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Melissa Adaptation for Space

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TECHNICAL NOTE 72.9.1
Functional test plan and procurement

Identification of functional tests

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1. Introduction

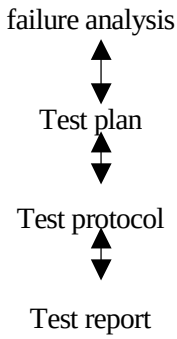
In the framework of the project MELiSSA – Adaptation for Space, a breadboard had to be constructed that demonstrates the continuous harvest and washing of *Arthrospira* from Compartment IVa in the MELiSSA loop.

Based on experimental work and a trade-off reported in TN72.6 and TN72.7.3, it was decided that the harvesting system should consist of an ultrasonic separation system, followed by an ultrafiltration step to recover the cells which pass through the ultrasonic system. In the overall concept, the permeate of the ultrafiltration step is further desalinated by electrodialysis, generating two streams: a desalinated diluate which can be used to wash the concentrated cell suspension and a concentrate enriched in salts which can be recycled to the photobioreactor in which *Arthrospira* is grown. It was agreed however, not to include the electrodialysis step in the breadboard and to perform washing steps with distilled water.

In the present technical note the following items are dealt with:

- General requirements specifications at the level of hardware and operation phases
- Test principles to evaluate the different failures
- Test plan for hardware and operation phase (process validation and hardware evaluation during run)

1.1 Architecture of the functional test procedure



2. Test organisation

2.1 Test phases

Hardware

Operation

2.2 Test team

Hardware phase: Vito

Operation phase : EPAS

3. System requirements

The response to requirements analysess are performed at two major levels: in the hardware phase and in the operation phase. The hardware phase concerns all the tests related to the instrumentation at start-up of the prototype. The operation phase consists in testing the instrumentation in a long-term running (1 month).
For an extensive overview of the breadboard components we refer to TN 72.8 Development of breadboard and the associated manual.

3.1 Requirements specifications

The breadboard is designed to cultivate, wash and concentrate *Arthrospira platensis*. To fulfill this objective, the reactor, the ultrasound system and the filtration unit must satisfy a number of criteria. These criteria vary from one phase to the other.

3.1.1 Criteria for Hardware phase

Table 1. Requirements of the breadboard components for the hardware phase

Requirements	Related instrumentation
1. Are units/instruments liquid/gas proof?	reactors, connections, valves, pumps, sensors, membranes
2. Do the instruments give correct/stable measurements?	Sensors, lamps photoreactor, controller
3. Is the mixing sufficient and homogeneous?	Mixers
4. Does the control answer in a correct way?	Controller

3.1.2 Criteria for Operation phase

Table 2. 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

3.2 Listing of possible failures

Table 3 lists the general failures, related to the instrumentation, for the hardware phase and operation phase. These failures correspond to events potentially responsible of dissatisfaction for one or several requirements. They are limited to functional aspects of both phases.

Table 3. General failures list

Nber	Failures	Related instrumentation										
		Reactor/tank	Cooler	Pumps	Mixers	Valves	Sensors	Connections	Controller	Membranes	Lamps photoreactor	
Hardware phase												
1	Insufficient mixing force or non homogeneous mixing				■							
2	Incorrect cooling of the reactors		■									
3	Liquid/gas leakage	■		■		■	■	■		■		
4	Erroneous measurement/control						■		■		■	
5	Unstable measurement/control						■		■			
6	Reactor/tank break	■										
Operation phase												
7	Clogging	■		■		■		■		■		
8	Corrosion	■	■	■				■				
9	Deterioration of measurement/control						■		■			
10	Instrument break		■	■	■	■	■	■	■	■	■	
11	Reactor/tank break	■										

3.3 failures in hardware phase

The Failures and associated test plans related to the hardware phase can be divided in 3 different groups: the hardware related to the growth reactor (photoreactor) and buffer tank (Part 1), to the ultrasound system (Part 2) and to the filtration unit (Part 3). Reference is made to Figure 1.

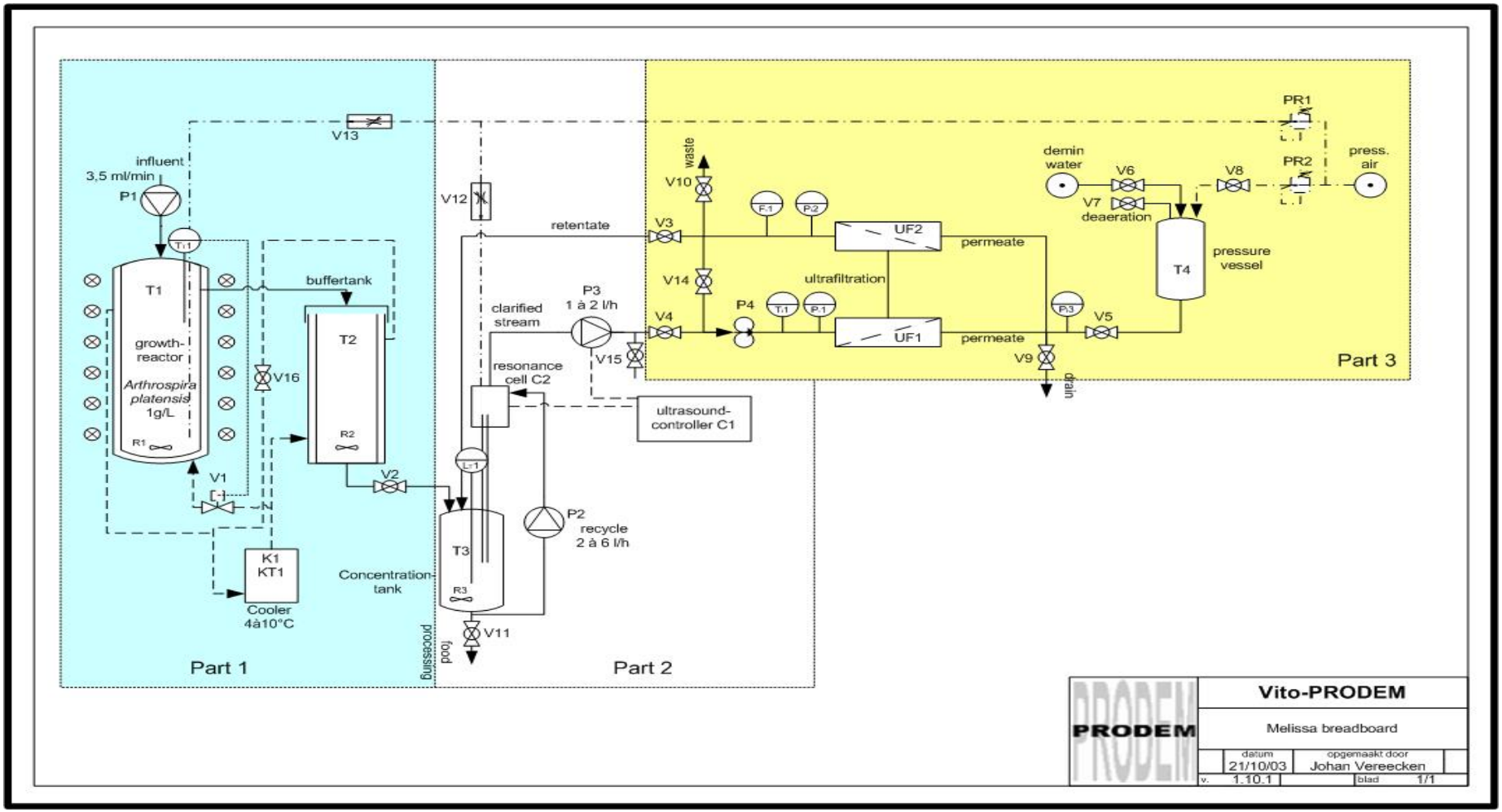


Figure 1. Conceptual design of the breadboard

3.3.1 Part 1: Photoreactor and buffer tank

Table 4 presents the different instruments and critical points of the photoreactor and buffer tank hardware and their related Failures.

Table 4. Failures listing for photoreactor and buffer tank in construction phase

Instruments		Failures					
Reference	**Description**	Insufficient mixing force or non homogeneous mixing	Incorrect cooling of the reactor/tank	Liquid/gas leakage	erroneous measurement/control	Unstable measurement/control	Reactor/tank break
T1	Photoreactor						
T2	Buffer tank						
R1	Mixer for photoreactor						
R2	Mixer for buffer tank						
T_T1	Temperature sensor						
K1	Cooler						
KT1	Cooler temperature control						
P1	Pump to feed reactor						
V1	Temperature controlled valve between reactor and cooler						
V2	Manually controlled valve between buffer tank and ultrasound system						
V13	Manually controlled valve for air supply to photoreactor						
V16	Manually controlled valve between cooler and buffer tank						
	Connections						
	Lamps						

3.3.2 Part 2: Ultrasound system

Table 5 presents the different instruments and critical points of the ultrasound unit hardware and their related Failures.

Table 5. Failures listing for ultrasound unit in construction phase

Instruments		Failures					
Reference	**Description**	Insufficient mixing force or non homogeneous mixing	Incorrect cooling of the reactors	Liquid /gasleakage	erroneous measurement/control	Unstable measurement/control	Reactor/tank break
T3	Concentration tank						
P2	Recycle pump						
P3	Harvest pump ultrasound unit						
C1	Ultrasound controller						
C2	Resonance cell						
L_T1	Level sensor						
R3	Mixer for the concentration tank						
V11	Manually controlled valve to remove concentrated cells from the concentration tank						
V12	Manually controlled valve for air cooling of resonance cell						
V15	Manually controlled valve after ultrasound unit for sampling						
	Connections						

3.3.3 Part 3: Filtration unit

Table 6 presents the different instruments and critical points of the Filtration unit hardware and their related Failures.

Table 6. Failures listing for filtration unit in construction phase

Instruments		Failures					
Reference	**Description**	Insufficient mixing force or non homogeneous mixing	Incorrect cooling of the reactors	Liquid/gas leakage	erroneous measurement/control	Unstable measurement/control	Reactor/tank break
UF1	Membrane						
UF2	Membrane						
T4	Demineralised water tank						
P4	Pump in recirculation loop						
Ti1	Temperature sensor						
Pi1	Pressure sensor before membranes						

Pi2	Pressure sensor after membranes						
Pi3	Pressure sensor on permeate flow						
Fi1	Flow meter						
V3	Manually controlled valve in retentate recycle to ultrasound unit						
V4	Manually controlled valve between ultrasound and filtration unit						
V5	Manually controlled valve between demineralised water tank and membranes						
V6	Manually controlled valve in water supply pipe to demineralised water tank						
V7	Manually controlled valve for deaeration of demineralised water tank						
V8	Manually controlled valve for air supply to demineralised water tank						
V9	Manually controlled valve for permeate drain						
V10	Manually controlled valve for retentate sampling						
V14	Manually controlled valve in filtration recycling loop						
PR1	Pressure reducer						
PR2	Pressure reducer						
	Connections						

3.4 Operation phase : functional aspects

The failures and associated test plan related to the functional aspects of the operation phase can also be divided in the same 3 groups as was done for the hardware phase (see point 3).

3.4.1 Part 1: Photoreactor and buffer tank

Table 7 presents the different instruments and critical points of the photoreactor and buffer tank in operation phase and their related Failures.

Table 7. Failures listing for reactor in operation phase

Instruments		**Failures**				
Reference	**Description**	Clogging	Corrosion	Deterioration of measurement/control	Instrument break	Reactor/tank break
T1	Photoreactor					
T2	Buffer tank					
R1	Mixer for photoreactor					
R2	Mixer for buffer tank					

T_T1	Temperature sensor					
K1	Cooler					
KT1	Cooler temperature control					
P1	Pump to feed reactor					
V1	Temperature controlled valve between reactor and cooler					
V2	Manually controlled valve between buffer tank and ultrasound system					
V13	Manually controlled valve for air cooling of photoreactor					
V16	Manually controlled valve between cooler and buffer tank					
	Connections					
	Lamps photoreactor					

3.4.2 Part 2 : Ultrasound system

Table 8 presents the different instruments and critical points of the ultrasound unit in operation phase and their related Failures.

Table 8. Failures listing for ultrasound unit in operation phase

Instruments		**Failures**				
Reference	**Description**	Clogging	Corrosion	Deterioration of measurement/control	Instrument break	Reactor/tank break
T3	Concentration tank					
P2	Recycle pump					
P3	Harvest pump ultrasound unit					
C1	Ultrasound controller					
C2	Resonance cell					
L_T1	Level sensor					
R3	Mixer for the concentrate tank					
V11	Manually controlled valve to remove concentrated cells from the concentration tank					
V12	Manually controlled valve for air supply to resonance cell					
V15	Manually controlled valve after ultrasound unit for sampling					
	Connections					

3.4.3 Part 3: Filtration Unit

Table 9 presents the different instruments and critical points of the filtration unit in operation phase and their related Failures.

Table 9. Failures listing for filtration unit in operation phase

Instruments		Failures				
Reference	**Description**	Clogging	Corrosion	Deterioration of measurement/control	Instrument break	Reactor/tank break
UF1	Membrane					
UF2	Membrane					
T4	Demineralised water tank					
P4	Pump in recirculation loop					
Ti1	Temperature sensor					
Pi1	Pressure sensor before membranes					
Pi2	Pressure sensor after membranes					
Pi3	Pressure sensor on permeate flow					
Fi1	Flow meter					
V3	Manually controlled valve in retentate recycle to ultrasound unit					
V4	Manually controlled valve between ultrasound and filtration unit					
V5	Manually controlled valve between demineralised water tank and membranes					
V6	Manually controlled valve in water supply pipe to demineralised water tank					
V7	Manually controlled valve for deaeration of demineralised water tank					
V8	Manually controlled valve for air supply to demineralised water tank					
V9	Manually controlled valve for permeate drain					
V10	Manually controlled valve for retentate sampling					
V14	Manually controlled valve in filtration recycling loop					
PR1	Pressure reducer					
PR2	Pressure reducer					
	Connections					

4. Test principles

Table 10. 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

5. Test plan for hardware phase

5.1 Part 1: Photoreactor and buffer tank

Table 11. Test plan 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			
	R2	Mixer for buffer tank			
Check correct cooling of the reactor/tank	K1	Cooler			
Check absence of liquid/gas leakage	T1	Photoreactor			
	T2	Buffer tank			
	T_T1	Temperature sensor			
	P1	Pump to feed reactor			
	V1	Temperature controlled valve between reactor and cooler			
	V2	Manually controlled valve between buffer tank and ultrasound system			
	V13	Manually controlled valve for air supply to photoreactor			
	V16	Manually controlled valve between cooler and buffer tank			

		connections			
Check accuracy and stability of measurement	T_T1	Temperature sensor			
	KT1	Cooler temperature control			
		Lamps photoreactor			
Check absence of reactor/tank break	T1	Photoreactor			
	T2	Buffer tank			

5.2 Part 2: Ultrasound unit

Table 12. The test plan 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			
Check absence of liquid/gas leakage	T3	Concentration tank			
	P2	Recycle pump			
	P3	Harvest pump ultrasound unit			
	C2	Resonance cell			
	L_T1	Level sensor			
	V11	Manually controlled valve to remove concentrated cells from the concentration tank			

	V12	Manually controlled valve for air cooling of resonance cell			
	V15	Manually controlled valve after ultrasound unit for sampling			
		connections			
Check accuracy and stability of measurement	C1	Ultrasound controller			
	L_T1	Level sensor			
Check absence of reactor/tank break	T3	Concentration tank			
	C2	Resonance cell			

5.3 Part 3: Filtration unit

Table 13. Test plan 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			
	UF2	Membrane			
	T4	Demineralised water tank			
	P4	Pump in recirculation loop			
	Ti1	Temperature sensor			
	Pi1	Pressure sensor before membranes			
	Pi2	Pressure sensor after membranes			

	Pi3	Pressure sensor on permeate flow			
	Fi1	Flow meter			
	V3	Manually controlled valve in retentate recycle to ultrasound unit			
	V4	Manually controlled valve between ultrasound and filtration unit			
	V5	manually controlled valve between demineralised water tank and membranes			
	V6	Manually controlled valve in water supply pipe to demineralised water tank			
	V7	Manually controlled valve for deaeration of demineralised water tank			
	V8	Manually controlled valve for air supply to demineralised water tank			
	V9	Manually controlled valve for permeate drain			
	V10	Manually controlled valve for retentate sampling			
	V14	Manually controlled valve in filtration recycling loop			
	PR1	Pressure reducer			
	PR2	Pressure reducer			
		Connections			
Check accuracy and stability of	Ti1	Temperature sensor			

measurement	Pi1	Pressure sensor before membranes			
	Pi2	Pressure sensor after membranes			
	Pi3	Pressure sensor on permeate flow			
	Fi1	Flow meter			
Check absence of reactor/tank break	T4	Demineralised water tank			

6. Test plan for operation phase

6.1 Test plan for functional aspects of the operation phase

6.1.1 Part 1: Photoreactor and buffer tank

Table 14. Test plan for functional aspects of operation phase for photoreactor and buffer tank

Test objective	Instrument ref	Instrument description	Test perfomed (Y/N)	Test result	Comments
Check absence of clogging	T1	Photoreactor			
	T2	Buffer tank			
	P1	Pump to feed reactor			
	V1	Temperature controlled valve between reactor and cooler			
	V2	Manually controlled valve between buffer tank and ultrasound system			
	V13	Manually controlled valve for air cooling to photoreactor			

	V16	Manually controlled valve between cooler and buffer tank			
		Connections			
Check absence of corrosion	T2	Buffer tank			
	K1	Cooler			
	P1	Pump to feed reactor			
		Connections			
Check absence of deterioration of measurement/control	T_T1	Temperature sensor			
	KT1	Cooler temperature control			
Check absence of instrument break	R1	Mixer for photoreactor			
	R2	Mixer for buffer tank			
	T_T1	Temperature sensor			
	K1	Cooler			
	KT1	Cooler temperature sensor			
	P1	Pump to feed reactor			
	V1	Temperature controlled valve between reactor and cooler			
	V2	Manually controlled valve between buffer tank and ultrasound system			
	V13	Manually controlled valve for air cooling to photoreactor			
	V16	Manually controlled valve between cooler and buffer tank			
		Connections			
		Lamps photoreactor			
Check absence of reactor/tank break	T1	Photoreactor			
	T2	Buffer tank			

6.1.2 Part 2: Ultrasound unit

Table 15. Test plan for functional aspects of operation phase for ultrasound unit

Test objective	Instrument ref	Instrument description	Test perfomed (Y/N)	Test result	Comments
Check absence of clogging	T3	Concentration tank			
	P2	Recycle pump			
	P3	Harvest pump ultrasound unit			
	C2	Resonance chamber			
	V11	Manually controlled valve to remove concentrated cells from the concentration tank			
	V12	Manually controlled valve for air supply to resonance cell			
	V15	Manually controlled valve after ultrasound unit for sampling			
		Connections			
Check absence of corrosion	T3	Concentration tank			
	P2	Recycle pump			
	P3	Harvest pump ultrasound unit			
	C2	Resonance cell			
		Connections			
Check absence of deterioration of measurement/control	C1	Ultrasound controller			
	L_T1	Level sensor			

Check absence of instrument break	P2	Recycle pump			
	P3	Harvest pump ultrasound unit			
	C1	Ultrasound controller			
	C2	Resonance cell			
	L_T1	Level sensor			
	R3	Mixer for the concentration tank			
	V11	Manually controlled valve to remove concentrated cells from the concentration tank			
	V12	Manually controlled valve for air supply to resonance cell			
	V15	Manually controlled valve after ultrasound unit for sampling			
		Connections			
Check absence of reactor/tank break	T3	Concentration tank			

6.1.3 Filtration unit

Table 16. Test plan for functional aspects of operation phase for filtration unit

Test objective	Instrument ref	Instrument description	Test perfomed (Y/N)	Test result	Comments
Check absence of clogging	UF1	Membrane			
	UF2	Membrane			
	P4	Pump in recirculation loop			

	V3	Manually controlled valve in retentate recycle to ultrasound unit			
	V4	Manually controlled valve between ultrasound and filtration unit			
	V5	Manually controlled valve between demineralised water tank and membranes			
	V6	Manually controlled valve in water supply pipe to demineralised water tank			
	V7	Manually controlled valve for deaeration of demineralised water tank			
	V8	Manually controlled valve for air supply to demineralised water tank			
	V9	Manually controlled valve for permeate drain			
	V10	Manually controlled valve for retentate sampling			
	V14	Manually controlled valve in filtration recycling loop			
		Connections			
Check absence of corrosion	P4	Concentration tank			
		Connections			
Check absence of deterioration of measurement/control	Ti1	Temperature sensor			
	Pi1	Pressure sensor before membranes			

	Pi2	Pressure sensor after membranes			
	Pi3	Pressure sensor on permeate flow			
	Fi1	Flow meter			
	PR1	Pressure reducer			
	PR2	Pressure reducer			
Check absence of instrument break	UF1	Membrane			
	UF2	Membrane			
	P4	Pump in recirculation loop			
	Ti1	Temperature sensor			
	Pi1	Pressure sensor before membranes			
	Pi2	Pressure sensor after membranes			
	Pi3	Pressure sensor on permeate flow			
	Fi1	Flow meter			
	V3	Manually controlled valve in retentate recycle to ultrasound unit			
	V4	Manually controlled valve between ultrasound and filtration unit			
	V5	Manually controlled valve between demineralised water tank and membranes			
	V6	Manually controlled valve in water supply pipe to demineralised water tank			
	V7	Manually controlled valve for deaeration of demineralised water tank			

	V8	Manually controlled valve for air supply to demineralised water tank			
	V9	Manually controlled valve for permeate drain			
	V10	Manually controlled valve for retentate sampling			
	V14	Manually controlled valve in filtration recycling loop			
	PR1	Pressure reducer			
	PR2	Pressure reducer			
		Connections			
Check absence of reactor/tank break	T4	Demineralised water tank			

6.2 Test plan for operational aspects of the operation phase

The requirements for this phase have been listed in 3.1.2. The following items have to be evaluated in the tests:

- algal growth in the photoreactor
- evolution in biomass quality in the cooled buffer tank
- optimisation of algae concentration by ultrasonic separation
- for membrane filtration unit :
    - is the cell concentration of the retentate acceptable
    - is the biomass recovery acceptable
    - optimal membrane cleaning procedure
- evaluation of the biomass quality in terms of:
    - salinity
    - macromolecular composition

The breadboard will not be operated in sterile/axenic conditions.

6.2.1 Algal growth in the photoreactor

Optimal growth conditions for *Arthrospira* are well-known in the MELiSSA consortium.

Feed: Zarrouk medium at alkaline pH pumped to the photoreactor at a flow of 5 l/d to achieve a retention time of 1 d

Photoreactor:

- Calibration of light intensity in empty reactor
- Choice of light intensity to achieve a 1 g/l algae suspension (100 W/m²)
- Inoculation with *Arthrospira platensis* PCC 8005
- CO_2 supply by continuous aeration
- Mixing of reactor content to homogeneity
- Temperature of 35°C

Analysis:

- daily biomass concentration samples from photoreactor to be measured as dry weight after sufficient washing of the samples to remove salts. Sampling occurs through an opening on the top lid of the reactor.
- daily pH measurements

6.2.2 Biomass quality in the cooled buffer tank

- 4 samplings spread over the experimental period
- for each sampling:
    - sample from buffer tank after 24 h of storage
    - measurement of polysaccharide and protein content
    - microscopic evaluation

6.2.3 Algae concentration by ultrasonic separation

In principle, several paramaters need to be optimised:

- Harvest flow rate: in TN 72.8 we described that 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: general experience with the ultrasound system and our own experiments indicated that the ratio has to be between 1:2 and 1:3.

- Power input or field intensity: based on our own tests reported in TN 72.7, a field intensity of 5 W should give optimal results. If separation efficiencies are below 95%, higher field intensities have to be used. If separation efficiencies are 99%, lower intensities should be evaluated to reduce overall power consumption.
- 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 is 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 started 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. Settings used before were 600 s or 180 s 'on' and 3 s 'off'.

Per testing day, the algae suspension collected will be concentrated, washed and then concentrated again for food processing. Since 2 washing steps are probably needed, 4 ultrasound treatment runs will have to be evaluated in terms of separation efficiency by OD and dry weight measurements.

From the above the following analyses planning is proposed:

Analysis	**Concentration tank content (via V11)**	**Harvest flow (via V15)**
Dry weight	Beginning once/run end	 once/run end
OD 750 nm	Beginning once/run end	5/run spread in time
Polysaccharide content	once/week on the final concentrated suspension, only when separation has been optimised	/
Protein content	once/week on the final concentrated suspension, only when separation has been optimised	/

The number of washing cycles needs to be determined from the decrease in conductivity of the cell suspension (see below).

6.2.4 Membrane filtration unit

The membranes are backflushed by demineralised water after each concentration cycle. When the membranes are too much fouled, a thorough cleaning step should be applied. The fouling of the membranes can be followed by following the pressure drop over the membrane (manometers Pi1 and Pi2) as the pressure drop will increase with increasing membrane fouling.
A possible chemical cleaning procedure, advised by the membrane manufacturer, is cleaning with a NaOH solution (85°C, 15-20 g/l, 30 min) followed by cleaning with HNO_3 solution (50°C, 4.5 g/l, 15 min) with a clean water rinsing after each step. Experimental results showed that several washing steps were needed to fully recover the initial flux.
Another possible cleaning procedure is heat treatment, as ceramic membranes can withstand high temperatures.
Additional tests should be performed on membrane cleaning to determine what is the optimal cleaning procedure and frequency.

The cell concentration efficiency will have to be evaluated as well as the recovery of biomass or the irreversible binding of algae cells to the membranes. To be able to make the mass balances, samples have to be taken from the feed to the membranes (identical to the samples of the harvest flow of the ultrasound system), the concentrate and the permeate.

The cell quality of the concentrate will also be checked by taking a concentrate sample and analysing it for polysaccharide and protein content. This will be done after the separation has been optimised.

The following set of analyses is proposed:

Analysis	**Concentrate (via V10)**	**Permeate (via V9)**
Dry weight	Once at the end of a run	Once at the end of a run for a mixed sample
OD 750 nm	Once at the end of a run	Idem
Polysaccharide content	Once per week , only when separation has been optimised	/
Protein content	Once per week , only when separation has been optimised	/

6.2.5 Evaluation of the biomass quality during processing

The biomass ideally has to be desalinated to a final salinity of 0.3 g/l. The best way to follow up on the salinity of the suspension is to measure

- the conductivity of the permeate of the membrane filtration unit: once per run
  As long as the conductivity is larger than 500 µS/cm, an additional washing step is required.

- the conductivity of the cell suspension in the filtration unit: once per run

Permeate and concentrate samples will be taken after each run to determine the conductivity after the first concentration step and after each washing cycle.

For the permeate sample, also the elemental composition will be determined once a week for each run. This should be done after separation has been optimised. When this composition is compared to the initial composition of the Zarrouk medium, it is possible to determine what salts are used cell growth and processing.

Analysis	**Concentrate (via V10)**	**Permeate (via V9)**
Conductivity	Once at the end of each run	Once at the end of each run for a mixed sample
Elemental medium composition	/	Once per week at the end of each run for a mixed sample

Sensors will need to be calibrated regularly to certain proper measurements.