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1 GENERAL INTRODUCTION

The general concept of MELiSSA includes the use of the biomass generated in the two photosynthetic reactors, *Spirulina* in compartment IV and *Rhodospirillum* in compartment II, as food supply. Previous studies have shown that both microorganisms can be used as supplement in the food diet of rats. On the other hand, *Spirulina* has been used as an important source of proteins in children suffering from malnutrition and different types of food and pills based on *Spirulina* are commercialised widely.

In order to be used as food supply, the biomass obtained in the photosynthetic bioreactors has to be first harvested. Basically, the system already proposed consisted of two units: a centrifuge and a membrane module. The centrifuge is envisaged to recover the cells, producing a paste with a high percentage of liquid elimination (at least between 75% - 80%). The clear liquid obtained in the centrifuge would be then passed through a membrane module, with the objective to provide a complete clear liquid stream to be pumped to the next compartment. As the continuous centrifuge uses liquid to discharge the solid paste retained in it, that is the cells; there is a certain final degree of dilution of the cells.

In this TN 43.21 different aspects mainly concerning harvesting operational mode and water purification will be treated as stated in WP 43.2. In addition, another aspects related to the 75 litres bioreactor from *Spirulina* is also considered like interface with a Higher Plant Compartment and the corresponding interface with the Crew Compartment.

Harvesting procedure has been proposed to be carried out in two consecutive steps. First step includes a centrifugation system and the second step a membrane system, which can be conducted either in continuous or batch operational mode.
Both steps have been performed and results are presented in the second part to this TN.

Water purification was carried out with membrane modules and results are presented in the third part of this TN.

Before centrifugation step, biomass quality analyses were made in order to define or determine the maximum residence time for the biomass in holding tanks. This maximum residence time is such as allows the preservation of the main quality biomass properties. Both microbiological and nutritive properties have been considered in the whole tests conducted.

Results related to the tests about this residence time determination for the holding tanks are presented in the first part to this TN.

2 HARVESTING HOLDING TANKS

2.1 INTRODUCTION

Prior to the centrifugation step, biomass is stored into holding tanks (20 litres) when goes out form the bioreactor. The purpose of those holding tanks is to store enough biomass to allow the centrifugation step to work at the best conditions.

Residence time of biomass in the holding tanks can directly affect biomass quality that will be used in this process because of its degradation. To determine the maximum allowed residence time and other operational conditions, an experiment was planned. In the experiment some product samples were taken and preserved during different time periods in order to analyse biomass degradation related to time. Additionally, two samples were taken, one sample was kept at ambient temperature and the other sample was kept refrigerated at 4º C. Both samples were stored different time periods and then also analysed. An additional goal of this test was to find a measurement, easy and simple to perform at the final implementation, which either directly or indirectly indicates biomass quality conditions. There is, obviously, an organoleptic factor that could indicate something about biomass conditions, but this is not an easy measurement to be done by some standard equipment (electronic nose).
Looking for the most important compounds in *Spirulina* and which ones can be degraded first, proteins, carbohydrates and intracellular pigments quantity were analysed from the samples. Proteins and ammonium presents in the culture medium were also analysed. Dry weight and pH were measured for every sample as it was collected. These are two parameters that also may change depending on biomass conditions.

Work conditions were the following:

- All samples were kept in 1 litre bottles which were light preserved in both cases (ambient and refrigeration temperature), in order to keep both samples under the same light conditions.
- Biomass quantity referred to dry weight was measured before after the holding (ageing) period. pH measurements were also made to both times.
- Samples were freeze dried and kept in the refrigerator before being analysed.
- Six-day-sample at ambient temperature was not even analysed due to conditions observed at first sight (degradation, odour, etc). Only ammonium and protein quantity measures in the culture medium were made.
- Every analysis was made for all samples at the same time; for example, protein determination was made for all samples simultaneously and, in the same way with carbohydrates, etc. This was done in order to reduce to the minimum the analysis variability, and in the same way, samples were all freeze dried after the ageing period was achieved.
- In order to measure pigments quantity (chlorophyll and phycocianine) a preliminary assay was made in order to verify how much sonication time could modify the measurement (due to different cell disruption). After sonication, samples were filtrate and absorbance was measured.

2.2 RESULTS

When residence time in the bottles increases degradation of *Spirulina* biomass is expected and, therefore, losses in the quality due to a decrease of the main macronutrients. At the same time, as cells degrade they break and some compounds (proteins, carbohydrates, etc.) can be transferred to the culture medium. Those compounds may be found either as they were initially or as degradation products (ammonium, lactic acid). Initial microbial contamination in the sample also affects the results, the more time the biomass is stored the more contamination shows up.

Differences between samples kept at ambient temperature and samples kept refrigerated have been found. The most important difference was related to proteins, one of the most important parameters when quality of the sample is wanted to be stated.

It has been noticed that as ageing increases intracellular protein decreases and, on the contrary, the presence of extracelular protein increases in the culture medium. This also implies an increase of ammonium in the medium.

Relating to carbohydrates, changes are observed following similar behaviour as for proteins, that is, in the first days the more elapsed time the more decreasing appeared. After that, a slight increase becomes, which may be due to microorganism proliferation/contamination according to an increase in absorbance.

The presence of lactic acid in the medium was also measured but as far as it was not found, data analyses are not included in the results table.

Also, important pH differences were observed between initial and aged samples, although the difference depends on the buffer capacity of the medium

In reference to intracellular pigments, a decrease is expected as ageing in the bottles increase. Additionally, the fact that samples were light preserved increased their degradation. Results obtained showed some differences between samples at ambient temperature and refrigerated samples (in reference to chlorophyll), probably due to release produced by partial cell disruption.

The results compiled for all samples on those experiments are presented in Table 1 and in the Figure 1.

Results corresponding to pigments quantification assay are presented in Figure 2. Effect of time sonication was studied due to the possible interaction between cell disruption and pigments releasing. It was observed that if total sonication time increases absorbance also increase whenever sonication is applied in a discontinuous way. However, if sonication is applied for a continuous period of 40 seconds, absorbance is not improved, and a low absorbance is obtained.

2.2.1 TABLE OF RESULTS

	MEASURED PARAMETER	SAMPLE 0	SAMPLE 2	SAMPLE 4	SAMPLE 6
Ambient	**Dry weight variation (%)**	0	-6.72	-28.42	*n.d.
	Absorbance at 750 nm	0.2742	0.2583	0.1898	*n.d.
	pH variation (%)	0.0	-6.7	-5.6	*n.d.
	Proteins %	47.2	40.3	41.8	*n.d.
	Carbohydrates %	11	9.3	5.2	*n.d.
	Chlorophyll (g/l)	0.002	0.0011	0.00083	*n.d.
	Phycocianine (g/l)	0.033	0.0031	0.0035	*n.d.
	Ammonium medium (ppm)	0	5.5	8.7	11.6
	Proteins medium (mg /ml)	23.2	40.1	27.7	27.1
Refrigerated 4º C	**Dry weight variation (%)**	0	-8.21	-29.47	-24.96
	Absorbance at 750 nm	0.2742	0.2692	0.1694	0.1972
	pH variation (%)	0.0	-1.7	-2.4	-2.1
	Proteins %	47.2	42.4	42.46	46.9
	Carbohydrates %	11	9.1	6.8	7.8
	Chlorophyll (g/l)	0.002	0.0007	0.00091	0.00089
	Phycocianine (g/l)	0.033	0.016	0.0031	0.0047
	Ammonium medium (ppm)	0	5.3	6.1	7.6
	Proteins medium (mg/ml)	0	23.2	28.4	22.5

Being:

Sample 0	reference
Sample 2	sample with 2 days of residence time
Sample 4	sample with 4 days of residence time
Sample 6	sample with 6 days of residence time

*n.d.: not determined because of their significant degradation indirectly observed by organoleptic properties.

Table 1. Results of biomass analysis related to residence time in holding tanks

2.2.2 RESULTS ON GRAPHICS

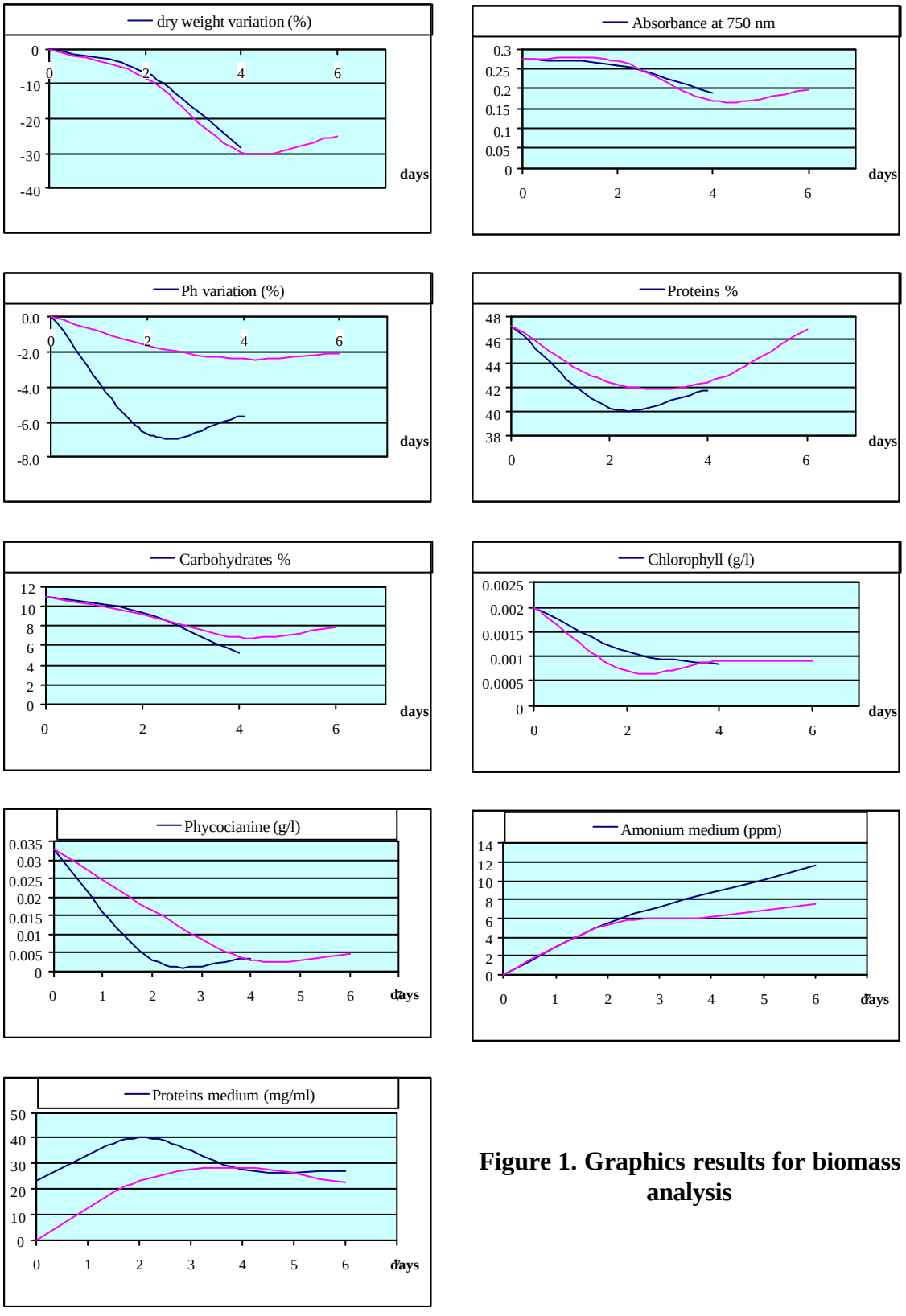


Figure 1. Graphics results for biomass analysis

2.2.3 PIGMENTS ASSAY GRAPHIC

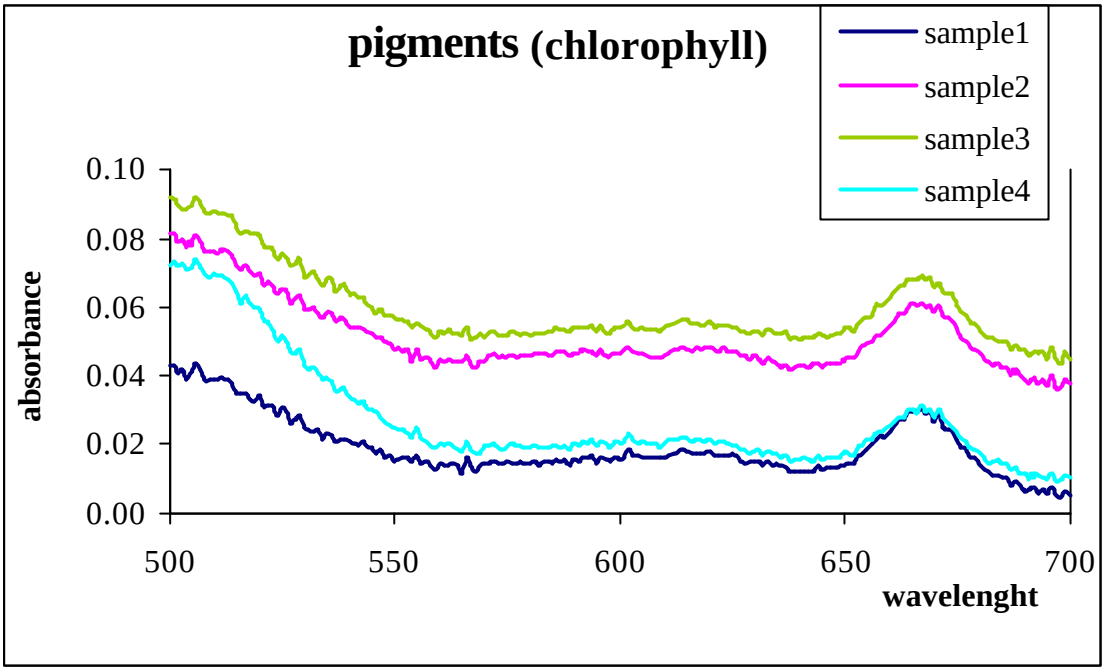


Figure 2. Pigments assay

Sample 1: sonication time =10 seconds.	**Sample 2**: sonication time = 20 seconds
Sample 3: sonication time = 30 seconds	**Sample 4**: sonication time = 40 seconds.

For samples 1, 2 and 3, a sonication time of 20 seconds with a duty cycle of 50% was used (10 s work +10 s stop). For the sample 4, sonication time was 40 seconds.

2.3 CONCLUSIONS

According to results, *Spirulina* is progressively degraded when is out of the reactor and optimal growth conditions are loosed. It is difficult to define a reasonable residence time for holding tanks because, ideally, *Spirulina* had to be consumed as it was being produced.

Initial contamination present in the reactor outlet may highly influence the results so it is necessary a low or null microbial contamination. Probably the contamination is the reason that lies to higher protein and carbohydrates quantities when ageing increases.

As it was pointed out, the most important changes are related to proteins, which appear in the culture medium as a degradation compound when cells are broken.
Taking into account organoleptic characteristics (odour, colour, biomass aspect), and the obtained results, it is recommended not to keep the biomass in the tanks more than two days and use a low temperature (refrigeration at 4ºC) as far as possible.

3 HARVESTING PROCEDURE

3.1 INTRODUCTION

The proposed configuration for the whole harvesting system involves two units: a centrifuge and a membrane module. The centrifuge is envisaged to recover the cells, producing a paste with a high percentage of liquid elimination (75% - 80%).

As stated in the results concerning to the centrifugation step in TN37.30, the higher the flow-rate the higher the efficiency and yields are attained. If necessary, the global harvester system can operate in a semi-continuous mode, that is, with repeated operating cycles using a reservoir tank to provide enough volume to be fed to the centrifuge at higher flow-rates, and thus, to obtain high efficiencies and yields.
In this way, the total treated volume per unit of time remains the same, without any significant loss of efficiency and/or yield.

The second step of the harvesting system, the membrane filtration, is not supposed to be much affected by the flow-rate. Thus, the overall yields and efficiency will be kept within satisfactory values.

Anyway, if necessary, a reservoir tank can be used to increase the feed flow-rate to the harvesting system in order to obtain a higher performance of the system without changing the treated volume per unit of time, as described above.

From the first studies on the biomass harvesting using a centrifugation system it was concluded that an extra dilution of the solids recovered occurred due to the discharge mechanism of the centrifuge. Additional experiments allowing minimisation of this phenomenon have been carried out and are presented in this report.

3.2 CENTRIFUGATION SYSTEM

The centrifuge selected is from *Westfalia Separator AG* and the type is a CSA 1-06-475 as well as stated in TN37.30. The system is steam sterilisable with flowing steam at a maximum pressure of 1.5 bar. Technical data is presented as follows: Solids discharge time, 0 s - 10000 s; Rotation speed, 0 rpm - 10000 rpm; Feed flow, 0 l/h - 50 l/h.

Considering the results reported in TN37.30 the rotation speed was fixed at 10000 rpm in all the tests because of the higher separation efficiency and yields observed. As operational variables the following ones were selected: inlet flow-rate, intermittent solids discharge time and discharge time duration. Volumes, concentrations, weights, and other additional measurements were taken from the inlet and outlet streams. All this information permitted to calculate key parameters as yields, efficiencies, recoveries, and to reveal possible inaccuracies or discrepancies from mass balances.
The centrifugation system is schematised in Figure 3

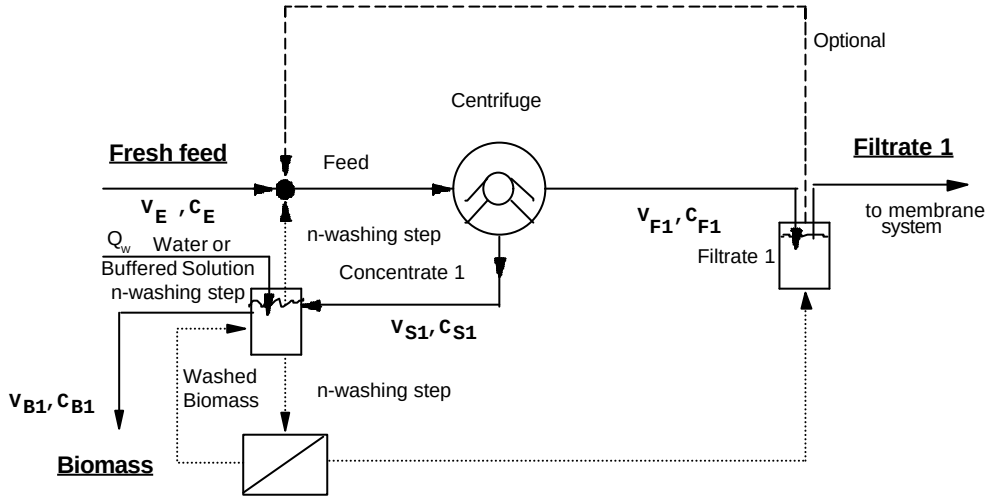


Figure 3 Scheme of the centrifugation system

One of the most important parameters to follow concerning the overall performance of the harvesting system is the concentration ratio that can be obtained between inlet and outlet streams from the centrifuge. If solids rejection in the clarified stream is almost total, then this concentration ratio theoretically takes the value corresponding to the quotient of inlet volume and discharge volume. As the discharge volume for the centrifuge is rather constant the water elimination is mainly governed by the discharge time and inlet flow-rate.
Thus, the higher discharge time and inlet flow-rate the higher concentration factor is obtained. If low flow-rates and/or low discharge times are used the solids concentration at the concentrated stream will be rather low or at least moderate. This effect was observed in earlier tests developed in TN37.30 producing a poor concentration factor and an excess of dilution in the solids discharge. The dilution of solids discharge is due to the addition of “extra” water to the system, mainly when low flow-rates and short time discharges are used.

3.2.1 KEY VARIABLES AND CALCULATIONS

A list of the nomenclature and units of the key variables and experimental data used in the study of the centrifugation system and the calculations for different variables and parameters are detailed as follows:

Q (l/h)	Feed flow
t_i (h)	Intermittent solids discharge time
t_d (s)	Discharge time duration
V_e (l)	Feed volume
V_f (l)	Clarificate volume
V_w (l)	Discharge water volume (non-measured)
Vs_1 (l)	Solids volume (first discharge)
Vs_2 (l)	Solids volume (second discharge)
C_e (g/l)	Feed concentration
C_f (g/l)	Clarificate concentration
Cs_1 (g/l)	Solids concentration (first discharge)
Cs_2 (g/l)	Solids concentration (second discharge)
Abs.e (750 nm)	Feed absorbance
Abs f (750 nm)	Clarificate absorbance
Abs.s (750 nm)	Centrate absorbance
DW f .s.a	Clarificate dry weight (calculate from Abs f)
R_{H2O} (%)	Water yield
f_w	Water discharge to feed volume ratio
Rs_1 (%)	Solids yield (calculated from solids mass balance)
Rs_2 (%)	Solids yield (experimental ratio)
Rt_1 (%)	Total solids outlet (first discharge)
Rt_2 (%)	Total solids outlet (first+second discharge)
fs_1	Solids concentration factor (first discharge)
fs_2	Solids concentration factor (first+second discharge)
fs_3	Volumetric concentration factor (first discharge)
fs_4	Volumetric concentration factor (first+second discharge)
fs_{th}	Theoretical volumetric concentration factor
Cd.e (ms/cm)	Feed conductivity
Cd.f (ms/cm)	Clarificate conductivity
Cd.s (ms/cm)	Concentrate conductivity

3.2.2 CALCULATED DATA

The key parameters in order to characterise the overall performance of the centrifugation system has been calculated using the following equations:

3.2.2.1 R_{H2O} (%). Water yield

The knowledge of this value allows determining the recovery of water from the solids solution fed to the separation system. At least, a value ranging from 75%-80% is required. It can be calculated considering that X_c , X_f and X_s are the mass fraction of solids at the clarified, feed and centrate respectively as well as density differences between streams are negligible in the final calculation.

$$R_{H_2O}(\%) = \frac{Vf(1-Xf)}{Ve(1-Xe)+Vw} = \frac{Vf(1-Xf)}{Vf(1-Xf)+Vs(1-Xs)}$$

3.2.2.2 f_w . Water discharge to feed volume ratio

This parameter represent the amount of "extra" or external water that is added to the solids to produce the solids discharge. It is very important to minimise its value, so that it produces an undesirable dilution of the solids decreasing the concentration factor.

$$f_w = \frac{Vw}{Vf} = \frac{Ve+Vs-Vf}{Vf}$$

3.2.2.3 Rs_1 (%). Solids yield (calculated from solids mass balance)

If there is no accumulation of solids in the separator, an overall mass balance can apply. Therefore, the solids yield can be determined from any two of the three material streams involved.

$$Rs_1 = \frac{VeCe-VfCf}{VeCe} = 1-\left(\frac{Vf}{Ve}\frac{Cf}{Ce}\right)$$

3.2.2.4 Rs_2 (%). Solids yield (experimental ratio)

This parameter is directly calculated from the measurement of solids concentration and volume, and is referred to the solids fed to the separation system. Differences between Rs_2 and Rs_1 indicate that solids accumulation in the centrifugation system occurs.

$$Rs_2 = \frac{VsCs}{VeCe}$$

3.2.2.5 Rt_1 (%). Total solids recovery (first discharge)

It relates to the recovery of the solids from the clarificate and concentrate streams and also indicates solids accumulation inside the centrifuge and so, accomplishment of the solids mass balance.

$$Rt_1 = \frac{VfCf + VsCs}{VeCe}$$

3.2.2.6 Rt_2 (%). Total solids recovery (first+second discharge)

It represents the same as Rt_1 but, in this case, also takes into account a second possible discharge of the solids in order to recover almost all of them.

$$Rt_2 = \frac{(VfCf + VsCs_1) + Vs_2Cs_2}{VeCe}$$

3.2.2.7 fs_1. Concentration factor (first discharge)

One of the main goals to be achieved is to obtain a value of this concentration factor as high as possible. This implies a high elimination of water and high efficiency of the system.

$$fs_1 = \frac{Cs_1}{Ce}$$

3.2.2.8 fs_2. Concentration factor (first+second discharge)

This concentration factor is calculated when a second discharge is forced to recover as much as possible the solids probably resting inside the centrifuge. This second discharge increases the solids recovery but diminishing solids concentration and so, concentration factor.

$$fs_2 = \frac{Cs_t}{Ce} = \frac{\frac{Vs_1Cs_1 + Vs_2Cs_2}{Vs_1 + Vs_2}}{Ce}$$

3.2.2.9 fs_3. Volumetric concentration factor (first discharge)

It is the relation between fed volume and recovered solids volume. If the separation presents high efficiency, that is to say, few solids appear in the clarificate stream, this factor will be similar to the concentration factor fs_1, except when mass balances are not accomplished.

$$fs_3 = \frac{Ve}{Vs_1}$$

3.2.2.10 fs_4 Volumetric concentration factor (first + second discharge)

It is concerned to the same effect that fs_3 but when a second discharge is done.

$$fs_4 = \frac{Ve}{Vs_1 + Vs_2}$$

3.2.2.11 $fs_{th.}$ Theoretical volumetric concentration factor.

It is calculated considering the solids volume discharge is fixed at a volume of 0.50 L. (nominal solids discharge)

$$fs_{th} = \frac{Ve}{Vs(=O.5L)}$$

3.2.3 RESULTS AND DISCUSSION

Several tests were conducted in this phase. The different tests differed from the inlet flow-rates, intermittent solids discharge time, discharge time duration, feed volume and concentration as the operational variables. The main objective of these experiments were to mainly study the effect of the inlet flow-rate, intermittent solids discharge time and discharge time duration on the recuperation factors of water and solids.
The intermittent solids discharge time were fixed at approximately 1 hour when low flow-rates were applied, increasing it up to about 2 hours for higher flow-rates in order to drastically minimise the additional consumption of water. In this way, higher solids concentration was obtained.

In reference to the discharge time duration, it was observed that, especially for high flow-rates and discharge time, if values were not high enough, accumulation of solids inside the centrifuge appeared. In the last test this operational condition was changed from 1.8s to 2 s. Accumulation of solids was minimised, but decreasing the solids concentration in the concentrated stream. Obviously a compromise between recuperation and concentration factor had to be solved in a final application. However, due to the high recovery of water attained, it is possible to anticipate that the best strategy would be to increase solids recovery although the solids concentration may decrease.
Moreover, in order to recover the low fraction of solids remaining inside the centrifuge a second discharge was immediately made after the first one. The solids recovery increased, but practically diluting by a factor of two the final solids concentration (1^{st} + 2^{nd} discharge). As a consequence, it can be concluded that a second discharge is not recommended for a standard operation.
The results corresponding to these tests are presented in the Table 2. In general, water yields increase with the flow-rate presenting values always above 95 %. The separation efficiency of the system is satisfactory. The solids concentration factor is not very different from the volumetric concentration factor in the major part of the tests.

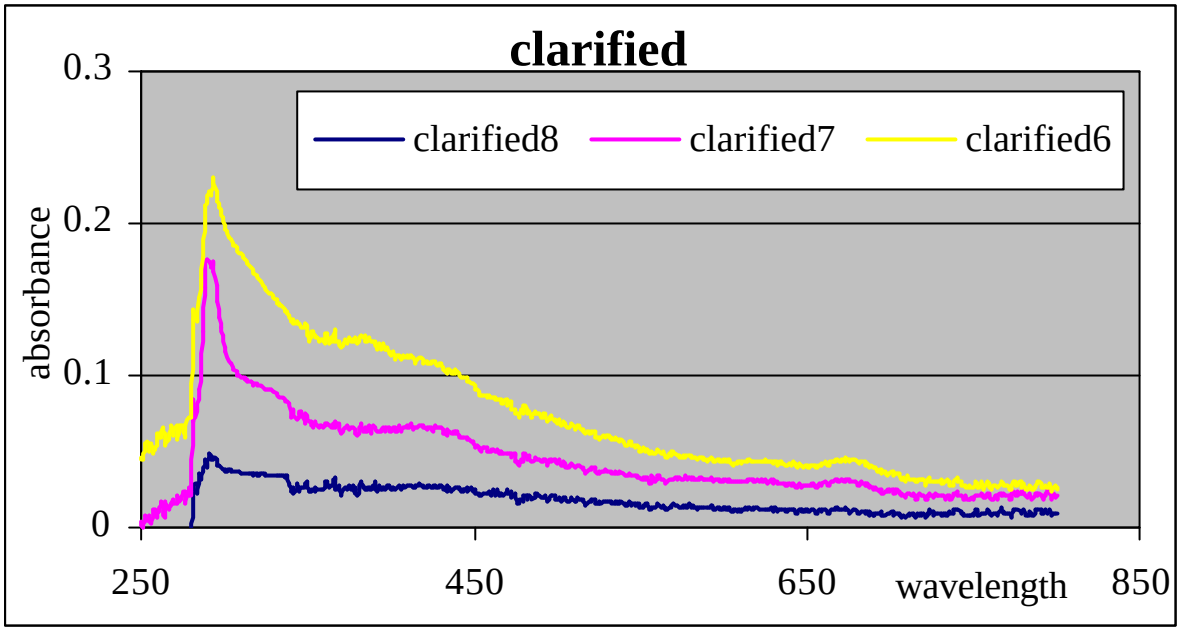
A concentration factor of 24 was obtained in the final test, with a high recovery of water and solids, reaching the objectives for this harvesting system prior to a further solids treatment of water purification that will follow.
In order to observe if significant disruption of the cells was produced measures of conductivity and absorbance of different streams were made. Conductivity can be used as indirect measurement of ionic species either released from the cells or resulting from concentration of solids. The results obtained can be interpreted as only slight salting concentration effect of the biomass is produced. This fact is very important in order to plan the washing step of the biomass.
Absorbance measurements between 250-800 nm of the clarified and concentrated stream can be related to the biomass integrity and status. If cells suffer from important disruption, an increasing of extracelular protein (260-280 nm) is expected as well as pigments (about 400 nm). These two measurements additional variables that could be considered in further studies about biomass harvesting. Figure 4 shows the absorbance measurements of the last three experiments.

The integrity of *Spirulina* cells was also followed by microscopic observation. In a single centrifugation cycle cell disruption was observed, estimating cell disruption not being higher than 5-10% of the total amount of cells treated.

Q(l/h)	ti.s.d(h)	td.d(s)	Ve(l)	Vf(l)	Vs_1(l)	Vs_2(l)	Wf(gr)	Ws(gr)	Ce(g/l)	Cf(g/l)	Cs_1(g/l)	Cs_2(g/l)	Abs.e (750nm)	Abs f	Abs.s	DWf.s.a	R_{H2O}(%)	f_w
2.60	1.0	1.8	2.6	1.855	0.43	----	1,753	400	0.54	0.141	2.77	----	0.0120 (*10)	0.0166	0.4271 (*10)	0.00997	81.22	--
2.60	1.0	1.8	2.6	1.860	0.46	----	1,753	448	0.54	0.090	2.51	----	0.0120 (*10)	0.0122	0.4685 (*10)	0.0072	80.21	--
3.50	1.0	1.8	3.50	3.200	0.45	----	3,054	400	0.54	0.127	3.24	----	0.0120 (*10)	0.0153	0.5697 (*10)	0.0091	87.71	4.3
3.50	1.0	1.8	3.50	3.100	0.41	----	2,984	398	0.77	0.113	2.82	----	0.0652 (*10)	0.0143	0.4819 (*10)	0.0085	88.35	0.3
4.48	1.0	1.8	4.48	3.500	0.47	----	3,300	448	0.77	0.094	3.74	----	0.0652 (*10)	0.0128	0.7727 (*10)	0.0076	88.20	--
10.70	≈ 2.0	1.8	18.03	18.500	0.25	0.94	17,350	248	0.59	0.123	28.53	0.95	0.0769 (*10)	0.0263	0.7683 (*60)	0.0157	98.70	4.0
8.96	≈ 2.0	1.8	13.00	11.500	0.31	0.55	11,650	280	0.75	0.020	26.00	1.20	0.1269 (*10)	0.0194	0.8001 (*60)	0.0116	97.45	--
9.67	≈ 2.0	2.0	14.50	13.500	0.53	0.55	13,300	519	0.65	0.018	15.60	0.42	0.1436 (*10)	0.0083	0.5062 (*60)	0.0049	96.28	--

Q(l/h)	ti.s.d(h)	td.d(s)	Vf(l)	Vc(l)	Vs_1(l)	Vs_2(l)	Rs_1(%)	Rs_2(%)	Rt_1(%)	Rt_2(%)	fs1	fs2	fs3	fs4	fs_{th}	Cd.e(ms/cm)	Cd.f(ms/cm)	Cd.s(ms/cm)
2.60	1.0	1.8	2.6	1.855	0.43	----	81.36	84.84	103.48	----	5.13	----	6.05	----	5.2	8.86	---	8.90
2.60	1.0	1.8	2.6	1.860	0.46	----	88.08	82.24	94.16	----	4.65	----	5.65	----	5.2	8.86	---	8.90
3.50	1.0	1.8	3.50	3.200	0.45	----	78.50	77.14	98.65	----	6.00	----	7.78	----	7.0	8.86	---	9.93
3.50	1.0	1.8	3.50	3.100	0.41	----	87.00	42.90	55.90	----	3.66	----	8.54	----	7.0	8.39	---	8.40
4.48	1.0	1.8	4.48	3.500	0.47	----	90.46	50.96	60.49	----	4.86	----	9.53	----	9.0	8.39	---	8.54
10.70	≈ 2.0	1.8	18.03	18.500	0.25	0.94	78.61	67.05	88.44	96.83	48.36	11.43	72.12	15.15	36.1	7.59	7.55	7.15
8.96	≈ 2.0	1.8	13.00	11.500	0.31	0.55	97.64	82.67	85.03	91.80	34.67	13.52	41.94	15.12	26.0	5.79	5.73	6.12
9.67	≈ 2.0	2.0	14.50	13,500	0.53	0.55	97.36	87.72	90.36	92.81	24.00	12.11	27.36	13.43	29.0	5.72	5.59	5.72

Table 2 Results obtained in the different harvesting tests with the centrifuge



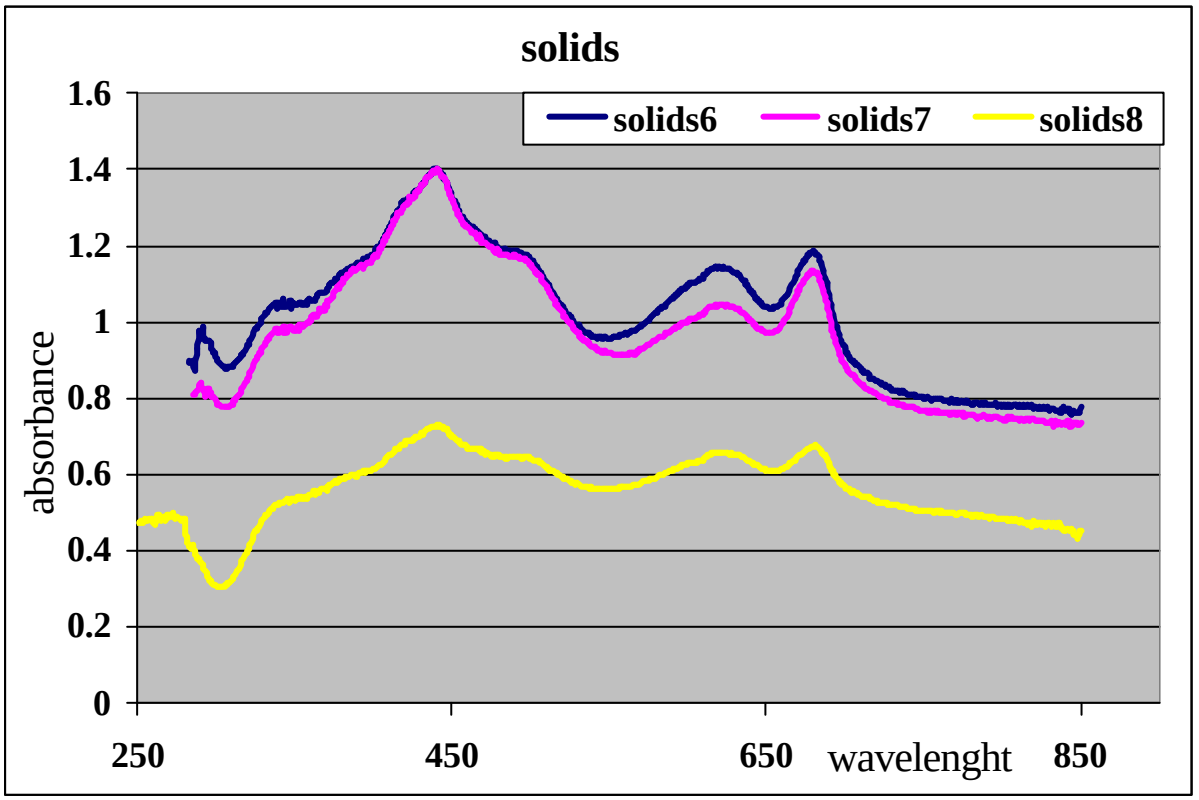


Figure 4 Absorbance measurements of clarified and solids

3.2.4 CONCLUSIONS

The concentration factor, that is to say, paste concentration/feed concentration, have been increased to values up to 20-30, that implies a net recovery of water higher than 95 %. This factor is fixed in ideal conditions by the ratio between solids discharge volume and feed volume. As the solids discharge volume is rather constant independently of the operational conditions, the most important variable to take into account is the feed volume. From the fact that the feed volume is the product of the discharge time and the flow-rate, best results will be obtained for both high discharge time and flow-rate.

However, high discharge times has the disadvantage of producing excessive breakage of the cells and consequently a decrease on the product quality. As a consequence, from a global point of view the best strategy is to use high flow-rates with moderate discharge times (0.5-2 h). From the moment that the recommended flow-rate is about 10 L/h this implies that a strictly continuous operation is not possible. For instance *Spirulina* bioreactor works with an outlet flow-rate ranging from 0.5 to 2.5 L/h. The possibility to recirculate the clarificate obtained from the centrifuge allows to increase feed flow-rate and then to reach high concentration factors (always related to entrance/exit of the centrifuge). However, the fresh feed solution is diluted by the recirculation and then, the overall efficiency of the system is kept low (solids concentration). Thus, an operation in batch or at least semi-continuous mode is recommended for this centrifugation step.

3.3 FINAL CENTRIFUGATION EXPERIMENTS

In order to fully characterise the centrifuge system further studies on this step were performed. The studies were concentrated in the determination of what and how are the effects of changing intermittent discharge time duration and flow rates on yields, concentration factors and additional variables related to the biomass quality as conductivity, extracelular proteins, pH, pigments and so. Mathematical functions of the relations among variables were be obtained allowing to predict the behaviour of the system in future operational conditions that may be necessary to optimise any aspect of the system performance.
In Table 3 planned experiments are presented.

	Flow-rate = 2.5 l/h	**Flow-rate = 5.0 l/h**	**Flow-rate =10. L/h**
Disch. Time = 0.5 h		✓	
Disch. Time= 1.0 h	✓	✓	✓
Disch. Time= 2.0 h		✓	Preliminary experiments

Table 3. Planned experiments for the centrifuge system.

3.3.1 KEY VARIABLES AND CALCULATIONS

The key variables and experimental data used in the final experiments of the centrifugation system have been previously described.
Some variables (all concerning to second discharge) has been withdrawn and some variables have been added, like pH, extracelular proteins and pigments to include variables that may give qualitative biomass quality information.
Used nomenclature is described as follows:

pH.e	Feed pH
pH.f	Clarificate pH
pH.s	Solids pH
Ex.prt.e (mg / ml)	Extracelular protein in the feed medium
Ex.prt.e (g prt./ g bm.e)	Extracelular protein in the feed medium referred to feed biomass concentration
Ex.prt.f (mg / ml)	Extracelular protein in the clarified medium
Ex.prt.f (g prt./ g bm.e)	Extracelular protein in the clarified medium referred to feed biomass concentration
Ex.prt.s (mg / ml)	Extracelular protein in the solids stream
Ex.prt.s (g prt./ g bm.e)	Extracelular protein in the solids stream referred to feed biomass concentration
Prt.dif (%)	Protein difference between feed stream and clarified plus solids stream (protein balance in the liquid phase).
Ex.clor.e (g/l)	Extracelular chlorophyll in the feed medium
Ex.clor.e (g clor./g bm.e)	Extracelular chlorophyll in the feed medium referred to biomass concentration
Ex.phy.e (g/l)	Extracelular phycocyanin in the feed medium
Ex.phy.e (g phy./g bm.e)	Extracelular Phycocyanin in the feed medium referred to feed biomass concentration
Ex.clor.f (g/l)	Extracelular chlorophyll in the clarificate
Ex.clor.f (g clor./g bm.e)	Extracelular chlorophyll in the clarificate referred to feed biomass concentration
Ex.phy.f (g/l)	Extracelular phycocyanin in the clarificate
Ex.phy.f (g phy./g bm.e)	Extracelular phycocyanin in the clarificate referred to feed biomass concentration
Ex.clor.s (g/l)	Extracelular chlorophyll in the solids stream
Ex.clor.s (g clor./g bm.e)	Extracelular chlorophyll in the solids stream referred to feed biomass concentration
Ex.phy.s (g/l)	Extracelular phycocyanin in the solids stream
Ex.phy.s (g phy./g bm.e)	Extracelular phycocyanin in the solids stream referred to feed biomass concentration

3.3.2 CALCULATED DATA

The following parameters have been added to the final experiments to characterise the overall performance of the centrifugation system. They have been calculated using the following equations:

3.3.2.1 Ex. prt.e (g prt. / g bm.e)

This value shows the relation between extracelular protein quantity found in the feed stream against the initial biomass concentration. The same equation is used to determine the extracelular protein in the clarified and solids stream. This is made just replacing Ve volume for Vf and Vs respectively.

$$Ex.prt.e = \frac{\frac{ex.prt.e(mg/ml)*Ve}{Ce*Ve}}{1000}$$

3.3.2.2 Prt.dif (%)

The knowledge of this value allows to determine the difference between the extracelular protein value in the feed flow versus the total output flow (clarificate + solids) from centrifugation system.

$$prt.dif\ (\%) = \frac{((Vf*ex.prt.f)+(Vs*ex.prt.s))(-(ex.prt.e*Ve))}{ex.prt.e*Ve}$$

3.3.2.3 Ex. clor.e (g clor./g bm.e)

This parameter represents the quantity of extracelular chlorophyl present in the feed stream. The same equation is used to determine the extracelular chlorophyl in the clarified and solids stream. The same criteria are applied for phycocianine.

$$Ex.clor.e = \frac{ex.clor.e(g/l)*Ve}{Ve*Ce}$$

3.3.3 RESULTS AND DISCUSSION

A total of six new tests were conducted in this phase (see Table 3).
The different test are based on the combination of different inlet flow-rates and intermittent solids discharge time.
Discharge time duration was fixed to 2s, because, as commented previously, the best strategy is increase solids recovery although the solids concentration decreases.
By the same reason, in these final experiments, no second discharge to recover the low fraction of solids remaining inside the centrifuge was made. If a second discharge is made, the solids stream dilution will be much higher.
The results corresponding to these tests are presented in Table 4.

In fact, the hypotheses made in previous experiments are confirmed by current results. Taking into account only two operational variables as flow-rate and the intermittent solids discharge time, important relationships were verified.

Relating to the feed flow-rate, it directly affects to water yield (RH_2O), solids yield (Rs_1, Rs2) and solids concentration (fs_1, fs_3, fth) values. For a given intermittent solids discharge time but with different flow-rate when flow-rate increases values obtained for those parameters increases (4.8 l/h in test 4 in front of 9.8 l/h in test 6; 2.8 l/h in test 1 in front of 5.3 l/h in test 3). Solids recuperation is better at low flow rate when low solids discharge time is applied (1h), despite being water yield is worse (test 3 vs. test 5).

In relation to the intermittent solids discharge time, it was observed that if it increases, water yield and concentration factor values also increase. This can be verified by directly comparing some of the test performed: test 2 vs. test 3, test 3 vs. test 4 and test 5 vs. test 6.

Results of variables related to the biomass quality, indicate that centrifugation step produce some cell disruption, as can be observed in Picture 1 and Picture 2.

Conductivity values are slightly higher in solids stream, because salts concentration increases when biomass increases, afterwards cell concentration is much higher in this stream than in the clarified stream and that can be interpreted as an increment in the salts content by some concentration effect due to total volume reduction. On the contrary pH value is lower in the concentrated stream, mainly due to two effects: water used in the centrifuge discharge produces a dilution effect and also cell disruption affect to pH.

Extracelular protein and pigments quantity was measured in order to verify whether cell disruption was important.
Measures were taken, in the feed stream, in the clarified and in the concentrate stream. To make a better comparison of the results, values were all referred to initial biomass concentration in the feed stream
In relation to feed stream, higher values for extracelular protein (test 2 and 3), were observed on those samples where initial biomass concentration was higher (test 2 and test 3).
In the clarified stream, low extracelular protein concentration due to low biomass concentration in it was expected. However, results were not too different from the ones observed in the concentrate stream. The results obtained show that, if cell disruption takes place, extracelular compounds will be present at both, concentrate and clarified streams.

An important fact is that higher values for extracelular protein in the clarified stream (test 1 and test 6) were observed, when feed flow-rate was low (2.8 l/h) and when feed flow-rate was high as well (9.8 l/h).
In the solids stream extracelular protein concentration was high whenever solids concentration was high (test 6). This was expected as far as cell disruption takes place more easily.

In general, it can be argued that low flow-rate (2.8 l/h) or high intermittent discharge time (2h.) produces an important effect in the disruption of cells, and this fact, produces protein losses to the medium, as we observed when protein difference (prt.dif (%)) were measured.
Pigments, (chlorophyll and phycocyanin) are difficult to quantify because they easily appear in the medium (clarified stream is slightly yellowish coloured) and their degradation occurs quickly.

No strong relation was found between extracelular pigments concentration and flow-rate and intermittent solids discharge time. In the clarified stream there are present at low quantity due to low biomass concentration but in the solids stream values are so high that measures are not reliable.

Some test values were correlated in order to model the most important variables in those tests. The modelled variables were: water yield (RH_2O), solids concentration factor (fs_1), solids yield (Rs_2), extracelular protein in the clarified (ex.prt.f) and protein difference (prt.dif). In this sense, mathematical functions satisfactorily describing relations among some process variables were obtained. Two general types of equations were investigated; finally selecting which provided a better concordance between predicted and experimental values for each process variable studied.
The general functions used are detailed herein after.

3.3.3.1 General Equation 1 used for modelling

$f = a*X^b*Y^c$

3.3.3.2 General Equation 2 used for modelling

f = a + (bX)+(cY) +(dXY). This type of equation is the most commonly used in factorial experimental design.

Where X = flow-rate, Y = intermittent solids discharge time and a,b,c are equation parameters that vary depending on the process variable. Thus, functions can be expressed in these forms:

$$\mathbf{f = a*(Q_L)^b*(ti._s)^c}$$

$$\mathbf{f = a + (bQ_L)+(c\ Q_L\ ti._s) +(d\ ti._s)}$$

Experimental results obtained for each variable and predicted values for the mathematical functions applied are represented from Figure 5 to Figure 9.

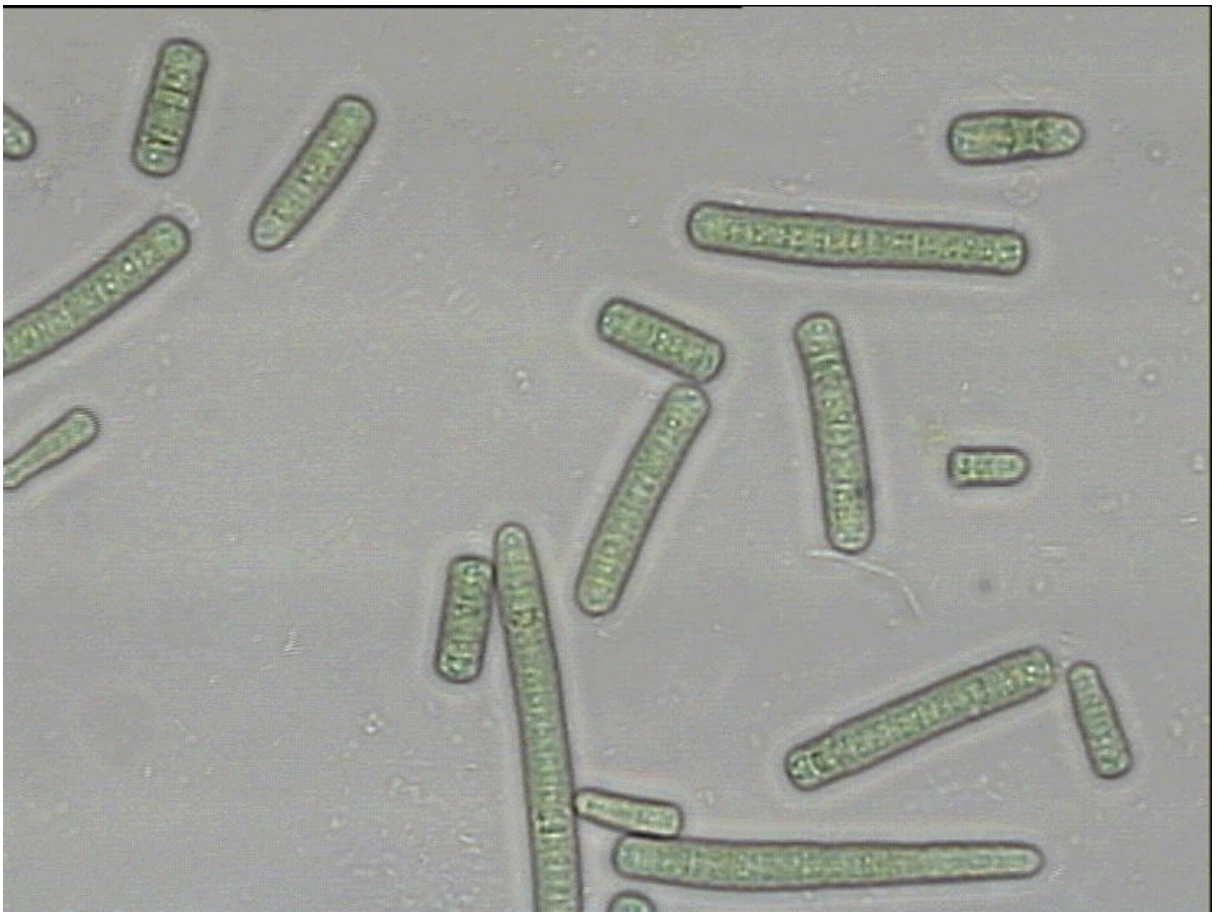
Test	Q (l/h)	ti.s.d (h)	td.d (s)	disch num.	Ve (l)	Vf (l)	Vs (l)	Wf (gr)	Ws (gr)	Ce (g/l)	Cf (s.a) (g/l)	Cs (g/l)	Abs.e (750nm)	Abs f	Abs.s	RH2O (%)	fw
1	2.80	1.0	2.0	2	5.6	4.560	1.18	4,419	1,104	1.04	0.0193	4.23	0.1710 (*10)	0.0322	0.3030 (*30)	79.52	0.49
2	5.00	0.5	2.0	2	5.0	3.800	1.22	3,450	1,092	1.40	0.0365	4.67	0.2740 (*10)	0.061	0.2211 (*50)	75.79	0.64
3	5.30	1.0	2.0	2	10.60	9.500	1.1	8,800	1,088	1.47	0.0126	13.40	0.2462 (*10)	0.021	0.5281 (*50)	89.75	0.23
4	4.80	2.0	2.0	1	9.60	8.900	0.52	8,400	504	0.89	0.0104	14.13	0.1480 (*10)	0.0174	0.4950 (*50)	94.56	0.14
5	9.12	1.0	2.0	2	18.24	17.000	1.3	16,550	1,300	1.24	0.0126	15.46	0.2080 (*10)	0.0211	0.5100 (*50)	93.00	0.15
6	9.80	2.0	2.0	1	19.60	19.000	0.58	18,800	560	1.31	0.0057	40.14	0.2520 (*10)	0.0096	1,2018 (*60)	97.16	0.06

Test	Rs1 (%)	Rs2 (%)	Rt (%)	fs1	fs3	fsth	Cd.e (ms/ cm)	Cd.f (ms/ cm)	Cd.s (ms/ cm)	pH. e	pH. f	pH. s	Ex.prt. e (mg/ ml)	Ex.prt. e (g prt./ g bm.e)	Ex.prt. f (mg/ ml)	Ex.prt. f (g prt./ g bm.e)	Ex.prt.s (mg/ml)	Ex.prt.s (g prt./ g bm.e)
1	98.49	86.03	87.55	4.08	4.75	11.2	6.39	5.61	6.49	8.13	7.93	7.20	83.8	0.0809	187.9	0.1477	201	0.0408
2	98.02	81.39	83.37	3.34	4.10	10.0	6.06	5.52	6.07	8.01	8.00	7.53	134.5	0.0961	115.2	0.0625	468	0.0816
3	99.23	94.60	95.36	9.12	9.64	21.2	5.49	5.34	6.23	9.63	8.81	7.16	131.7	0.0896	119.9	0.0731	1015	0.0716
4	98.92	86.00	87.08	15.88	18.46	19.2	5.52	5.49	5.83	8.02	7.76	6.64	55.2	0.0620	53.4	0.0556	982	0.0597
5	99.05	88.86	89.81	12.47	14.03	36.5	5.66	5.65	5.50	8.63	7.67	6.78	87.7	0.0707	69.9	0.0525	594	0.0341
6	99.58	90.67	91.10	30.64	33.79	39.2	5.94	6.02	6.49	8.97	8.81	7.15	61.4	0.0469	148.1	0.1096	2516	0.0568

Test	Prt.dif (%)	Ex.clor.e (g/l)	Ex.clor.e (g clor./ g bm.e)	Ex.phy.e (g/l)	Ex.phy.e (g phy./ g bm.e)	Ex.clor.f (g/l)	Ex.clor.f (g clor./ g bm.e)	Ex.phy.f (g/l)	Ex.phy.f (g phy./ g bm.e)	Ex.clor.s (g/l)	Ex.clor.s (g clor./ g bm.e)	Ex.phy. s (g/l)	Ex.phy. s (g phy/ g bm.e)
1	133.0	5.65E-05	5.45E-05	6.69E-04	6.46E-04	7.72E-05	6.07E-05	8.88E-04	6.98E-04	1.40E-03	2.85E-04	0.268	0.0546
2	50.0	1.55E-04	1.10E-04	1.34E-03	9.58E-04	2.76E-04	1.50E-04	2.54E-03	1.38E-03	3.13E-03	5.45E-04	1.41	0.2461
3	61.5	6.71E-05	4.56E-05	8.78E-04	5.97E-04	1.71E-04	1.04E-04	2.60E-03	1.59E-03	8.17E-03	5.77E-04	5.75	0.4058
4	86.0	3.88E-05	4.35E-05	5.79E-04	6.50E-04	8.16E-05	8.50E-05	1.03E-03	1.08E-03	9.09E-03	5.53E-04	5.13	0.3120
5	22.6	8.76E-05	7.06E-05	1.19E-03	9.57E-04	1.92E-05	1.44E-05	3.16E-04	2.37E-04	5.89E-03	3.38E-04	2.75	0.1578
6	255.	8.44E-05	6.45E-05	1.10E-03	8.40E-04	2.56E-04	1.89E-04	3.64E-03	2.69E-03	2.53E-02	5.73E-04	13.6	0.3066

Table 4 Results obtained in the different harvesting tests with the centrifugation system

Picture 1 *Spirulina* cell in the culture medium before centrifugation (*40).



Picture 2 *Spirulina* cells after applying severe centrifugation conditions (*40).

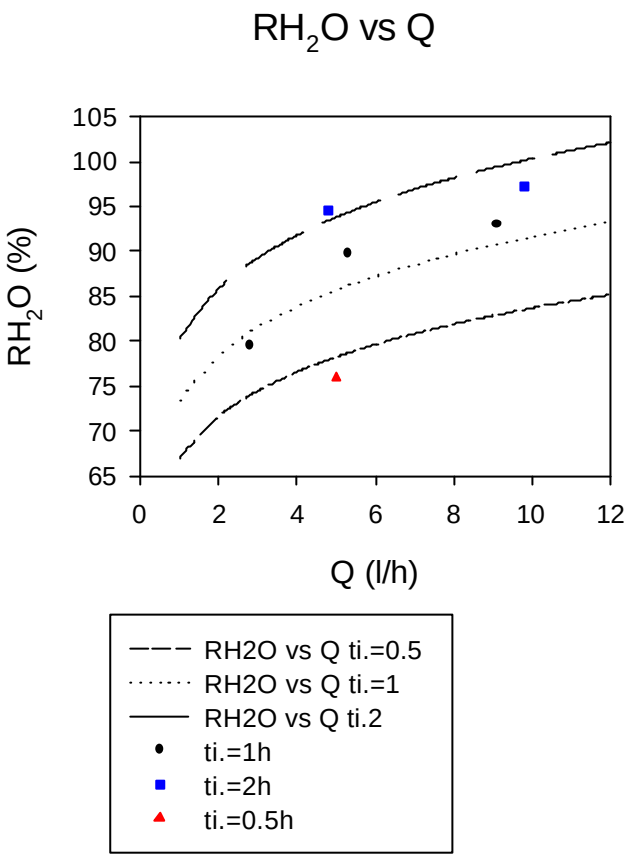


Figure 5 Water yield

Modelled mathematical function:

$$f = a*Q^{b}*ti^{c}$$

Parameters values:

a = 73.27
b = 0.09
c = 0.13

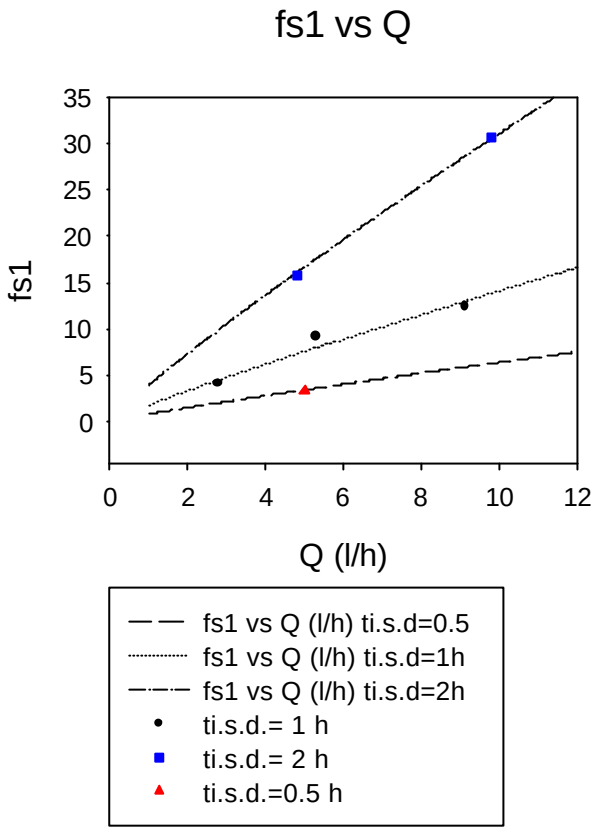


Figure 6 Solids concentration factor

Modelled mathematical function:

$$f = a*Q^{b}*ti^{c}$$

Parameters values:

a = 1.78
b = 0.89
c = 1.14

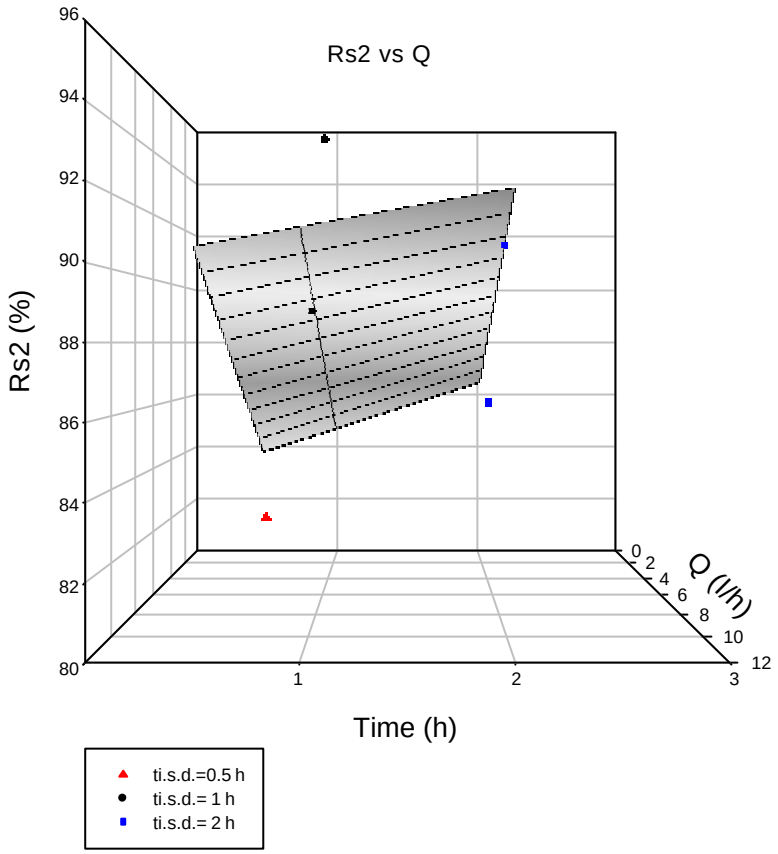


Figure 7 Solids yield

Mathematical function:

$$f = a + (bQ) + (cti) + (dQti)$$

Parameters values:

a = 82.98
b = 0.52
c = 0.68
d = 0.68

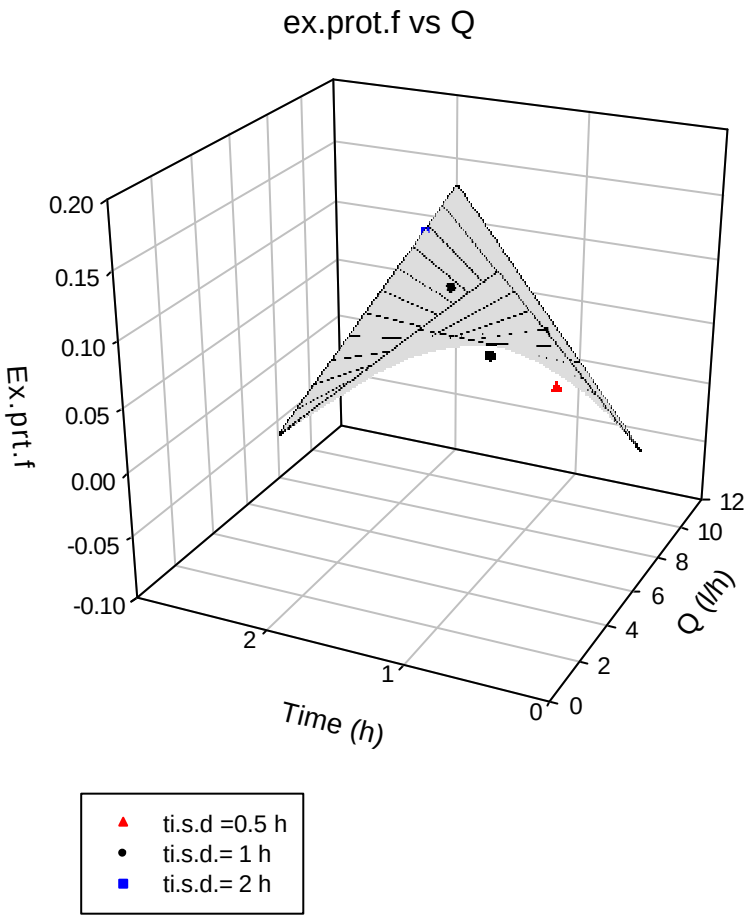


Figure 8 Extracellular protein in the filtrate stream

Modelled mathematical function:

$$f = a + (bQ) + (cti) + (dQti)$$

Parameters values:

a = 0.26
b = (-0.03)
c = (-0.11)
d = 0.02

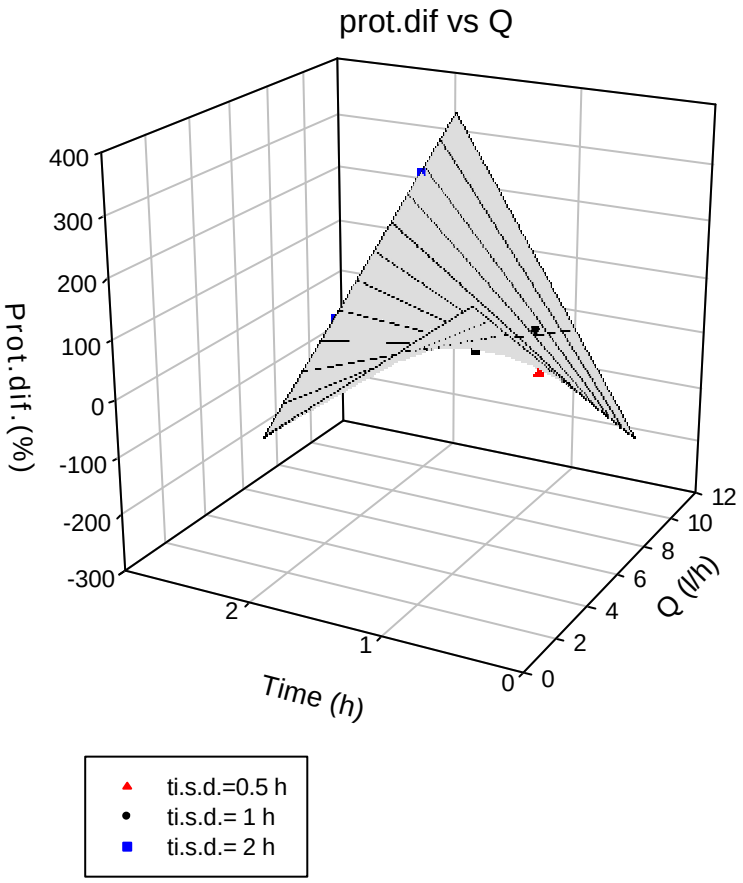


Figure 9 Protein difference

Modelled mathematical function:

$$f = a + (bQ) + (cti) + (dQti)$$

Parameters values:

a = 392.73
b = (-65.02)
c = (-232.07)
d = 49.62

3.3.4 CONCLUSIONS FROM FINAL CENTRIFUGATION TESTS

Hypotheses made in previous experiments were confirmed. High flow-rates and high intermittent solids discharge time implies a net recovery of water higher that 95% (99% in test 6) and a solids concentration factor about 30, although a partial cell disruption appears in the most severe centrifugation conditions.

Partial breakage of the cells was confirmed, as expected at higher retention time and rotation speed, by extracelular protein and pigments measurements as well as by microscopic observation. This effect might only be transcendent in case the whole integrity of the cells would be necessary, because all the main nutrients remain in the concentrate phase despite cells are partially disrupted.

An increase of discharge time duration (2s) allow to obtain high solids recuperation values Experimental values were higher than for previous experiments (> 90% in some cases).

Therefore, it can be concluded that the best strategy in order to plan the washing step of the biomass might not be an additional centrifugation system. As an alternative a microfiltration membrane system could be used in order to minimise “extra” breakage of the cells. Obviously, operation time, flow-rates, filtration area, pore size, type of microfiltration and operational mode, among other operational variables, had to be fixed in order to reduce as far as possible membrane clogging and concentration polarisation.

4 WATER PURIFICATION

4.1 GENERAL INTRODUCTION

The present part covers the second step of the harvesting system, the membrane modules section.
The clear liquid obtained from the centrifuge (filtrate 1) in Figure 10 is fed, under pressure, through different membrane modules, with the objective to provide the purest possible liquid, in order to be recycled for a higher plant and crew compartment.

The process selected for water recycling is expected to be dependent on the source of wastewater and the quality of water needed for a given application. The main problem for regenerating water quality is to eliminate compounds that make it non-potable and/or non-hygienic, which are mainly microorganisms (bacteria, viruses, protozoan, yeast, etc) and both organic and mineral compounds.

Thus, taking into account that the membrane filtration methods for water recovery aboard a space habitat would be basically process to obtain hygiene and potable water (Eckart 1994), the filtration processes that can be used are:
- reverse osmosis(RO)
- microfiltration (MF)
- electrodialysis

The major competing regenerative subsystems for potable and hygiene water processing of the manned space program of the United States are the Microfiltration (MF) and Reverse Osmosis (RO) processes. The quantitative resources for the microfiltration and reverse osmosis processes are similar, with MF having slightly lower power requirements and re-supply weight and volume. The three major issues of reliability, integration, and complexity all favour the MF because of its single pass operation which leads to a less complex, more reliable design (Eckart 1994). However, if water with reduced mineral content is desired, RO process is strictly necessary to be implemented.

The conclusions of the previous studies undertaken by ESA dealing with water recovery, have demonstrated the excellent adaptation of membranes technologies to purify low polluted waste waters (Corryl, Techno-Membranes, 1998).

The tangential filtration was shown as the best alternative, once the centrifugation step was performed (TN 37.30). A more detailed description of the different filtration processes follows below:

Microfiltration and Ultrafiltration: (MF and UF)

What distinguishes the most commonly used membrane processes -microfiltration and ultrafiltration- is the application of hydraulic pressure to speed up permeate and the transport processes. However, the nature of the membrane itself controls which components are retained.
Microfiltration (MF) and Ultrafiltration (UF) are similar; primarily differing in the size of the pores needed to reject the suspended species. Ultrafiltration retains only macromolecules or particles larger than about 10-200 Å.
Microfiltration processes are designed to retain particles in the "micron" range, that is, suspended particles in the range from 0.10µm to approximately 10 µm.

Ultrafiltration can be looked at as a method for simultaneously purifying, concentrating, and fractionating molecules or fine colloidal suspensions. Microfiltration is also a method for essentially separating suspended particles from dissolved substances in a feed stream, provided the particles meet the size requirements for microfiltration membranes.
Ultrafiltration, together with reverse osmosis constitute the first continuous molecular separation processes that do not involve a phase change or interphase mass transfer, this should result in considerable savings in energy.

Pyrogens cannot be eliminated by autoclaving or microfiltration, but have been successfully removed by ultrafiltration. Ultrafiltration modules of about 10,000 molecular weight “cut-off” (MWCO) appear to be optimal. UF stage can simultaneously remove pyrogens and others microorganisms (Munir Cheryan, Ph.D, 1986).

Reverse osmosis: (RO)

In the standard osmotic process, water moves from a compartment with a less concentrated to a compartment with a more concentrated solution driven by osmotic pressure. In RO, the process is truly reversed, pressure is applied to the wastewater until this osmotic pressure is exceeded, forcing water to pass across a semipermeable membrane and leaving most ions and larger organic compounds behind. The RO unit rejects all suspended solids, all macromolecules, and most low molecular weight salts, although typical membranes are unable to remove small organics. The result is a large volume of relatively pure permeate and a small volume of very concentrated fluid. Common RO membranes require pre-treatment by ultrafiltration (UF) to remove suspended solids and large molecules to prevent membrane fouling and concentration polarisation.

Ultrafiltration is a process that filters most suspended solids and macromolecules, while allowing low molecular weight salts and water to permeate the membrane.
As the first stage of the RO system, the primary function of UF is to remove large contaminants that would otherwise foul the RO membranes.

Two membranes have received the most attention for RO in space; the inside skinned hollow fibre membrane and the dual layer membrane. The membrane itself is the key element of the process. The most attractive features of RO for space habitat water recovery are low energy consumption compared with other physicochemical alternative treatments and no requirement for a solid-liquid phase separator in zero gravity (Eckart 1994).

4.2 GENERAL SCHEME OF WATER PURIFICATION

Both potable and hygiene water have to match certain quality requirements. Limits for physical parameters, chemical and biological (including microorganisms) constituents have to be accomplished. A membrane module step has as a major objective to adequate these parameters in order to mainly recycle this water for the higher plant and crew compartment.

The general scheme presented in Figure 10 includes a microfiltration step to decrease the cell and suspended solids concentration of the clarified liquid obtained from the centrifugation step (filtrate 1). In the second step, ultrafiltration is necessary to eliminate macromolecules such as proteins, some pyrogens and microbial contamination. Finally, a reverse osmosis module is necessary to reject low molecular weight salts.

Depending on water utilisation, crew or higher plant compartment, the number of reverse osmosis modules to be used in the final step will increase, because the quality standards in both cases are not the same. For drinking water, the process has to be extremely efficient in order to accomplish the high quality requirements and prevent microbial risk contamination.

In the general scheme of water purification there are some critical points to be taken into account such as water pH, carbonate/bicarbonate concentration (alkalinity), harness (Ca^{2+} and Mg^{2+})metals presence and microbiological contamination.

PH: liquid obtained from *Spirulina* culture medium has an alkaline pH. In order to minimise possible problems with microbial contamination and substantial accumulation of OH^- radical from bicarbonate ion as a carbon source before microfiltration step it may be necessary a stabilisation step where pH value is lowered to 4. Stabilising agents candidates are: sulphuric acid, peracetic acid, hydrogen peroxide and iodine. A good treatment for bacteria elimination was tested by Corryl- Techno-Membranes with Oxonia and H_2SO_4 (Corryl, 1998).
Nevertheless this pH, is not accepted for drinking water and higher plant compartment and also could affected the ultimate disinfection step (chloronitation, ozone or U.V) and it will be necessary a final neutralisation step. However, with this low pH cations that contribute to water hardness are soluble and so must be eliminated by using chemical softening or even ion exchange if necessary.

Alkalinity: The most common constituents of alkalinity are bicarbonate (HCO_3^-), carbon (CO_3^{2-}) and hydroxide (OH^-). The relative quantities of the alkalinity species are pH dependent. Reactions involved in their chemical equilibrium are as follows:

- $CO_2 + H_2O \Leftrightarrow H_2CO_3^*$ (Dissolved CO_2 and carbonic acid) (1)
- $H_2CO_3^* \Leftrightarrow H^+ + HCO_3^-$ (Bicarbonate) (2)
- $HCO_3^- \Leftrightarrow H^+ + CO_3^{2-}$ (Carbonate) (3)
- $CO_3^{2-} + H_2O \Leftrightarrow HCO_3^- + OH^-$ (Hydroxide) (4)

The reaction represented by Eq (4) is a weak reaction, chemically spaking. However, utilisation of the bicarbonate ion as a carbon source by algae can drive the reaction to the right and result in substantial accumulation of OH^-

In large quantities, alkalinity imparts a bitter taste to the water. The principal objection to alkaline water, however, is the reactions that can occur between alkalinity and certain cations in the water (hardness).

Hardness that is equivalent to the alkalinity is termed carbonate hardness. At supersaturated conditions, the hardness cations will react with anions in the water to form a solid precipitate.

So, depending on the water hardness values, softening process is recommend before stabilisation step because is pH-dependent (the optimum pH for carbonates precipitation is between 9-11)

Softening processes commonly used are chemical precipitation and ion exchange.

Chemical precipitation: chemical precipitation converts calcium hardness to calcium carbonate and magnesium hardness to magnesium hydroxide. This can be accomplished by the lime-soda ash (CaO) process or by caustic soda (Na OH) process.

Ion exchange: As practised in water softening, ion exchange involves replacing calcium and magnesium in the water with another, nonhardness cation, usually sodium or even H^+. This exchange takes place at a solids interface. In similar quantities, calcium and magnesium are adsorbed more strongly to the medium than is sodium. As the hard water is contacted with the medium, the following generalised reaction occurs:

- $\{Ca, Mg\} + [anion] + 2Na[R] \rightarrow \{Ca, Mg\}[R] + 2Na + [anion]$

Ion exchanges produce softer water than chemical precipitation and avoid the large quantity of sludge encountered in the lima-soda process. The physical and mechanical apparatus is much smaller and simpler to operate. There are several disadvantages however. The water must be essentially free of turbidity and particulate matter or the resin will function as a filter and become plugged. Surfaces of the medium may act as an adsorbent for organic molecules and become coated. Iron and manganese precipitates can also foul the surfaces if oxidation occurs in, or prior to, the ion exchange unit. The water should not be chlorinated prior to ion exchange softening. (Peavy, 1985).

Metals: All metals are soluble to some extent in water. While excessive amounts of any metal may present health hazards, only those metals that are in relatively small amounts are commonly labelled toxic, other metals fall into the nontoxic group.

Nontoxic metals: In addition to the hardness ions, calcium and magnesium, other nontoxic metals commonly found in water include sodium, iron, manganese, aluminium, copper and zinc. Sodium, by the far most common nontoxic metal found in natural waters, is highly reactive with other elements. Although salts of sodium are very soluble in water, excessive concentrations cause a bitter taste in water. Sodium is also corrosive to metal surfaces and, in large concentrations, is toxic to plants

Iron and manganese quite frequently occur together and present no health hazards at concentrations normally found in natural waters. In very small quantities may cause colour problems.

The other nontoxic metals are generally found in very small quantities in natural water systems, and most would cause taste problems long before toxic levels were reached. However, copper and zinc are synergetic and when both are present, even in small quantities, may be toxic to many biological species.

Toxic metals: Toxic metals are harmful to humans and other organisms in small quantities. Toxic metals that may be dissolved in water include arsenic, barium, cadmium, chromium, lead, mercury, and silver. Cumulative toxins such as arsenic, cadmium, lead and mercury are particularly hazardous. (Peavy, 1985)

Chlorine compounds: When chlorine is added as a disinfection method, oxidizable substances, such as Fe^{+2}, Mn^{+2}, H_2S, and organic matter, react with the chlorine and reduce most of it to the chlorine ion. Chlorine can react with amonia to form chloramines (tòxic compound), dependent to pH and temperature. Recent work in the USA, has identified a possible link between bladder cancer and chlorinated water (Gray, 1994). Many substances will readily combine with chlorine, especially reducing agents and unsaturated organic compound. Suspended organic and inorganic matter absorbs chlorine, whereas iron and manganese neutralise chlorine by forming insoluble chlorides. Thus it is better to remove these problematic substances by appropriate treatment (adsorption) before disinfection rather than increasing the dose of chlorine.

Chlorine react with organic compounds to form chlorinates hydrocarbons, many of which are toxic. (Gray, 1994)

Pathogens: from the perspective of human use and consumption, the most important biological organisms in water are pathogens, those organisms capable of infecting or of transmitting diseases to humans. Many species of pathogens are able to survive in water and maintain their infectious capabilities for significant periods of time.

Although standard disinfection practises are known to kill bacteria and viruses, confirmation of effective viral disinfection is difficult, owing to the small size of the organism and the lack of quick and conclusive tests for viable virus organisms. (Peavy, 1985).

Organics and organic materia: Many organics materials are soluble in water. Dissolved organics in water are usually divided into two categories: biodegradable and nonbiodegradable. Biodegradable material usually consists of starches, fats, proteins, alcohols, acids, aldehydes, and esters. Although some of these materials can cause colour, taste, and odour problems, the principal problem associated with biodegradable organics is a secondary effect resulting from the action of microorganism of these substances. The amount of oxygen consumed during microbial utilisation of organics is called the biochemical oxygen demand (BOD).

Some organic materials are resistant to biological degradation. Tannic and lignic acids, cellulose, and phenols are often found in water systems. Molecules with exceptionally strong bonds (some of the polysaccharides) are essentially nonbiodegradable. Measurement of nonbiodegradable organics is usually by the chemical oxygen demand (COD). May also be estimated from a total organic carbon (TOC) analysis. (Peavy, 1985).

Some of this organics (polysaccharides) can produce a membrane concentration polarisation in the ultrafiltration and reverse osmosis steps. So, it is recommend a previous adsorption step before a reverse osmosis step. The most commonly used filtration process involved passing the water through a stationary bed of granular medium. Solids in the water are retained by the filter medium.

Taking into account all these last considerations the process must include a previous softening step (chemical precipitation or ion exchange) and a stabilisation step with chemical agents with bactericide effect and water acidification (Corryl, Techno-Membranes, 1998).
The finished water still contains pathogenic viruses and bacteria, which need to be removed or destroyed by using proper disinfection methods such as chlorination, ozone or ultraviolet radiation.

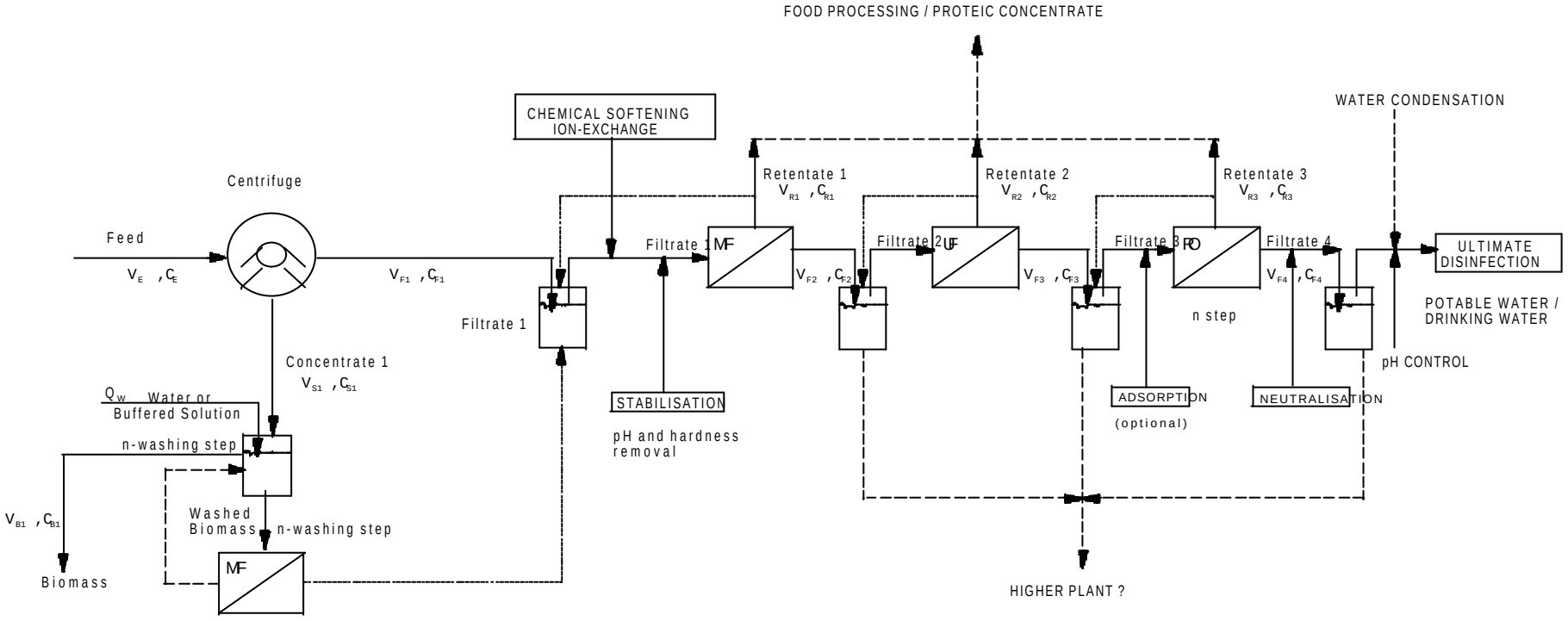


Figure 10. General Scheme for Water Purification

4.3 INTERFACE WITH THE HIGHER PLANT COMPARTMENT

It has to be taken into account that higher plants could contribute to a number of advanced life support issues like atmosphere and water and waste management.
Complete closure of life support systems using higher plants requires the development of low-mass hydroponic nutrient delivery systems in which all materials, including degraded human waste water and inedible plant biomass, are recycled.
Plant water transpiration can easily be recuperated (condensation) and considered as potable (Eckart, 1994).

The role of higher plants in life support systems was already preliminarly summarised by Tamponnet (1993). This summary remarks an important issue because some plants may directly absorb nutrients from wastewater. Therefore, water obtained from membrane modules, can be recycled into a nutrient solution for plants. Plant transpiration water can be condensed and collected for drinking water for crew compartment because of their low salt and contaminants contents expected.

However related to higher plant application, potential contaminants may be carried in the water, and the culture system should be free of pathogenic organisms. Membrane process should assure the quality of water, principally concerning the microbial contamination (viruses, enteric bacteria, etc.). On the other hand; limits for some bacteria could be obtained using a reference drinking water quality. However, relating to salt and mineral compounds less strict limits are adequate comparing to potable water requirements. So, reverse osmosis step, according to quantity and kind of salts in the water after ultrafiltration step, may be not strictly necessary.
Table 5 summarises the nutrient content ranges for nutrient solutions in hydroponics as indicated in several references:

Component	Amount (g/l)
Potassium nitrate	0-1.1
Calcium nitrate	0-1.29
Ammonium nitrate	0-0.1
Calcium biphosphate	0-0.31
Potassium sulfate	0-0.63
Calcium sulfate	0-0.76
Magnesium sulfate	0.17-0.54
Ammonium sulfate	0-0.14
Total Salts	0.95-3.17

Table 5 Composition of nutrient solutions for Higher Plants (Shepelev, 1972)

A total of 17 elements are considered to be essential for higher plants. Adequate supply of each of these nutrients can be maintained by matching nutrient uptake and delivery.

Essential elements for higher plants and internal concentrations considered adequate are summarised in Table 6 (T.N 40.1,University of Guelph)

Element	**Typical Concentration in Dry Tissue (ppm)**
Micronutrient	
Chlorine (Cl^-)	100
Iron (Fe^{3+}, Fe^{2+})	100
Manganese (Mn^{2+})	50
Boron (H_3BO_3)	20
Zinc (Zn^{2+})	20
Copper (Cu^+, Cu^{2+})	6
Molybdenum (MoO_4^{2-})	0.1
Nickel (Ni^{2+})	--
Macronutrient	
Carbon (CO_2)	450000
Oxygen (O_2, H_2O, CO_2)	450000
Hydrogen (H_2O)	60000
Nitrogen (NO^-_3, NH_4^+)	15000
Potassium (K^+)	10000
Calcium (Ca^{2+})	5000
Magnesium (Mg^{2+})	2000
Sulfur (SO_4^{2-})	1000
Phosphorus ($H_2PO_4^-$, HPO_4^{2-})	2000

Table 6 Essential elements for higher plants

Nutrient solution has to be supervised for composition and pH. When analysis indicates low nutrients levels in the solution (salts), constituents have to be added as required.
For most of higher plants, the pH nutrient solution has to be in the range of 5.5-6.5. So it needs to be constantly adjusted and buffer added.

Most of these essential micronutrient and macronutrient are presented in the feed culture medium used in the bioreactor compartments, and therefore, it is expected to have the major part of them in enough quantities as exit streams.

4.3.1 CULTURE BROTH CONDITIONS AND TREATMENT

Water for higher plant compartment has to accomplish minimum parameters of quality and be microbiologically suitable for its use. Critical points to be taken into account have been explained in the general scheme of water purification.

Maybe, the most important parameters are salts quantity and total amount of microorganisms in the water. Other parameters are also important but not so critical, than for drinking water.

All nutrient solutions for higher plants contain macro and microelements necessary for them to grow. Water that comes from the centrifugation step (filtrate 1) can contain an important quantity of salts, although it will be necessary to test that they are within correct limits.

Proposed treatment is the described in the first part of this T.N, this is the membrane modules, and the conditions of final water obtained in the final step (ultrafiltration) will be described in the results section.

Liquid obtained from centrifuge (filtrate 1) is fed, through a microfiltration module in order to eliminate the small biomass quantity and suspended solids that remains in the liquid.

Liquid obtained in the microfiltration step (filtrate 2) is then processed by using an ultrafiltration module in order to eliminate molecules such as proteins and carbohydrates and eventual microbial contamination still present in the filtrate 2.

Salts are not eliminated by microfiltration and ultrafiltration systems. Thus, if water analyses indicate too high concentration of some salts, an osmosis reverse step will be necessary.

pH of final liquid obtained will be important, because recommended pH value for higher plants is not the same than for drinking water for the crew compartment. So, after the neutralisation step, pH has to be adjusted according to the higher plant requirements (it depends in any case on the crop considerate).

4.4 INTERFACE WITH THE CREW COMPARTMENT

Water recycling processes must provide hygiene and potable water for the crew compartment. Both potable and hygiene water have to match certain quality requirements. In addition to such factors as flavour and clarity, trace levels of organic and inorganic contaminants are of great concern. Certain pollutants are of greater concern for longer duration missions than for shorter ones due to the capacity of some compounds to accumulate in body tissues. Also of concern are microorganisms that may become pathogenic under some conditions.

Some data about potable and hygiene water requirements are presented as follows in Table 7 and Table 8:

Parameter	Input/Output (Kg/man-day)
Potable water	2.27-3.63
Hygiene water	1.36-9

Table 7 Metabolic values for Normal Spacecraft Operation of one Astronaut (Skoog, 1985)

Consumable	Design Load (Kg/man-day)
Drinking water	1.6
Shower water	2.7
Food preparation water	0.75
Hand wash water	4.1

Table 8 Average U.S. Space Station Design Loads for Water (Hienerwandel and Kring, 1988)

The potable and hygiene water quality requirements, limits for physical parameters, chemical and biological constituents, are shown in the next table.

Physical Parameters	Limits (Potable water)	Limits (Hygiene water)
Total solids (mg/ml)	100	500
Colour true (Pt/Co units)	15	15
Taste (TTN)	3	3
Odour (TON)	3	3
Particulates (max. size in microns)	40	40
pH	6.0-8.4	5.0-8.4
Turbidity (NTU)	1	1

Table 9 Potable and hygiene water quality requirements (Wieland, 1992)

In this TN 43.21 only drinking water characteristics and its corresponding quality parameters due to be the stronger requirements to be satisfied will be studied.

Drinking water has to accomplish some standards, as the EC Directive, 15 July 1980 relating to the quality of water intended for human consumption (see annexes) and the ESA standard.

Parameters	**Drinking water ESA standard**
pH	6.5-8.5
Conductivity (mS/cm)	0.75
Turbidity (NTU)	2.5
TOC (ppm)	0.5
Oxidative power (ppm)	-
F^- (ppm)	1
Cl^- (ppm)	200
NO_3^- (ppm)	25
PO_4^{3-} (ppm)	5
SO_4^- (ppm)	250
Na^+ (ppm)	150
K^+ (ppm)	12
NH_4^+ (ppm)	0.5
N-Kjeldahl (ppm)	1

Table 10 Drinking water ESA standard (Corryl Techno-Membranes, 1998)

Therefore, values for different parameters in drinking water have to be within the permissive values refereed.

4.4.1 CULTURE BROTH CONDITIONS AND TREATMENT

Same criteria that are applied to medium conditions at higher plant compartment can be applied for the crew compartment but, with stricter limits. Besides the microbiological, physical and chemical criteria, all of them very important, it has to be taken into account organoleptical characteristics.

So, treatment had to be probably longer in both terms of time and operational units, including reverse osmosis step and different final test of acceptance (taste, odour and colour).

Proposed treatment should be the same scheme proposed for the higher plant compartment but now, including an additional osmosis reverse step. The number of operations for each step will depend on analyses results from the specific reactors operational conditions and probably, it would be necessary several steps of reverse osmosis (Corryl Techno-Membranes, 1998).

4.5 DISINFECTION METHODS

Although membrane modules can be extremely efficient at removing bacteria (Corryl Techno-Membranes, 1998), the finished water may still contain pathogenic viruses and bacteria, which need to be removed or destroyed. In practice it is impossible to sterilise water, to kill off all microorganism present. Therefore the water is disinfected, by using disinfection methods such as chlorination, ozone or ultraviolet radiation to ensure that pathogens are kept below safe levels.

When applying disinfection agents, the following factors must be considered: 1) contact time, 2) concentration and type of chemical agent, 3) intensity and nature of physical agent, 4) temperature, 5) number of organisms, 6) types of organisms, and 7) nature of suspending liquid. (Metcalf & Eddy, 1991).

Disinfection with chlorine:
Chlorine is, perhaps, the most commonly disinfectant used throughout the world.
Chlorine and its compound are readily available in gas, liquid or solid forms. It is easy to add to water, has a high solubility (7000 mg/l) and is relatively cheap. The residues it leaves in solution continue to destroy possible pathogens after the water has left the treatment plant (residual protection). In the space habitat, solid forms are the best alternative, such as calcium hypochlorite ($Ca(OCl)_2$), sodium hypochlorite (NaOCl). Calcium and sodium hypochlorite are most often used in very small treatment plants, where simplicity and safety are far more important than cost. Sodium hypochlorite is often used at large facilities, primarily for reason of safety as influenced by local conditions (Metcalf & Eddy, 1991) .
Disinfection is much more effective at acidic pH.
Chlorine is not as aggressive as ozone and there are a number of pathogenic microorganisms, which are resistant to chlorination. Effectively eliminating all the coliforms present does not necessarily indicate that all other pathogenic microorganisms have also been destroyed. Factors such as temperature and pH also affect chlorination, its efficiency decreasing at lower temperatures and in more alkaline waters.
A major problem is the presence of ammonia. This reacts readily with chlorine to form a range of compounds known as chloramines, the exact nature of which depends on the relative concentrations of the two chemicals and the pH.
When the ammonia is present the dose of chlorine must be increased to ensure that sufficient excess chlorine is left in the water to destroy the pathogens (N.F. Gray, 1994).
The presence of additional compounds that with react with chlorine, such as organic nitrogen , may greatly alter the shape of the breakpoint curve. The amount of chlorine that must be added to reach a desired level of residual is called chlorine demand.
Certain organic constituents in wastewater interfere with the chlorination process. Many of these organic compounds may react with chlorine to form toxic compounds that can have long-term adverse effects on the beneficial uses of the waters to which they are discharged. To minimise the effects of these potentially toxic chlorine residuals on the environment, it has been found necessary in some cases, to dechlorinate wastewater treated with chlorine.
For dechlorination, sulfur dioxide is the most commonly used. Activated carbon has also been used as adsorption operation to remove chlorine compounds.(Metcalf & Eddy, 1991)

Disinfection with ozone:
Ozone has powerful oxidation properties and tends to be used where the natural water contains materials that would combine with chlorine to form unacceptable odours or tastes. Ozone, which is often used in combination with activated carbon (adsorption step), can eliminate all bacteria at a dose rate of 1 ppm within 10 minutes, and can also reduce colour, taste and odour (N: F Gray,1994)
Because ozone is chemically unstable, it decomposes to oxygen very rapidly after generation, and thus, must be generated on-site. The most efficient method of producing ozone today is by electrical discharge. This system could have some implementation problems in space habitats.
Ozone is an extremely reactive oxidant, and it is generally considered that bacterial kill through ozonation occurs directly because of cell wall disintegration.
Ozone is also a very effective virucide and is generally believed to be more effective than chlorine. Ozonation does not produce dissolved solids and is not affected by the ammonium ion or pH influent to the process. For these reasons, ozonation is considered a viable alternative to chlorination; especially where dechlorination may be required, despite being more expensive. (Metcalf & Eddy, 1991).

Disinfection with ultraviolet radiation:
Ultraviolet radiation has been shown to be an effective bactericide and virucide while not contributing to the formation of toxic compounds.
At present time, the low-pressure mercury arc lamp is the main method of generating UV energy used for disinfection. Operationally, the lamps are either suspended outside of the liquid to be treated or submerged in it.
Ultraviolet light is a physical rather than a chemical-disinfecting agent. Radiation with a wavelength of around 254 nm penetrates the cell wall of the microorganism and is absorbed by cellular materials including DNA and RNA, which either prevents replication or causes death of the cell to occur.
Because the only ultraviolet radiation effective in destroying bacteria is which reaches the bacteria, the water must be relatively free from turbidity that would absorb the ultraviolet energy and shield the bacteria.
It has also been reported that ultraviolet light is not an effective disinfectant on wastewater that contains high solid concentrations.
Because ultraviolet light is not a chemical agent, no toxic residuals are produced.
In general, is used at small plants or for institutions where the chance of contamination after treatment is unlikely (Metcalf & Eddy, 1991).

Comparison of ideal and actual characteristics of the most commonly used disinfectants are summarised in Table11 (Metcalf & Eddy, 1991).

Taking into account the characteristics of most commonly used disinfectants and the water final characteristics, a best alternative for disinfection could be combine an ozonation and chlorination treatment.
Ozone is high toxic with microorganisms and is a very effective virucide and it is not affected by the pH. Chlorination, on the other hand, produces a residual protection that can be desired in some cases. Ultraviolet light could be an alternative to ozone disinfection if water wouldn't contain high solids concentration (as water from harvesting and purification steps) and can be easily used at small water treatment plants.

Characteristic	Properties/ Response	Chlorine	Ozone	UV radiation
Toxicity to microorganisms	Should be highly toxic at high dilutions	High	High	High
Solubility	Must be soluble in water or cell tissue	High	High	N/A
Stability	Loss of germicidal action on place should be low	Stable	Unstable, must be generated as used	Must be generated as used
Non-toxic to higher forms of life	Should be toxic to microorganisms and non toxic to man and other animals	Highly toxic to higher life forms	Toxic	Toxic
Homogeneity	Solution must be uniform in composition	Homogeneous	Homogeneous	N/A
Interaction with extraneous material	Should not be absorbed by organic material other than bacterial cells	Oxidises organic matter	Oxidises organic matter	Slight
Toxicity at ambient temperatures	Should be effective within ambient temperature range	High	High	High
Penetration	Should have the capacity to penetrate through surfaces	High	High	Moderate
Non-corrosive and non-staining	Should not disfigure metals or stain clothing	Highly corrosive	Highly corrosive	N/A
Deodorising ability	Should deodorise while disinfecting	High	High	None
Availability	Should be available in large quantities and reasonably priced	Low cost	Moderately high cost	Moderately high cost

Table 11 Comparison of the most commonly used disinfectants in water treatment

4.6 WATER PURIFICATION PROCEDURE

4.6.1 INTRODUCTION

Following a centrifugation step (always in the best conditions, already described in the first part to this T.N) the clarified liquid obtained will pass continuously, under pressure, through a membrane module.
If batch operation is desired, the retentate is recirculated through the module, and the permeate (filtrate) is removed continuously. In principle, as both cells and permeate are continually removed from the module, there is no accumulation of either within the filtration system, but produces an increase of solids concentration in the retentate stream.
Tests were conducted for microfiltration and ultrafiltration steps. For the time been no test has been conducted for a reverse osmosis step due to limited equipment availability, but specific objectives and discussion were already presented in section 4 and 4.3.2.
Each filtration unit (every filtration test was conducted separately one from another) worked several hours in order to characterise the membranes and the performance of the system in terms of quantity and quality and to validate the concept for a long duration testing, always based on permeate flux basis (J= flow rate/ area).

4.6.2 TANGENTIAL FILTRATION SYSTEM

To perform the ultrafiltration and microfiltration test specific equipment was selected due to some advantages. The equipment selected is from *Millipore* and the model is *Minitan* ^{TM}. This equipment allows tangential flux over ultrafiltration and microfiltration membranes. The exclusive design of the filtration plates produces a retentate flux that keeps an effect of "cleaning" over the membrane surface. With the aid of a peristaltic pump the feed to be processed (liquid/solid) is pumped from a non-pressurised vessel.
Technical data is presented as follows

Technical data

Membrane surface net	60 cm^2/plate
Number of plates	4
Dead volume spiral channel	< 50 ml
Membrane pore size	0.45 µm (Millipore HVLP OMP 04-Durapore) for microfiltration . 10.000 (Millipore PTGC OMP 04-Polisulfone) for ultrafiltration.

In order to conduct tangential microfiltration and ultrafiltration, four plates were used. Thus, the filtration area used was 240 cm^2 for all the test.

4.6.2.1 Microfiltration tests

Tests were conducted for an initial *Spirulina platensis* cell concentration of 0.5 g/l dry weight going into the centrifuge. The cell concentration obtained from the centrifuge filtrate was 0.006 g/l dry weight (filtrate 1). The total filtrate 1 treated volume was about 20L, corresponding to a bioreactor operation time of approximately 24h, considered as appropriated for cycle operating time in batch mode.

As operational variables the following ones were selected: inlet pressure (**P_e**), retentate flow rate (**Q_r**), clarified or filtrate flow rate (**Q_f**), retentate stream concentration (**C_r**), and clarified concentration (**C_f**) being all measured. The feed flow rate was fixed (**Q_e**) during all tests and so, there were no significant variations.

The system is not operating in steady state conditions of flow until approximately 15 min. That is why data was normally not recorded until almost stable values are reached.

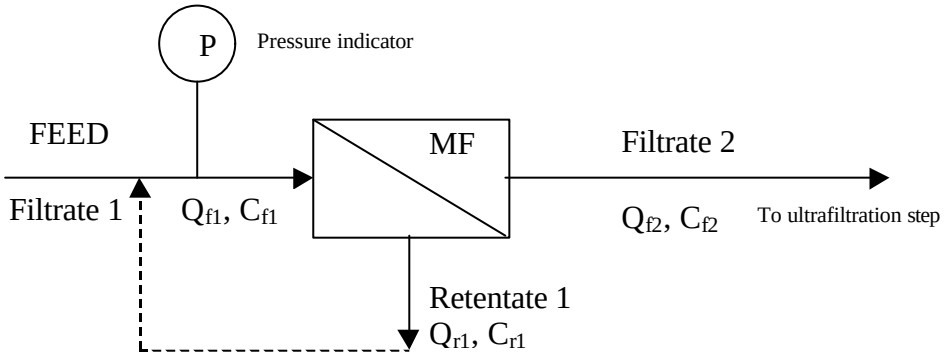


Figure 11 Microfiltration system

One of the most important parameters to follow to study the overall performance in the microfiltration system is the **Q_r/Q_f** ratio. This ratio shows the relation between both outlet flows. A ratio greater that 10 indicates that the system is working in good conditions because a good "cleaning" effect of the membrane surface is being produced by tangential filtration. To know whether the system works properly differences between **C_f** initial and final can be checked. A minimum **C_f** reduction of about 50% has been reached in this test. This reduction has been considered as a satisfactory result due to the very low concentration that represents the inlet **Cf_1** as well as the outlet **Cf_2**. At those ppm levels (µg/ml) common analytical methods -based on dry weight or absorbance-can not differentiate with enough precission between chemical solids (organic/inorganic) and microorganisms.

4.6.2.1.1 Results and discussion

Test was conducted during 5 hours uninterrupted. Every 15 min. $\mathbf{Q_f}$ and $\mathbf{Q_r}$ were measured and samples were collected to determine $\mathbf{C_r}$ and $\mathbf{C_f}$ concentrations.
From initial volume (20 litters) of filtrate 1, we obtained 10 litters of filtrate 2 and approximately 7L of retentate. During all test, the system runner successfully and operational time (5 hours) was considered adequate to confirm efficiency of the system.

Operating conditions are the following:

- Filtrate/retentate > 10
- Feed flow rate + Qr > 15 l/h
- Pressure < 1.4 bars
- Permeate flux rate > 60 l/h*m^2

Initial and final values for **Cf** and **Cr** are the following:

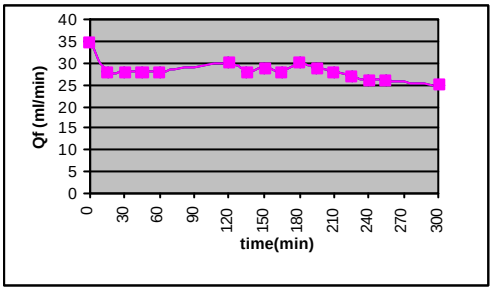
- Initial C_f = 9.9 mg/ml
- Final C_f = 4.5 mg/ml
- Final C_r = 13.1 mg/ml

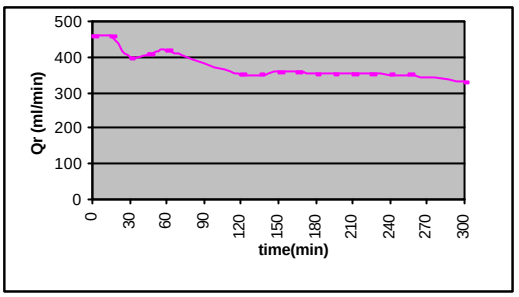
Obtained results are presented in Table12 and Figure 12.

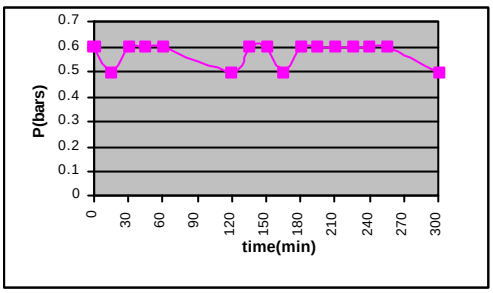
$\mathbf{P_e}$ increased and reached a steady value of about 0.6 bars after 30 min of operation.
During all test $\mathbf{Q_r}$ and $\mathbf{Q_f}$ decreased slowly. The $\mathbf{Q_r/Q_f}$ ratio was also decreasing during all test long The concentrations $\mathbf{C_r}$ and $\mathbf{C_f}$ were slightly variable during the test and $\mathbf{C_r}$ increased in the end of it.
Due to low cell concentration in the filtrate from centrifuge, measurements of cell concentration were made with the spectrophotometer measuring the absorbance at 750 nm.
Results were always < 0.009 g/l for C_r, and obviously lower in the filtrate stream (C_f).
The quotient between $\mathbf{Q_r}$ and $\mathbf{Q_f}$ always was kept above 10. This value increased at first and kept constant at the end.
The relation between $\mathbf{Q_f}$ and **A** (filtration area) decreased slightly during the test as expected.
Thus, obtained results demonstrated that microfiltration is a good alternative to reduce the remaining cells in the filtrate stream obtained from the centrifugation step.

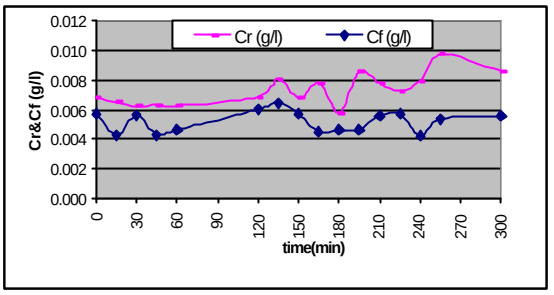
Time (min)	Q_f (ml/min)	Q_r (ml/min)	P (bars)	Abr	Abf	C_r (g/l)	C_f (g/l)	Q_r/Q_f	Q_f/A (l/h*m^2)
0	35	460	0.6	0.0115	0.0095	0.0069	0.0057	13.14	87.5
15	28	460	0.5	0.0109	0.0072	0.0065	0.0043	16.43	70.0
30	28	400	0.6	0.0104	0.0072	0.0062	0.0043	14.29	70.0
45	28	410	0.6	0.0106	0.0067	0.0063	0.0040	14.64	70.0
60	28	420	0.6	0.0105	0.0078	0.0063	0.0047	15.00	70.0
120	30	350	0.5	0.0115	0.0101	0.0069	0.0060	11.67	75.0
135	28	350	0.6	0.0135	0.0108	0.0081	0.0065	12.50	70.0
150	29	360	0.6	0.0113	0.0095	0.0068	0.0057	12.41	72.5
165	28	360	0.5	0.0131	0.0075	0.0078	0.0045	12.86	70.0
180	30	355	0.6	0.0097	0.0077	0.0079	0.0046	11.83	75.0
195	29	355	0.6	0.0144	0.0078	0.0086	0.0047	12.24	72.5
210	28	355	0.6	0.0130	0.0093	0.0078	0.0056	12.68	70.0
225	27	355	0.6	0.0121	0.0095	0.0072	0.0057	13.15	67.5
240	26	350	0.6	0.0133	0.0071	0.0080	0.0042	13.46	65.0
255	26	350	0.6	0.0163	0.0090	0.0097	0.0054	13.46	65.0
300	25	330	0.5	0.0144	0.0093	0.0086	0.0056	13.20	62.5

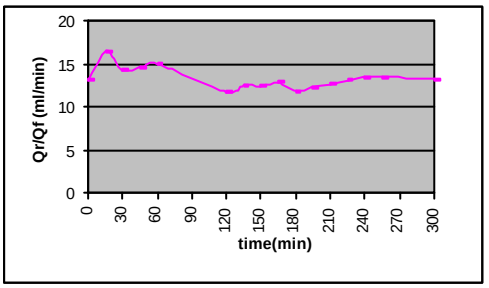
Table 12. Microfiltration test results











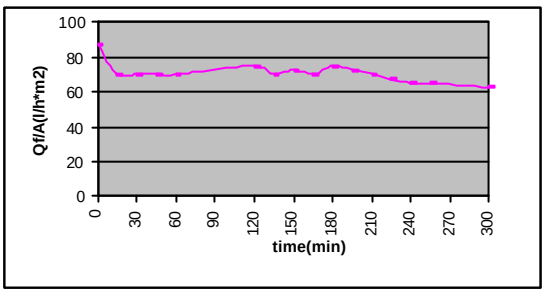


Figure 12 Microfiltration Test Plots

4.6.2.2 Ultrafiltration test

Water obtained in the microfiltration step, passed through an ultrafiltration module in order to retain the proteins and other possible macromolecules present in the medium.
Equipment used was the same that for microfiltration step but with adequate membranes. That is, membranes with porous size of 10.000 "cut-off"(polisulfone).
Approximate treated volume was 7L obtained from microfiltration step, and test was conducted for an initial protein concentration of 10 µg/ml. This value was a bit lower than the obtained from previous centrifugation tests, probably due to particular *Spirulina* bioreactor operational conditions or even eventual protein adsorption onto the microfiltration membranes previously used.

Carbohydrates were also measured to test whether membrane was efficient for carbohydrate removal.
Operational variables were the same as for microfiltration step. Measured variables were also $\mathbf{C_r}$ and $\mathbf{C_f}$ for the protein and carbohydrate concentration in both streams. The feed flow rate was also measured during the test.

The ultrafiltration system is schematised in the Figure13.

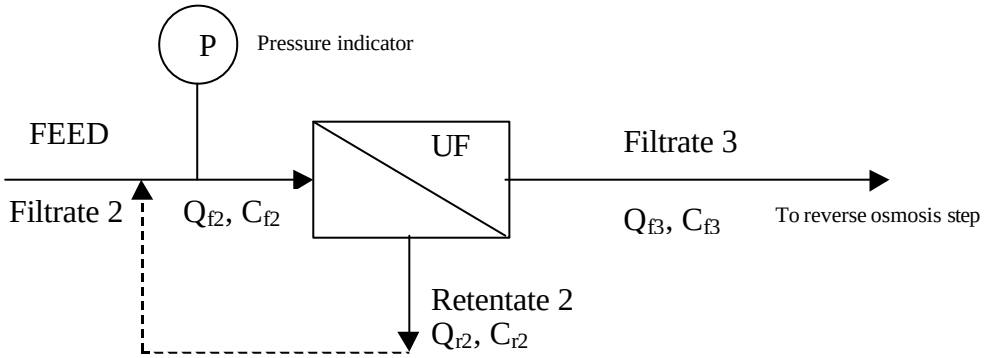


Figure 13 Ultrafiltration system

One of the most important parameters to follow in order to study the overall performance of the ultrafiltration system is the Q_r/Q_f ratio. This ratio may be used to optimise the process and the difference between the initial and final value of proteins and carbohydrates to check whether the test has been successfully conducted.

4.6.2.2.1 Results and discussion

Test was conducted during 5 hours uninterrupted. Every 15 min. $\mathbf{Q_f}$ and $\mathbf{Q_r}$ were measured and samples were collected to determinate proteins and carbohydrates concentrations.
Total ultrafiltrate volume was approximately 7L.

Operating conditions are the following:

Filtrate / retentate > 20
Feed flow rate (Q_{r2} + filtrate $_2$) > 15 l/h
Pressure < 1.4 bars
Permeate flux rate > 60l/h*m^2

Initial and final values for proteins and carbohydrates are the following:

Initial protein concentration: 10µg/ml
Final protein concentration: 5 µg /ml
Initial carbohydrates concentration: 38 µg /m l
Final carbohydrates concentration: 34 µg / ml

Obtained results are presented in Table13 and shown in Figure 14.

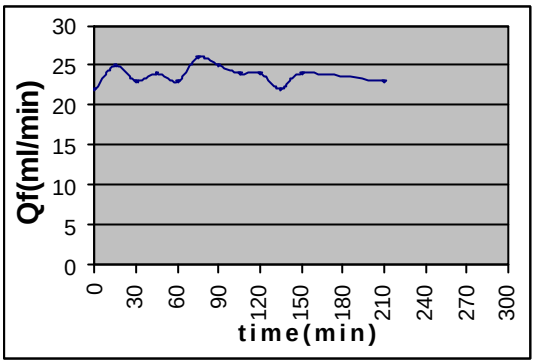
$\mathbf{P_e}$ reached a constant value of about 1.3 bars after 15 min of operation
$\mathbf{Q_r}$ and $\mathbf{Q_f}$ decreased slowly but not a lot. $\mathbf{Q_r}$ / $\mathbf{Q_f}$ relationship is higher than for microfiltration in order to increased the separation efficiency. This ratio decreased considerably at the end of process.
Protein concentration increased in the retentate steam and decreased in the filtrate steam, and initial value in the filtrate steam is higher than final value.
There are differences between carbohydrate concentration in the filtrate and in the retentate, although initial and final values are not very different.

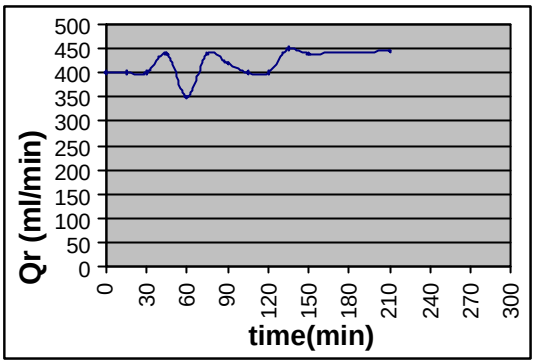
So, ultrafiltration test is effective to retain proteins but it does not seem to be effective with carbohydrates, maybe because molecular size is smaller than supposed due to partial molecule breakage.
Anyway, a protein concentration about 10-20 µg/ml is not high enough to justify the extensive use of any separation method, only if some toxic or pathogenic effects are likely to happen. If an additional reduction on both protein and carbohydrates was desired, an adsorption process with activated carbon could be suitable as previously commented.

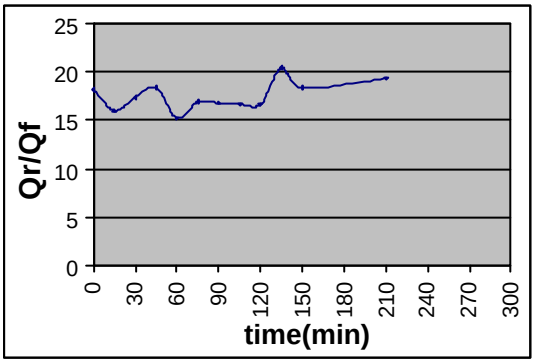
Time (min)	Q_f (ml/min)	Q_r (ml/min)	P (bars)	Q_r/Q_f	Q_f/A (l/h.m^2)	Prot.f (mg/ml)	Prot.r (mg/ml)	Carb.f (mg/ml)	Carb.r (mg/ml)
0	22	400	1.3	18.18	55.0	8.2	10.2	37.5	38.3
15	25	400	1.3	16.00	62.5	8.6	12.1	28.6	50.6
30	23	400	1.3	17.39	57.5	8.5	n.d.	38.8	n.d.
45	24	440	1.3	18.33	60.0	11.3	12.2	38.1	51.3
60	23	350	1.3	15.22	57.5	8.3	10.1	40.0	40.4
75	26	440	1.3	16.92	65.0	10.1	12.0	34.8	35.4
90	25	420	1.3	16.80	62.5	10.2	10.3	32.8	37.6
105	24	400	1.3	16.67	60.0	n.d.	11.0	n.d.	44.6
120	24	400	1.3	16.67	60.0	7.1	11.6	31.1	39.0
135	22	450	1.3	20.45	55.0	8.1	13.1	30.1	39.9
150	24	440	1.4	18.33	60.0	7.7	12.1	30.4	49.2
210	23	445	1.3	19.35	57.5	8.5	17.6	40.1	58.5
300	n.d.	n.d.	n.d.	n.d.	n.d.	5.9	13.8	35.0	49.5

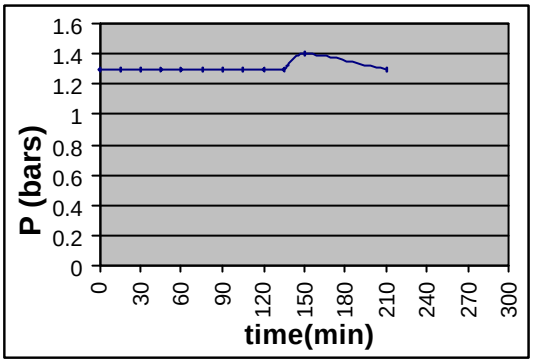
Table 13 Ultrafiltration Test Results

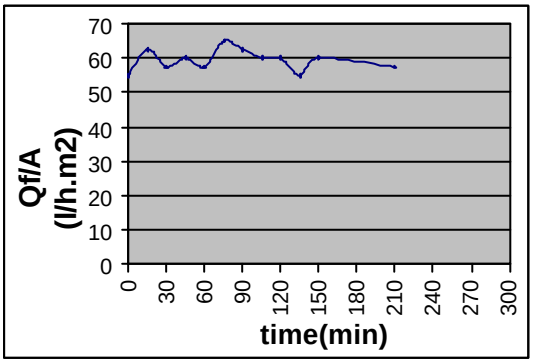
n.d. not determined.


Qf(ml/min)
0
5
10
15
20
25
30
0
30
60
90
120
150
180
210
240
270
300
time(min)


Qr (ml/min)
0
50
100
150
200
250
300
350
400
450
500
0
30
60
90
120
150
180
210
240
270
300
time(min)


Qr/Qf
0
5
10
15
20
25
0
30
60
90
120
150
180
210
240
270
300
time(min)


P (bars)
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
0
30
60
90
120
150
180
210
240
270
300
time(min)


Qf/A (l/h.m2)
0
10
20
30
40
50
60
70
0
30
60
90
120
150
180
210
240
270
300
time(min)

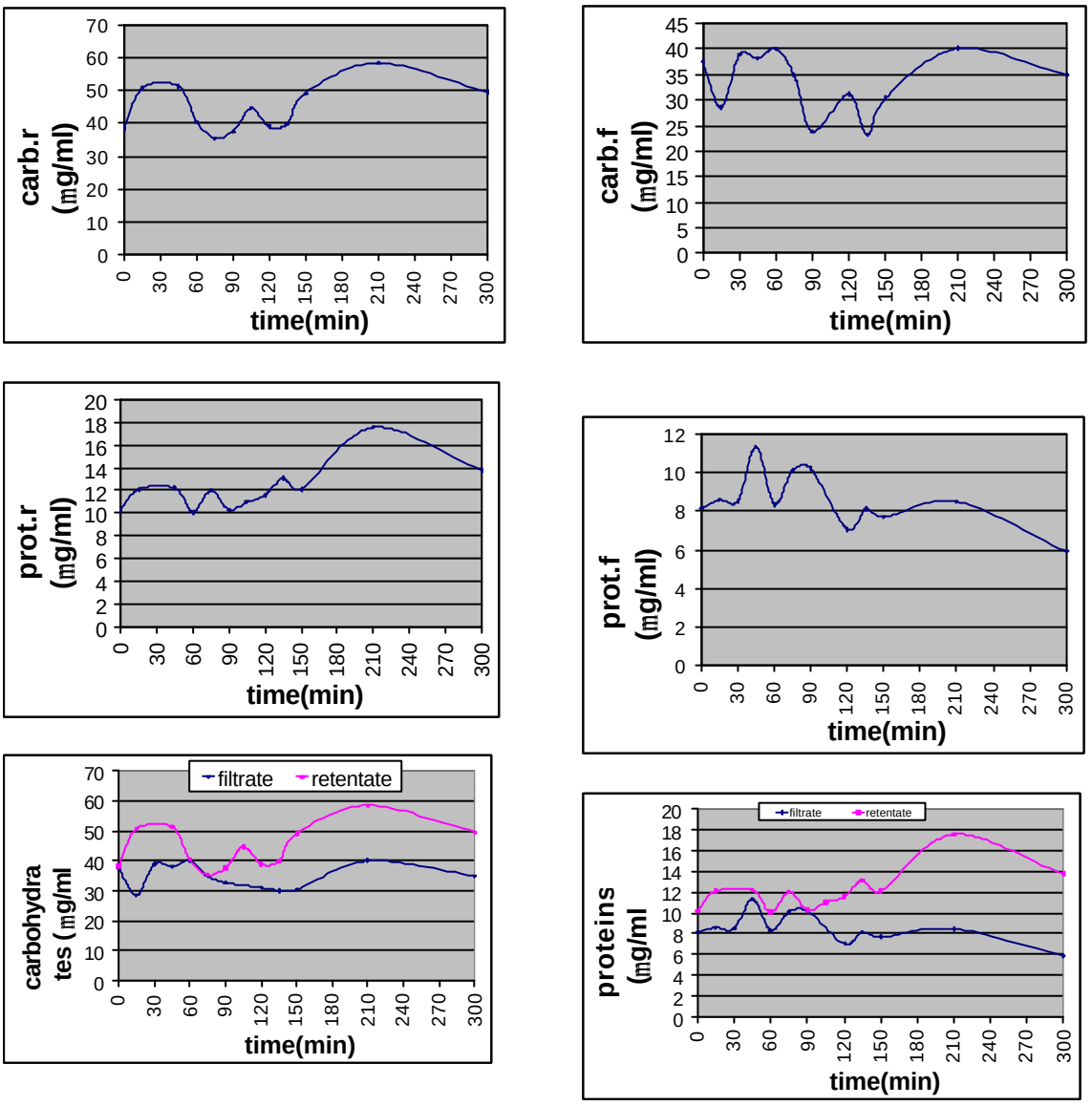


Figure 14 Ultrafiltration Test Results Plots

4.6.2.3 Conclusions to microfiltration and ultrafiltration steps

Both steps, accomplish the objectives proposed in the beginning and their efficiency has been demonstrated for water purification step.

Volume or amount of water that can be treated in both steps, will depend always on the relation between:

$$\frac{Qf}{A}$$

Where Q_f is the filtrate liquid flow (l/h) and A is the total membrane area in m^2 (that is 60 cm^2 to each plate, in this case it was used 4 plates in the microfiltration step and 4 plates in the ultrafiltration step).
Result is expressed in $l/h*m^2$ and this value come up to the process efficiency: about 60 $l/h*m^2$ (see the Table 12 and Table 13) in stationary state conditions.

The value of this quotient will not depend on the used area, but depends on the membrane retention capability. If we considered alternate cycles of operation/ washing, the previous relation can be expressed as:

$$\left(\frac{Q_f}{A}\right) * t * A_t = \text{TOTAL VOLUME} = V$$

Where t is the operation time and A_t is the total area to be used.

According this, capacity and efficiency of the system will be defined by this equation, so, modifying this parameters, we can obtained the changes desired in the system. For instance to know what the operation time will need to be to treat a total volume of 20 litters corresponding to the expected production volume from compartment IV, the previous equation can be arranged as follows:

$$t = \frac{V}{\left(\frac{Q_f}{A}\right) * A_t}$$

Thus, (Q_f/A) is always kept constant as experimentally determined for the studied configuration and A_t can be changed to obtain different operating times by only changing the number of membrane plates used.

In both cases (MF and UF) , (Q_f/A) was higher than $(60 l/h*m^2)$ which allows to process 20L of wastewater in approximately cycles of 12 hours, using 4 membranes plates of 60 cm^2 each one.

4.6.3 WATER ANALYSIS RESULTS AND DISCUSSION

According to the proposed general scheme for water treatment, after the ultrafiltration step, water should pass through an osmosis reverse step in order to eliminate salts.

This possibility was not tested in this TN, but mineral compounds in water were analysed after the ultrafiltration step in order to state both water quality prior reverse osmosis step and to determine specific needs to reach standards for the crew compartment supply as well as for the higher plant compartment.

To test the efficiency of each step, three samples were collected: one sample in the beginning of the ultrafiltration process (filtrate from microfiltration step), a second sample in the middle of the UF process (filtrate from ultrafiltration step) and a third sample at the end of the ultrafiltration process. In this way, possible adsorption or residual elimination could be detected.

4.6.3.1 Results:

Analyses of principals elements were obtained from the "Chemical Analysis Service" in the U.A.B, For parameters such as pH and conductivity, direct measures were made at the end of each step.

Analyses results of water after the purification process versus standard values for each parameter for both crew compartment and higher plants are presented in the Table 14.

Parameters /Element	Results Sample 1	Results Sample 2	Results Sample 3	Drinking water ESA standard	Higher plants
pH	8.56	8.99	-	6.5-8.5	5.5-6.5
Conductivity	5.66 mS/cm	6.01 mS/cm	-	0.75 mS/cm	-
TOC	70.93 ppm	30.74 ppm	-	0.5 ppm	-
TIC	48.72 ppm	46.17 ppm	-	-	-
F^-	0	0	-	1 ppm	-
Cl^-	348 ppm	369 ppm	-	200 ppm	100 ppm
NO_3^-	203 ppm	216 ppm		25 ppm	15000 ppm (NO_3, NH_4^+)
PO_4^{3-}	0	0	-	5 ppm	2000 ppm (HPO_4^{2}-)
SO_4^{2-}	434 ppm	471 ppm	-	250 ppm	1000 ppm
Na^+	1.2 g/l	1.1 g/l	1.1 g/l	150 ppm	-
K^+	0.7 g/l	0.6 g/l	0.7 g/l	12 ppm	10000 ppm
NH_4^+	0	0	0	0.5 ppm	-
Fe^{3+}	410 ppb	367 ppb	324 ppb	-	100 ppm
Mn^{2+}	563 ppb	433 ppb	375 ppb	-	50 ppm
Zn^{2+}	321 ppb	214 ppb	-	-	20 ppm
Cu^{2+}	104 ppb	67 ppb	78 ppb	-	6 ppm
Mo	33 ppb	31 ppb	33 ppb	-	0.1 ppm
Ni^{2+}	41 ppb	21 ppb	13 ppb	-	-
Ca^{2+}	18 ppm	14 ppm	10 ppm	-	5000 ppm
Mg^{2+}	24 ppm	20 ppm	25 ppm	-	2000 ppm
Cr^{3+}	60 ppb	16 ppb	2 ppb	-	-
Co^{2+}	7 ppb	6 ppb	6 ppb	-	-
Pb^{2+}	12 ppb	8 ppb	9 ppb	-	-

Table 14. Final water Analysis Results

Where:
Sample 1: collected in the beginnig of the ultrafiltration step
Sample 2: collected at the end of the ultrafiltration step.
Sample 3: collected in the middle of the ultrafiltration process.

4.6.3.2 Discussion

According to those results, it can be deduced that in the general scheme for water purification the followings factors have to be taken into account, as was advanced in the beginning of this document.

- The obtained value for the pH is above the permissive value for drinking water and higher plants so, after the stabilisation step (where pH decrease) a neutralisation step will be necessary, and afterwards adjusted until desired value for higher plants compartment (with acid or alkali addition).

- In order to minimise the carbonate hardness, (obtained values for TIC are high, due the carbonate presence in the culture medium), a softening step will be necessary. Ions like calcium and magnesium are present in small quantities and will not be a problem by themselves.

- A reverse osmosis step will be necessary in both cases (crew and plant compartment) due to the high values obtained for some parameters, principally those related to drinking water, such as conductivity, TIC, TOC, Cl^-, NO_3^-, SO_4^{2-}, Na^+ and K^+. Special attention has to be paid to Na^+ and K^+. These two ion concentrations are so high that and additional ionic-exchange step is recommended to reduce its content, depending on the RO efficiency. TOC values can be reduced before the reverse osmosis step, by adsorption with activate carbon. Micronutrients, which are necessaries for higher plant, are found in small quantities before the osmosis step, so after reverse osmosis step their concentration will be even lower and an additional remineralisation step will be necessary. This remineralisation can be made using either the "optional" stream drawn in the figure 3 in this TN or a partial bypass to the RO.

- Some toxic metals have been found in very small quantities, but their presence might produce some problems, principally those related to Pb^{2+}. This element was not added in the culture medium but it is present in the obtained water. It is likely that some reagents used in the culture medium contain heavy metals such as a Pb^{2+} in terms of ppb/ppm. Even so, heavy metals concentrations are far away from admissible limits, and an additional adsorption or ionic exchange process is advised.

Therefore, and taken into account all this remarks, final scheme of water purification for higher plants and drinking water are presented in Figure15 and Figure16 .

4.7 FINAL CONCLUSIONS

Even though microfiltration and ultrafiltration steps are effective in the whole water purification process, analyses results confirm that an osmosis reverse step is still necessary, principally to obtain drinkable water.

In order to obtain desired concentration of macronutrients and micronutrients and acceptable pH, an on line monitoring of these parameters is recommended.

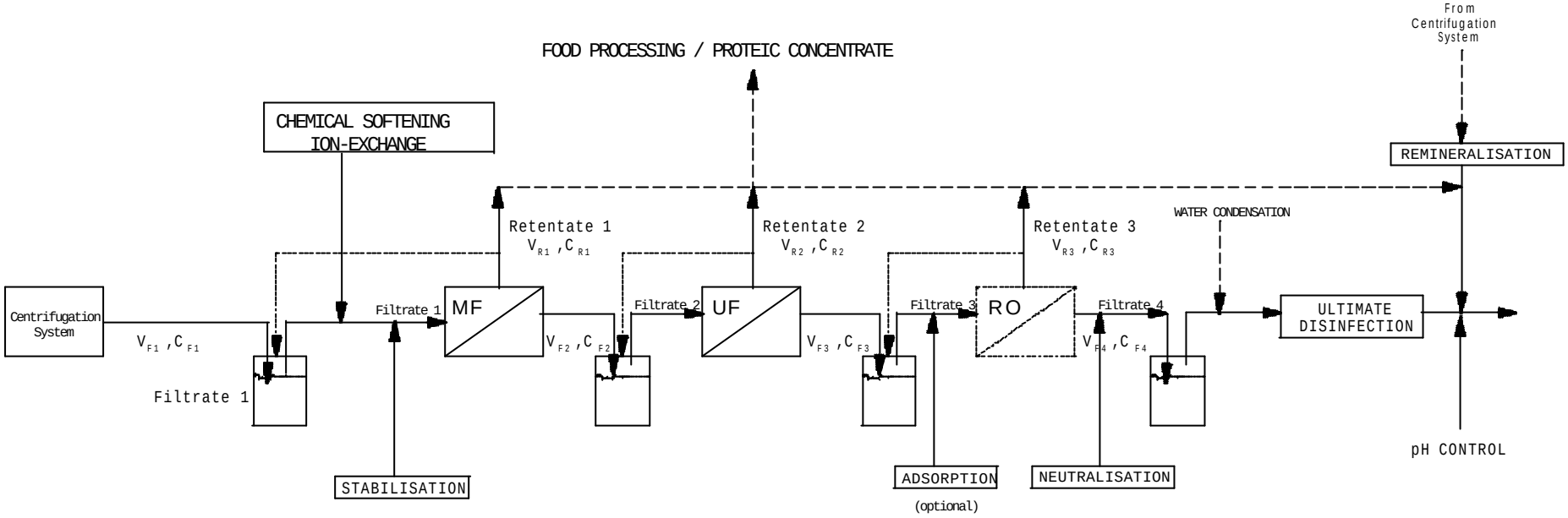


Figure 15. Water recycling scheme for the higher plants

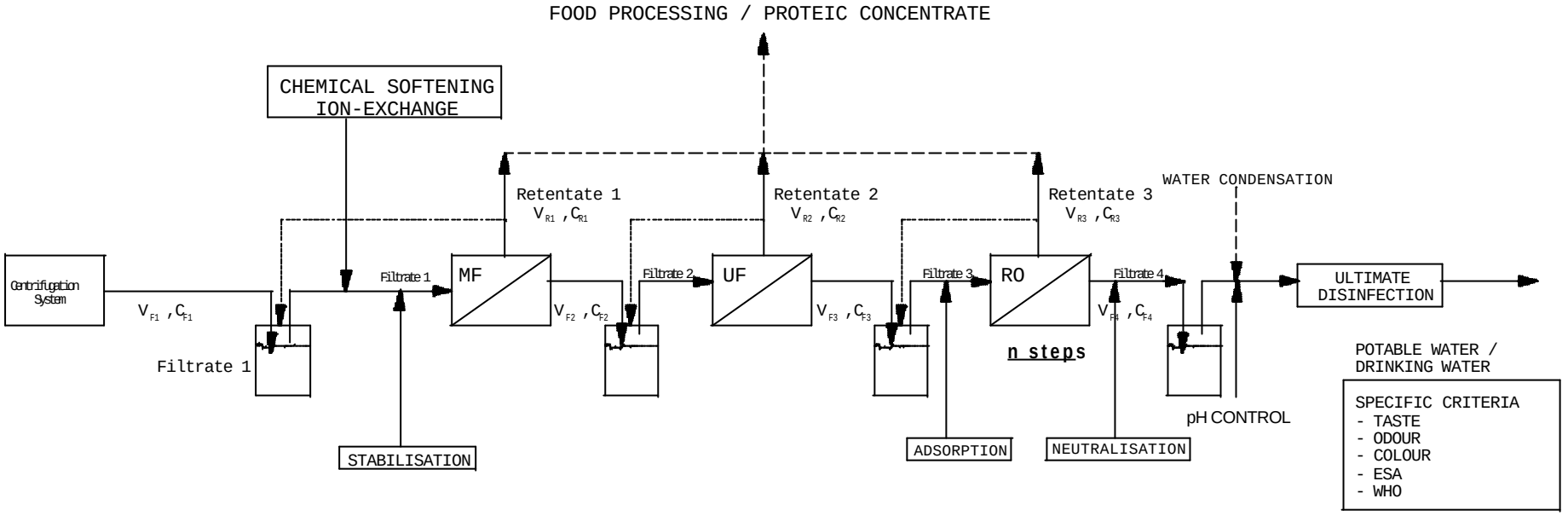


Figure 16. Water recycling scheme for crew compartment

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ANNEXES