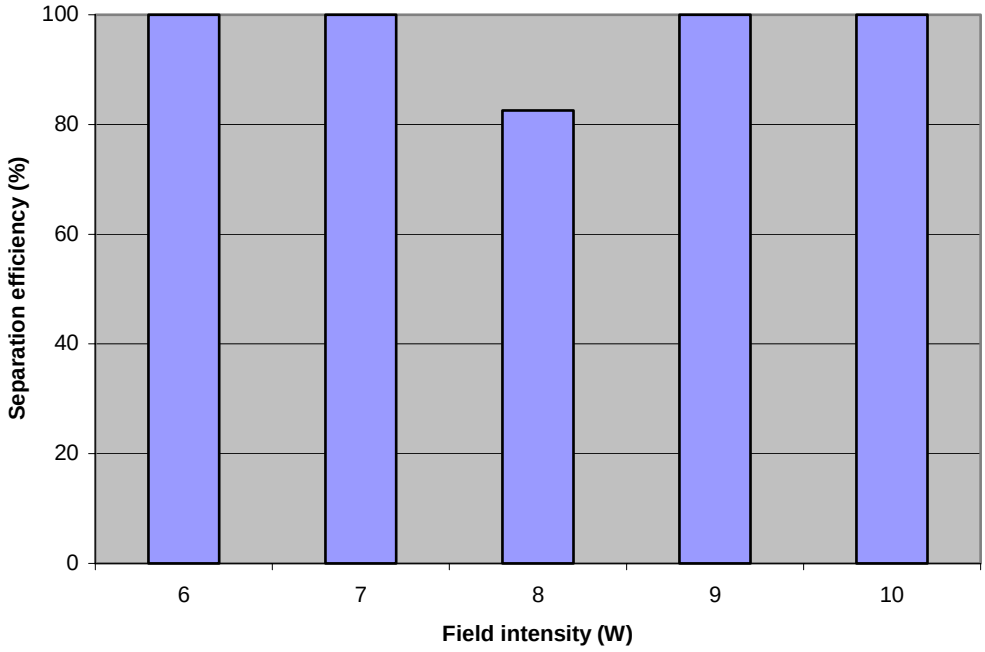


Figure 9. Effect of increasing field intensity on separator efficiencies of the whole breadboard. The recirculation flow was 4 L/h and the harvest flow 2 L/h.

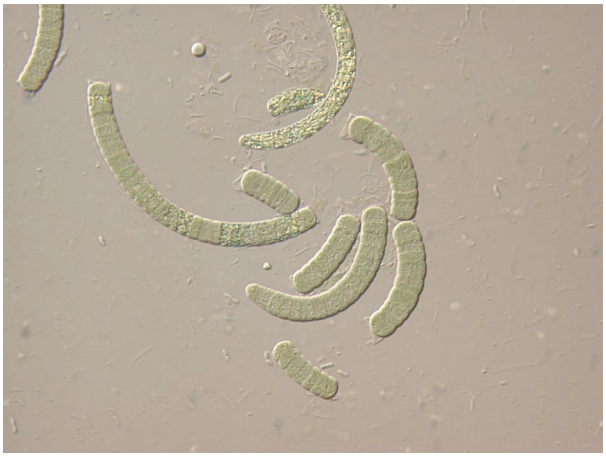


Figure 10. Microscopic view (400 X) of *Arthrospira platensis* in the concentrated stream of the filtration unit. Filaments fragmentation and dispersed cell inclusions are observed.

7. Adaptation of the breadboard

As already mentioned, the main objective of combining the ultrasound unit with the ultrafiltration unit for *Arthrospira platensis* harvesting is the insure a complete solid-liquid separation efficiency where the ultrasound should insure at least a 90 % biomass retention. The remaining 10% could significantly be retained by the ultrafiltration unit, avoiding by this mean biomass damage during the ultrafiltration.

In order to fulfil these requirements, some corrections have been made in the breadboard:

1. Pressure build up was initiated by placing a back pressure regulator at the outlet of the filtrate (after valve 10, Ref: Figure 1). As a result, filtrate production was possible after a pressure build up of 0.7 bar was reached and pump P4 was not overcoming the function of pump P3. The continuous suction of the biomass from the resonance chamber was not any more observed during the “stop” time of the ultrasound controller and the flow rate of the generated filtrate from the system was equal to the flow rate of algal suspension flowing through the ultrasound unit (2 L/h).

2. Pump P3 was over-dimensioned in its initial concept. It was running at less than 10% of its capacity leading to some losses in its performances. As shown in Figure 11, the pressure delivered by the pump is rather low for low flow rates. As indicated before, the harvesting flow rate of the alga was fixed at 2L/h (this is 33.3 ml/min). In this range, the pressure delivered by the pump is rather low. Therefore, it was decided to replace the pump by another with higher performances at lower flows.

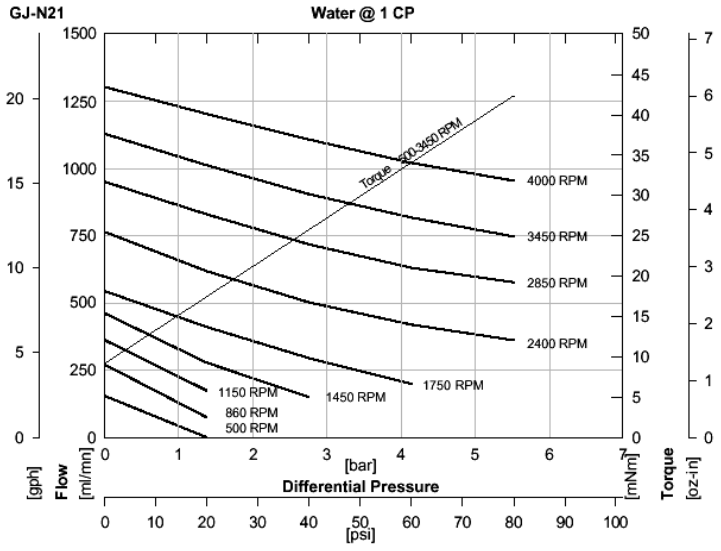


Figure 11. Performances of the Magnetic Drive Gear Pump P3

7.1 Results of separation efficiencies

7.1.1 Ultrasound separation system

After making some changes on the breadboard (see paragraph 7), some tests have been performed to evaluate the improvements in separation efficiencies. Since the most representative results have been obtained at field intensity of 10 W, the focus was done on this field to check the increase in efficiencies after the changes have been done. The results of the tests are presented in Figure 12.

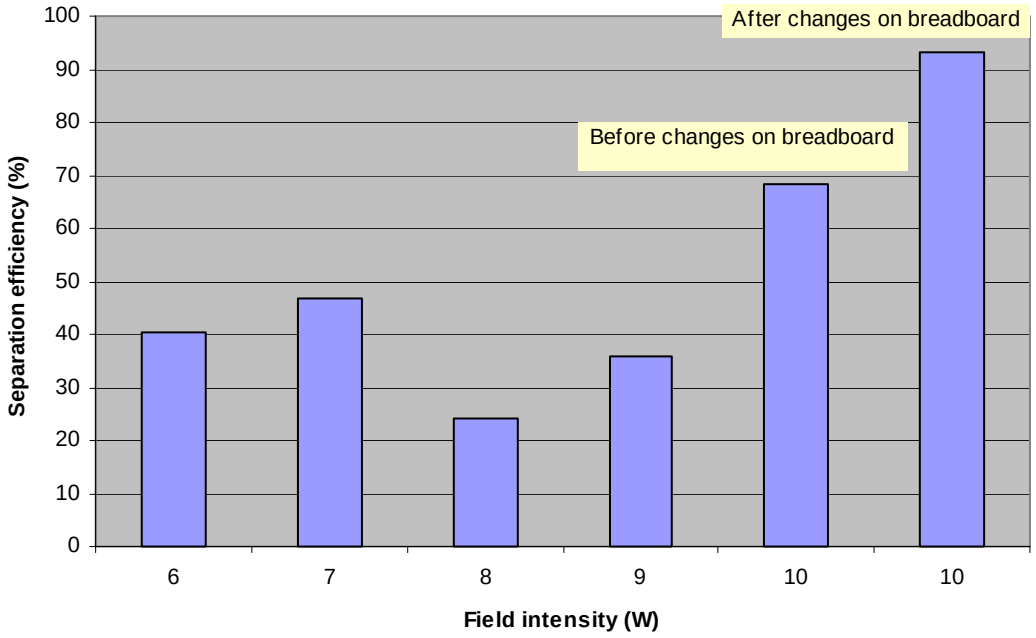


Figure 12. Effect of increasing field intensity on separation efficiency of the ultrasound unit after adaptation of the breadboard. The recirculation flow was set at 1L/h and the harvest flow at 2L/h.

Separation efficiencies of 93% were obtained with the ultrasound unit with the new adapted system. Based on the suggestion of Bosma (Agricultural University of Wageningen), the same experiment was performed with chlorella alga and the same separation efficiencies have been reported based on mass balances. These were satisfying results since the remaining 7% of the algal suspension were completely retained by the membrane filtration unit. No residues were obtained in the final collected filtrate. The remaining 7% suspension, in case they present some damages in the integrity of the filaments, could eventually be withdrawal (wasted) from the system by back washing membranes content in another tank than the concentration tank.

8. Desalination efficiency of the system

The objective of the desalination of the washing water in twofold: The first objective is to obtain a filtrate which contains high salt amounts and which could be used as culture medium for the algal cells. The second objective is to obtain a concentrate of alga which would be re-suspended in demineralised or low salts water decreasing significantly their salt contents. Previous tests, performed by VITO, showed that completely desalinated water leads to cell lysis (Osmotic pressure). Therefore, a certain amount of salts should to be present in the washing water. An option could be the further processing of the filtrated wasted by the system via electrodialysis and to use the desalinated stream to wash the alga rather than to use demineralised water.

The conductivity was measured in the influent (Zarrouk medium according to Zarrouk, 1966), in the photbioreactor, on the wasted filtrate at the end of a concentration cycle and on the cell suspension after the washing step. Some other complementary parameters (pH, dry matter, ashes and metals) have been also measured to determine the desalination efficiency of the breadboard. The analysis results are shown in Table 14.

Table 14. Analysis results of the major parameters and salts compounds at different levels of the breadboard performances.

Parameter	Influent	Photobioreactor	Biomass after first washing cycle	Filtrate wasted
pH	9.0	8.9	9.9	9.8
EC (mS/cm)	21.5	21	4.4	15.1
Na (mg/L)	7600	4600	1800	4700
Mg (mg/L)	25	14	6.2	21
Ca (mg/L)	12.5	9.5	2.5	4.0
K (mg/L)	1000	730	240	490
DM (mg/L)	18.7	22.3	7.2	8.5
Ashes (mg/L)	16.7	19.6	2.5	7

Based on these results, the desalination efficiency of the algal suspension was calculated after the first washing step. One of the desalination requirements was to study the option to desalinate the algal suspension down to very low salts contents (< 0.3 g/L) mainly through several washing steps. However, as already observed in our tests, the option to re-suspend the alga in demineralised water was not the most promising since the cell integrity was almost lost, certainly after applying two successive washing steps. Therefore, it was though to wash the algal suspension with the filtrate generated by the system, during the second washing cycle, which indeed, has a lower salts contents than the Zarrouk medium (Table 14).

Desalination efficiencies around 82% were obtained after only one single washing step. The conductivity in the suspended alga after washing was between 3.5 mS/cm and 4.5 mS/cm. This seems to be quite satisfying since the high salts content of the influent. It is doubtful that growth of *Arthrospira* on full strength Zarrouk medium will occur in the final MELiSSA concept. A reduction in the salinity of the feed to compartment IV will of course be beneficial in terms of chemical consumption and desalination efficiency which will give lower conductivity in the final re-suspended suspension but can only be applied when the growth pattern of *Arthrospira* is not disturbed.

9. Conclusions

The constructed breadboard was tested for its potential to harvest and desalinate suspension of *Arthrospira platensis* cultivated in Zarrouk medium. After some minor adaptations, separation efficiencies of 93% could be reached only at a level of the ultrasound unit. Without any doubt, a 100% separation efficiencies could be obtained when the ultrafiltration unit was coupled to the ultrasound unit.

Desalination of A. platenis was also possible using the same breadboard. By back washing the concentrate from the ultrafiltration unit with demineralised water, 82% desalination efficiencies were obtained. However, the integrity of the algal filaments was disturbed probably due to osmotic chocks. To avoid this disagreements, it was though to back wash the algal suspension with the filtrate wasted during the harvesting cycle after removal of salts . This is possible by adding an extra sub-system to the breadboard, consisting of an electrodialysis. The concentrated stream will be recycled to the photobioreactor to provide salts to the alga and the clarified stream will be used to wash the alga.

The quality of the produced biomass and of the generated suspension after washing seemed to be acceptable when evaluating its EPS, sugars and proteins content. However, more detailed investigations should be oriented on the physiology and the nutritional quality of *A.platensis* for consumption purposes using specific methods to study its nutritional aspects.

10. References

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