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Tests results evaluation

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LIST OF ABBREVIATIONS

AA: Acetate

BA: Butyrate

CA: Caproate

COD: Chemical Oxygen Demand

DM: Dry Matter

EC: Electroconductivity

FU: Filtration Unit

GL : Gas Loop

IBA: Isobutyrate

ICA: Isocaproate

IVA: Isovalerate

OM : Organic Matter

PA: Propionate

VA: Valerate

VFA : Volatile Fatty Acids

1 Introduction

The liquefying compartment of the MELiSSA loop is responsible for the biodegradation of human faecal material and other wastes (inedible parts of plant material) generated by the crew. The volatile fatty acids and ammonia produced during the anaerobic fermentation process are fed to the second photoheterotrophic compartment inoculated with the bacterium *Rhodospirillum rubrum*. The produced CO_2 is supplied to the photoautotrophic compartment inoculated with the algal strain *Arthrospira platensis* and to the higher plants compartment.

At the pilot plant of the University of Barcelona, the three compartments of the MELiSSA loop (photoheterotrophic compartment CII, nitrifying compartment CIII and photoautotrophic compartment CIVa) are already connected at lab scale and will be validated at pilot scale. In order to validate the whole MELiSSA loop, it is necessary to construct the first compartment at pilot scale (fermentation reactor) for the primary degradation of the waste produced by the crew.

However, between lab and pilot scales the construction of an intermediate prototype reactor represents an important step to evaluate and improve the theoretical concepts.

Once the prototype Waste Compartment is assembled, it is necessary to perform functional tests to evaluate the hardware and the process. A first series is performed to test individually the sensors, actuators and other instruments and equipment. After these functional tests the reactor is filled with the Melissa inoculum and fed with representative substrate. The next series of tests are performed for a period of four months.

This technical note presents the evaluation of the results of these functional tests. It includes moreover propositions for optimization.

2 Hardware tests evaluation

2.1 Bioreactor

2.1.1 General evaluation and propositions for optimization

Several comments could be made regarding the prototype bioreactor. Table 1 presents these remarks and some propositions for optimization, that were already carried out on the prototype or that will be included in the final design of the pilot.

Table 1. General evaluation of bioreactor hardware

Item	Remark	Proposition for optimisation	Tested on prototype & comments
Liquid port at bottom of the reactor	Frequent clogging	Add liquid port on side of reactor	Yes: OK
Connection to gas loop	Under liquid level: entrance of liquid in gas loop when volume fluctuates	Pay attention to place gas port higher than liquid with a sufficient safety range (N2 connection can be under liquid level)	No
pH probe	Unstable measurement (interference due to current)	Install probe proven to work	Yes: OK
pH control	Can initiate addition of lethal amount of base/acid in case of probe failure	Add a safety to stop addition after a certain time	Yes: OK
Level measurement	Fluctuates with pressure variation	Install capacitance level sensor independent of pressure variations or pressure sensors measuring negative pressures	No
Level measurement	The liquid volume can get too high in case of non-optimal operation: causes entrance of liquid inside the gas loop	Add safety to stop the influent feeding when the level gets too high	Yes: but not optimal due to uncertainties on level measurement
Influent port	Clogging	Ports must be big enough	Yes: diameter of 20mm tested and selected
Feeding pump	Clogging in the feeding loop	Install centrifugal pump of bigger capacity and circulate influent continuously through a separate loop	Yes: OK
Feeding pump	Clogging after 2-3 months	Pump tubing must be greased and replaced	Yes: but not optimal; tube size after pump

		regularly + plan regular pump cleaning	shall be reduced as much as possible
Tubes	Occasional clogging of the tube between bioreactor and V-F-001; cleaning difficult (presence of bends), unhandy and unsafe (contact with faecal material)	Always provide valves in tubes between bends; allow easy flushing of tubes with water or pressurized air (3-way-valves)	No

2.1.2 General conclusions

The bioreactor hardware satisfied its objectives. Propositions for optimization were tested and integrated to the pilot final design.

2.2 Gas Loop

2.2.1 General evaluation and propositions for optimization

The same approach was used to evaluate the gas loop hardware. Table 2 presents the remarks and some propositions for optimization, that were already carried out on the prototype or that will be included in the final design of the pilot.

Table 2. General evaluation of gas loop hardware

Sub system	Item	Problem / Remark	Proposition for optimisation	Tested on prototype
GL	Automatic liquid drain	Clogging: condensate accumulates in pressurized vessel	Use of a small pump	Yes: OK
GL	Valves	Impossibility to close independently every part of the loop to work on it	Add valves at each port of the bioreactor and between sensors and instruments	Yes: OK
GL	Pressurized buffer tank	Too big volume: too long time required to fill it with biogas, inaccurate gas production measurement	Minimize size of buffer tank or add system to measure gas production based on pressure increase (size must be big enough to provide gas to the bioreactor when its pressure decreases)	No
GL	Sampling port	Condensation: problems for gas measurements	Put sampling port after cooler, at least higher than the bioreactor	Yes: OK
GL	Gas analyser	Gets possibly high pressure and humidity when failure of compressor and gas backflow	Install a valve breaking in case of overpressure before and after analyser (perfect closure)	Yes: OK
GL	Gas analyser	Condensation can damage the analyser	Install safety filter at input of the gas analyser	Yes: OK
GL	Tubes, connections	Gas leaks	Install high quality plastic tubes and swagelok valves	No
GL	Configuration	Not clear	Prepare precise scheme of disposition before building and indicate tubes going under the board by drawn lines	No

2.2.2 General conclusions

The gas loop hardware satisfied most of its objectives. However the possibility of gas leakage was incompatible with an optimal running of process tests. Propositions for optimization were tested and integrated to the pilot final design. Particularly, the gas analyzer must be carefully protected from overpressure and condensation, which can be done by integrating humidity filters and safety pressure valves in the loop. This final design will thus be validated on pilot scale.

2.3 Filtration Unit

2.3.1 General evaluation and propositions for optimization

The same approach was used to evaluate the filtration unit hardware. Table 3 presents the remarks and some propositions for optimization, that were already carried out on the prototype or that will be included in the final design of the pilot.

Table 3. General evaluation of filtration unit hardware

Sub system	Item	Problem / Remark	Proposition for optimisation	Tested on prototype
FU	Tube for recirculation back to bioreactor	Risk of overflow in the buffer tank	Add intermediate non-return valve or place back liquid port higher than liquid level	Yes: OK
FU	Flow meter	Can give wrong high flow measurement when filled with air because of its location (higher than the pump)	Avoid air entrance and place flow meter before the pump at the same level	No
FU	Electrical cupboard	Hazardous due to its location under the buffer tank	Install electrical cupboard in a place protected from liquid projections	No
FU	Pressurizing valve	Clogging caused by too small diameter	Install other type of progressive valve with bigger diameter (system can work with pump flow variations but capacity is limited) or work with other strategy	Yes: OK
FU	Valve releasing filtrate	Occasional failure	Use more reliable valve and limit the time for filtration line in the software	No
FU	Pump	Noise due to non adapted stator/rotor size to temperature >30°C	Under size the stator	Yes: but still not optimal, stator should still be smaller
FU	Pump	Frequent electrical failure caused by short cut between wires in the motor	Protect carefully all electrical wires during installation	Yes: OK
FU	Pump	Must not run dry: impossible to empty completely FU and loss of sludge	Reduce the liquid volume in the unit in the design and reduce the dead volume	No
FU	Pump & pressure measurement	Pump type and capacity induces important pressure variations	Select other type of pump or add expansion for	Yes: OK (expansion installed and tested)

		(disturbing filtration process)	pressure stabilisation	
FU	Homogenisation with reactor	Cannot be controlled --> risk of emptying bioreactor or buffer tank	Add software controlled valve at back way junction between buffer tank and bioreactor or suppress buffer tank	Yes (by-pass buffer tank): OK
FU	Buffer tank	Not gas leak proof: problems of gas exchanges with bioreactor	Make it proof; install a by-pass to eliminate buffer tank during filtration	Yes: OK
FU	Buffer tank	Various problems (pressures, filling by gravity...)	Add a possibility for by-pass, and prevent using gravity force in the system	Yes: OK
FU	Cleaning	Absence of a port to fill with cleaning agent	Add clamp on the loop. Integrate a cleaning loop in the design	No

2.3.2 Operational aspects

Initially, a pressure control valve (valve V-F-006) was used to control the TMP (trans membrane pressure). Due to the nature of the feed solution, the valve did not work properly. The retentate contains indeed particles of vegetables that can be bigger (up to 2 mm) than the maximal opening of the valve. This caused frequent clogging of the valve while this was not wanted, consequently the control valve was removed form the filtration unit. All the experiments were therefore performed without the control valve. This means that the only parameter that can be used to regulate the TMP is the pump frequency. Other possibilities such as using a membrane valve were investigated. The pump frequency was used to regulate the TMP during the total test period.

In all experiments, the TMP (average TMP) was fixed at 0.4 bar. The TMP was regulated by the frequency of the pump. The flux is variable and the decrease in flux is an indication of membrane fouling.

Start up period (from 06/02/2004 to 01/03/2004) :

Figure 1,Figure 2 and Figure 3 show respectively the flux, TMP and cross flow velocity of the membrane filtration unit, starting with a new membrane on day 0. The gaps present on the figures correspond to stopping of the unit due to operational problems.

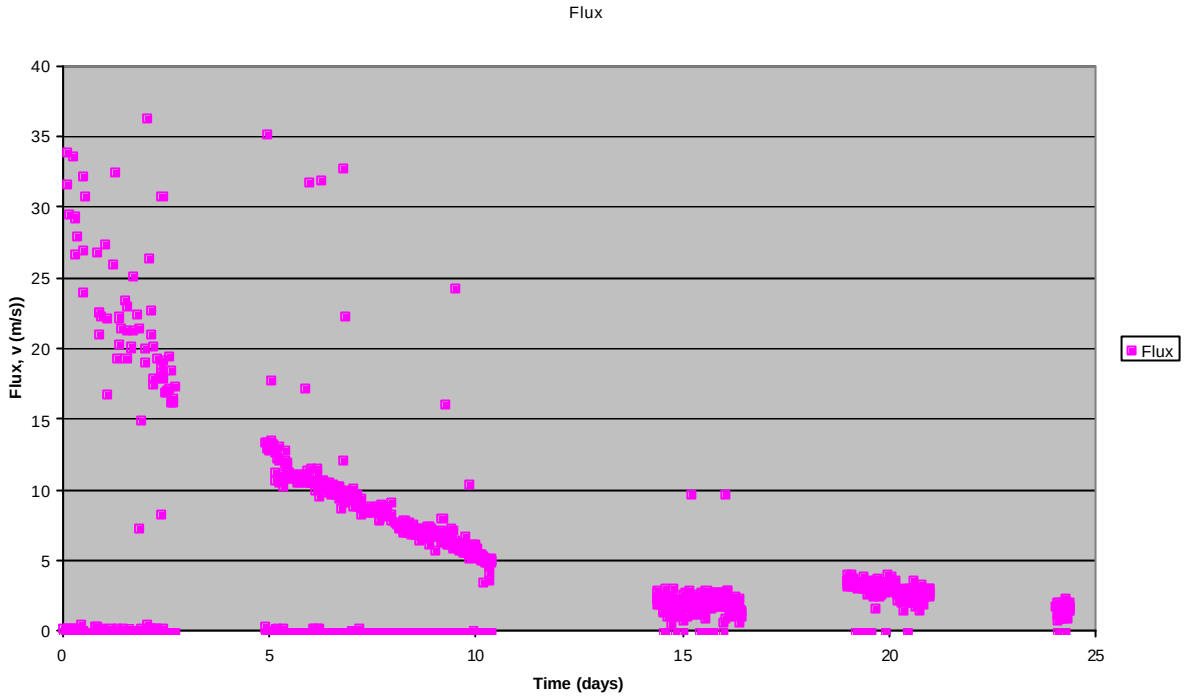


Figure 1 Flux during start up period

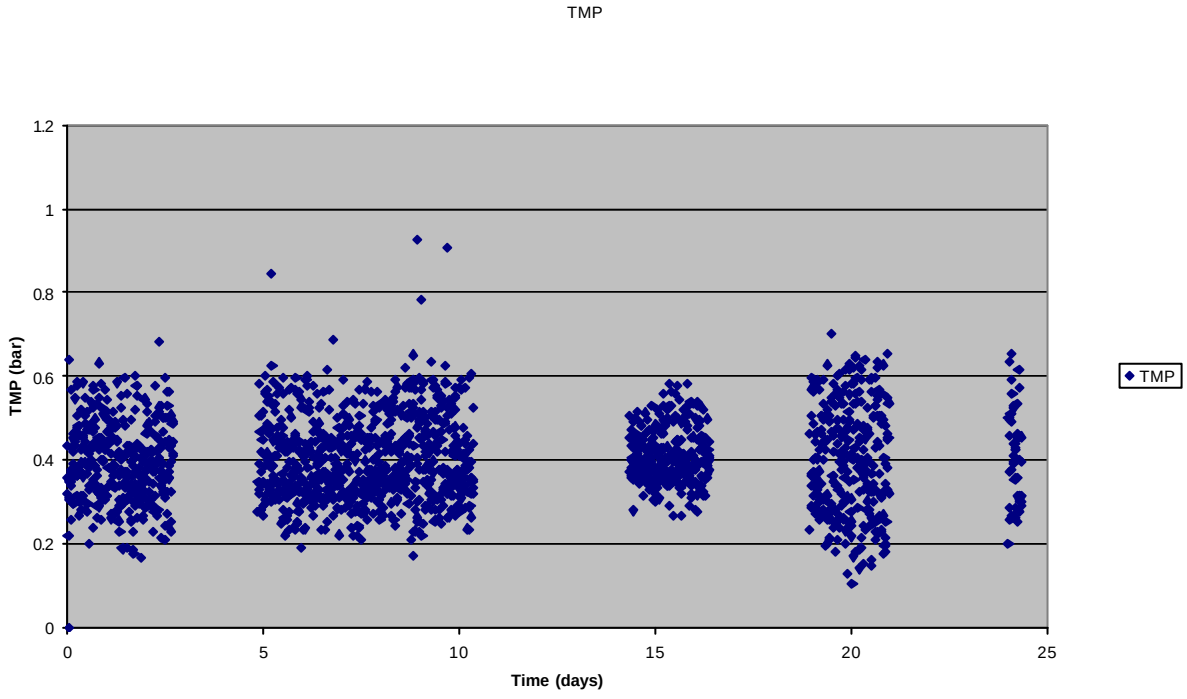


Figure 2 TMP during start up period

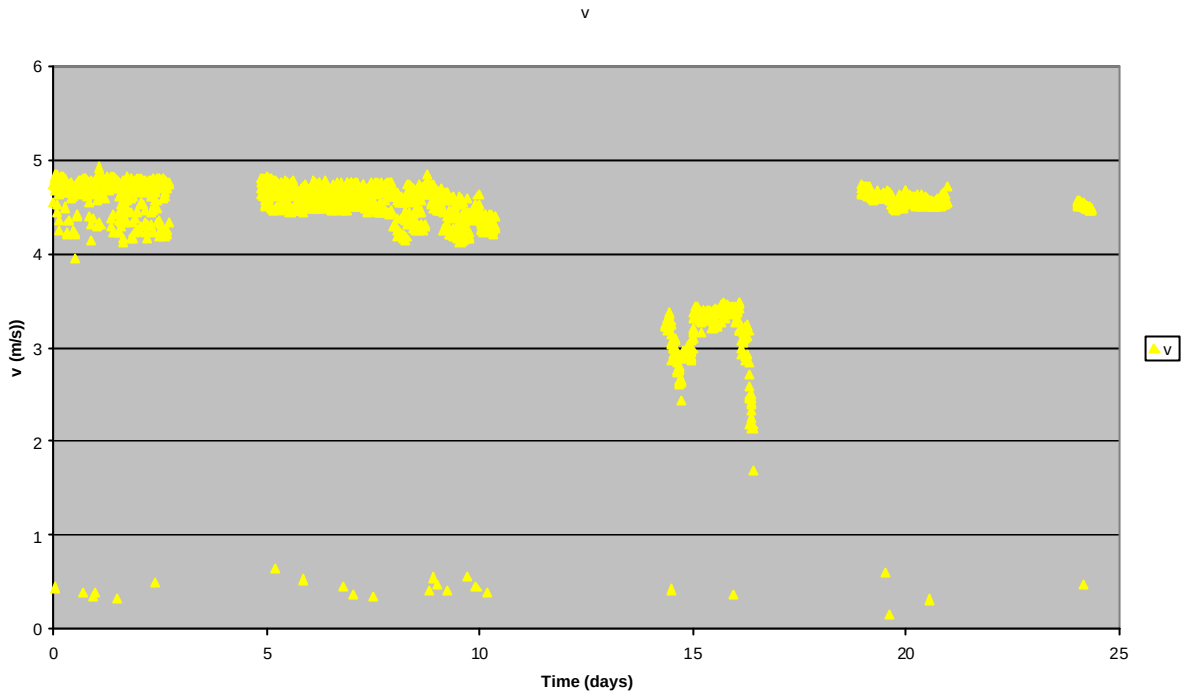


Figure 3 cross-flow velocity during start up period

The membrane flux decreases from 35 tot 3 l/hm^2 in 15 days, which shows that the membrane is dramatically fouled.

Test period (from 15/03/2004 to 20/07/2004):

The test period was started with a new membrane (membrane 1). Figure 4, Figure 5 and Figure 6 respectively show the flux, TMP and cross-flow velocity during this period. The TMP was set at 0.4 bar (average TMP). The TMP is controlled by the speed of the pump. Around day 100, the TMP was set at 0.6 bar in order to see its impact on the flux evolution.

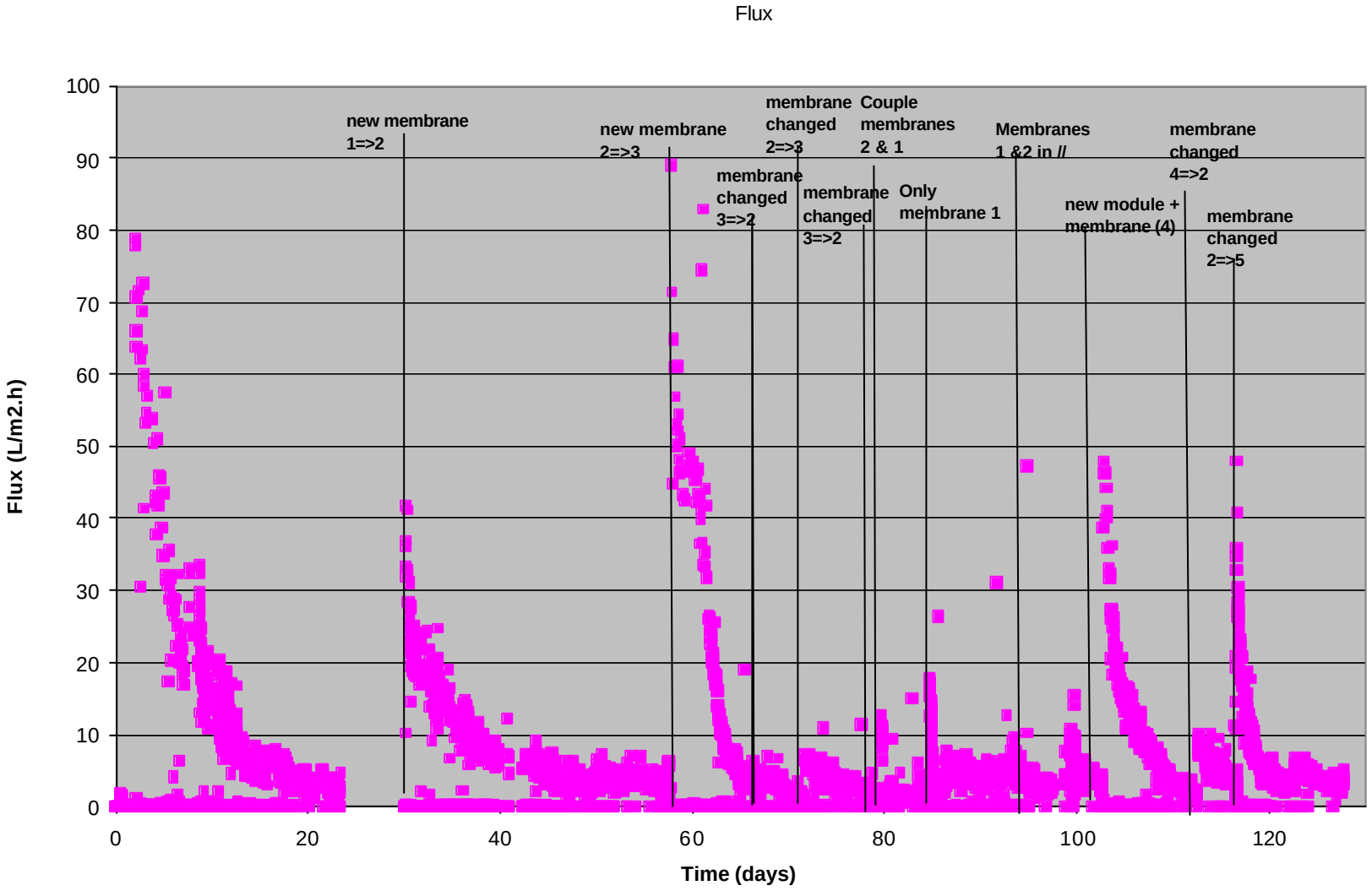


Figure 4 flux during test period

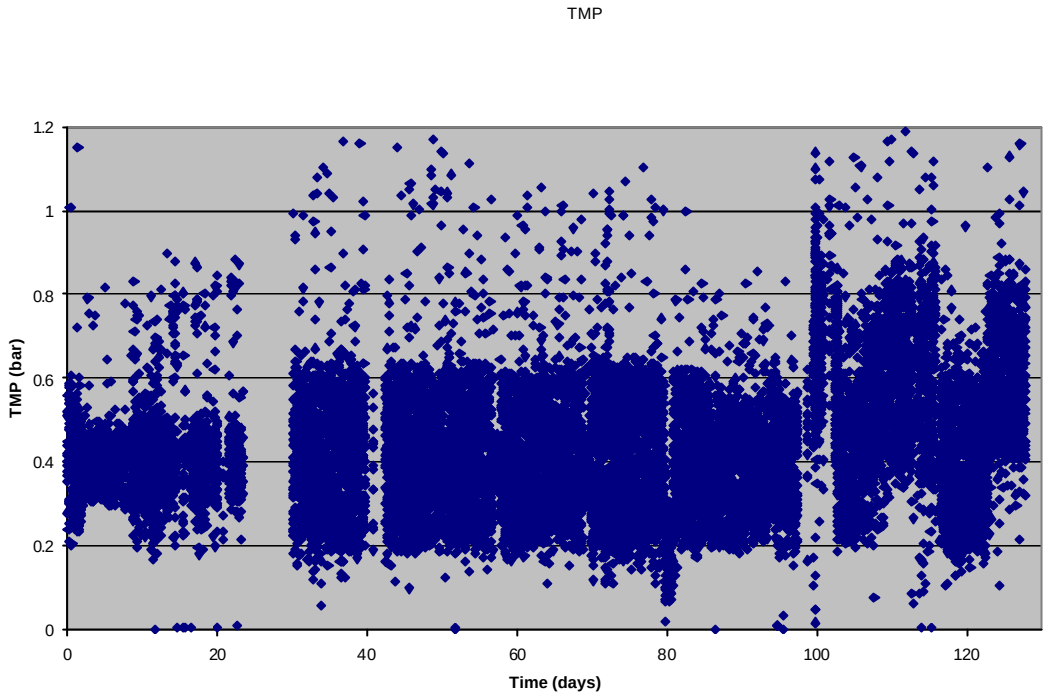


Figure 5 TMP during test period

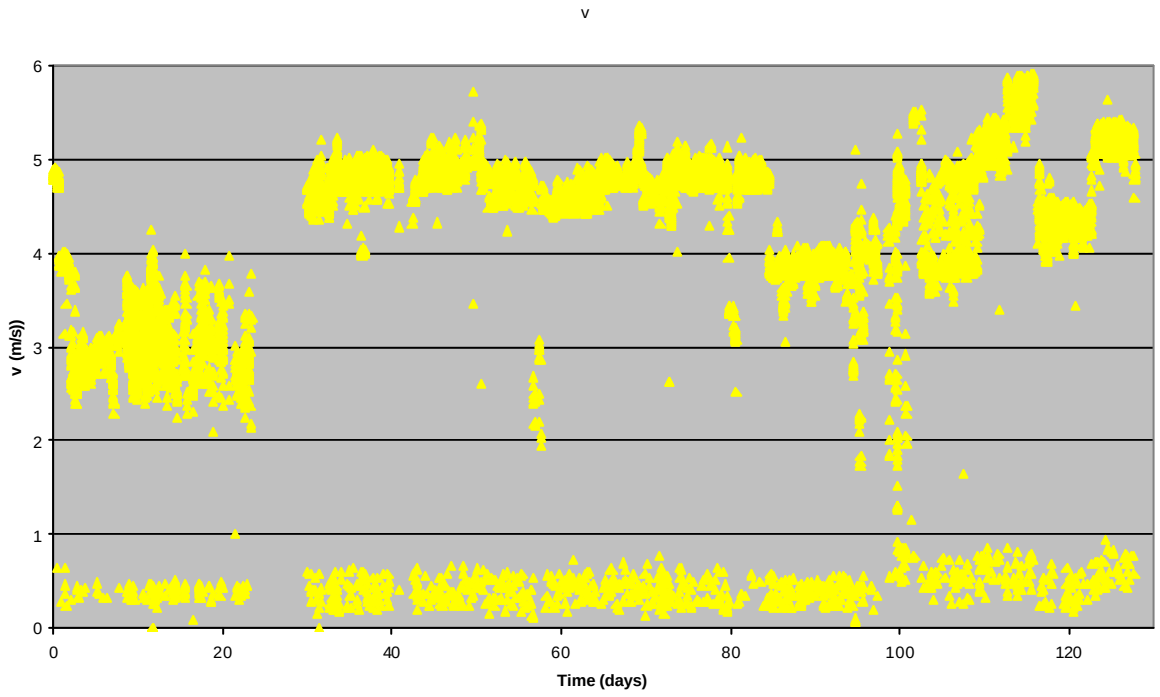


Figure 6 cross-flow velocity during test period

Table 4 gives an overview of the membrane history and the course of the experiments during the test period.

Table 4 Membrane history

Membrane					
	large module			small module	
Time (d)	1	2	3	a	b
0	Installed on filtration unit (FU)				
29	Removed from FU (flux <2L/h.m^2)	Installed on FU			
56		Removed from FU (flux<2L/h.m^2) Rinsed 2 days in NaOCl 250ppm Kept in water	Installed on FU		
64		Installed on FU (initial flux =5L/h.m^2)	Removed from FU (flux=8L/h.m2)		
			Rinsed 2 days in NaOCl 250ppm		
			Kept in water		
71		Removed from FU (flux = 2L/h.m^2)	Installed on FU (initial flux = 5L/h.m^2)		
		Rinsed 1 night in NaOCl 250ppm			
		Kept in water			
78		Installed on FU (initial flux = 5L/h.m^2)	Removed from FU (flux = 2L/h.m^2)		
			Rinsed 1 night in NaOCl 250ppm		
			Kept in water		
79	membrane 1 coupled with membrane 2 in parallel				
85	membrane was clogged=> rinsed with water	cleaning with BWTU procedure => no effect			
	FU runs with only membrane 1				
92	cleaning with BWTU procedure		used at VITO for test of different cleaning procedures		
	(flux: 5 =>13L/h.m^2)				
93	flux back to 5L/h.m^2	cleaning with BWTU procedure => no effect			
	test by pass FU				
93 ->99	Test 2 membranes in parallel with manual valve to increase TMP				

100	Removed from FU	[redacted]		new membrane installed in smaller module (smaller volume at permeate side to decrease bacterial regrowth on permeate side)	
112	[redacted]	tried again: flux increased, around 7; during 3d	[redacted]	cleaning with BWTU procedure	[redacted]
116	[redacted]	flux<3L/h.m^2 =>stopped	[redacted]	test back but flux<3 in a few hours	
	[redacted]		[redacted]	removed from FU	Installed on FU

The following cleaning procedures were tested at Epas during the testing period (see table 1) :

- Cleaning with 250 ppm NaOCl for 2 days :
  - rinse with tap water
  - immerse the membrane in NaOCl (250 ppm) for 2 days
  - rinse again with tap water
  - keep the membrane in tap water until it is used
- cleaning with 250 ppm NaOCl overnight : same procedure but immersion time is 1 night instead of 2 days
- X-Flow cleaning procedure (recommended for organic membranes):
  - Acid cleaning: a mixture of nitric acid and phosphoric acid (1/1) at pH 2 is recirculated for 20 minutes through the filtration module
  - Flushing with tap water: allows rinsing the module for 2 minutes
  - Alkaline cleaning: Sodium hydroxide at pH 11, with 200 ppm sodium hypochloride is recirculated for 20 minutes
  - Flushing with tap water: allows rinsing the whole filtration module in site
  - Sanitizing: for this purpose, 100 ppm of hypochlorite or 100 ppm dichloroisocyanurate can be used.
  - Flushing and keeping merged in water : it allows keeping the ultrafiltration module in solution until re-use to avoid drying the membrane.

From the results it is clear that these cleaning procedures were not effective as the fluxes increase only slightly or remain the same.

Membrane 3 was sent to Vito for cleaning. The MTC (Mass transfer coefficient = permeability) before cleaning was 3.5 l/hm^2 with water. During the cleaning procedures, the cleaning fluid was circulated over the membrane. Three cleaning procedures were tested :

- cleaning with P3-oxonia active (1%, at 1 bar, 45°C) : no effect
- cleaning with P3-ultrasil 141 (1% at 1 bar, 45°C) : no effect
- cleaning with NaOCl (600 ppm at 1 bar, 45°C for 30 minutes): **MTC >200 l/hm^2bar with water**

So cleaning with 600 ppm NaOCl during 30 minutes is effective. The difference between the NaOCl cleaning at Epas is the higher NaOCl concentration used and also the circulation of the NaOCl solution (for the cleaning procedure at Epas the membrane is immersed in the cleaning solution).

From the results it can be concluded that the initial flux of the membrane is high but the flux decreases to 5 l/hm^2 within 1 or 2 weeks. It further decreases to 3 l/hm^2 and it seems to stabilize between 2 and 3 l/hm^2. In the configuration of the prototype unit including one PVDF membrane, a minimum flux of 4.15 l/hm^2 is however needed to produce 2.5 L/d of filtrate.

Cleaning with NaOCl (125 ppm) only has a minor effect. The flux increases to approximately 5 l/hm^2 but decreases again within 1 to 4 days below 3 l/hm^2. Cleaning with the XFlow procedure also only has a minor effect. The NaOCl (600 ppm) cleaning performed at Vito was effective. However these conditions of cleaning are relatively aggressive and it should be checked that they do not damage the membrane. The membrane used for cleaning tests was not re-used in the prototype.

2.3.3 General conclusions

According to the filtration unit configuration, the choice of a sidestream concept is correct according to the high suspended solid concentration in the fermentor (5%) that favours the system compactness if the biological kinetics rates are proportional to the suspended solid concentration in the fermentor. The positive points are :

The choice of tubular membranes with large diameter (8mm) in accordance with this kind of suspension very concentrated in particles.

The hydrodynamics conditions imposed in the membrane channel (4m/s) should also limit any particle deposit on the membrane wall.

Nevertheless three points can be discussed. The choice of an organic membrane material is interesting taking into account the problem of the specific weight of the membrane material. According to its chemical resistance (2<pH<10 at 25°C) and thermal resistance (70°C at pH 7), the PVDF material is largely used in aerobic MBR where soft conditions are often developed (12 to 35 °C, pH 7). Nevertheless continuous thermophilic conditions of fermentation (55°C, pH 5) could contribute towards a gradual degradation of the membrane material. This point must be taken into account even the chosen membrane lifetime is only one year. So the choice of an inorganic membrane should be envisaged, an inorganic material being also easily sterilizable by steaming.

Concerning the operation at constant TMP, it is nowadays well known that it is better to work at constant permeate flux because of non reversible fouling and energy supply can be minimised. This filtration mode only imposes a specific pump installation on the permeate side.

The working conditions in the buffer tank, notably the range of temperature between 35 to 45°C, can greatly modify the biomass activity of thermophilic populations and generate some modifications of the suspension characteristics. If working at constant permeate flux the buffer tank is not necessary.

Though the important shear stresses applied along the membrane wall that should be sufficient to avoid any particle deposit and limit biofilm development, results show notable fouling of membrane and some great difficulty of regeneration. Great variations of pressure are also observed upstream and downstream the membrane module. The longitudinal pressure drop appears also largely greater than can be expected in such experimental conditions if the biological suspension has a newtonian behaviour (longitudinal pressure drop should not exceed 0.2 to 0.3 bar instead reaching 0.6 to 1 bar as observed during experimental running). In any way, such pressure variations must not be observed in a sidestream system and a so intensive fouling can not be explained by a classical reversible fouling phenomena.

The observed results oblige to have a better analysis of the fouling phenomena before defining any optimal configuration.

Additional tests for FU optimisation are presented in section 5.

3 Process tests evaluation

3.1 Method for data processing

3.1.1 Evolution of compounds

The data collected on influent, filtrate, reactor content and gas are compiled to visualise the mass evolution of the different components. All inputs and outputs are expressed in mg and can thus be visualized depending on the time (see Figure 7).

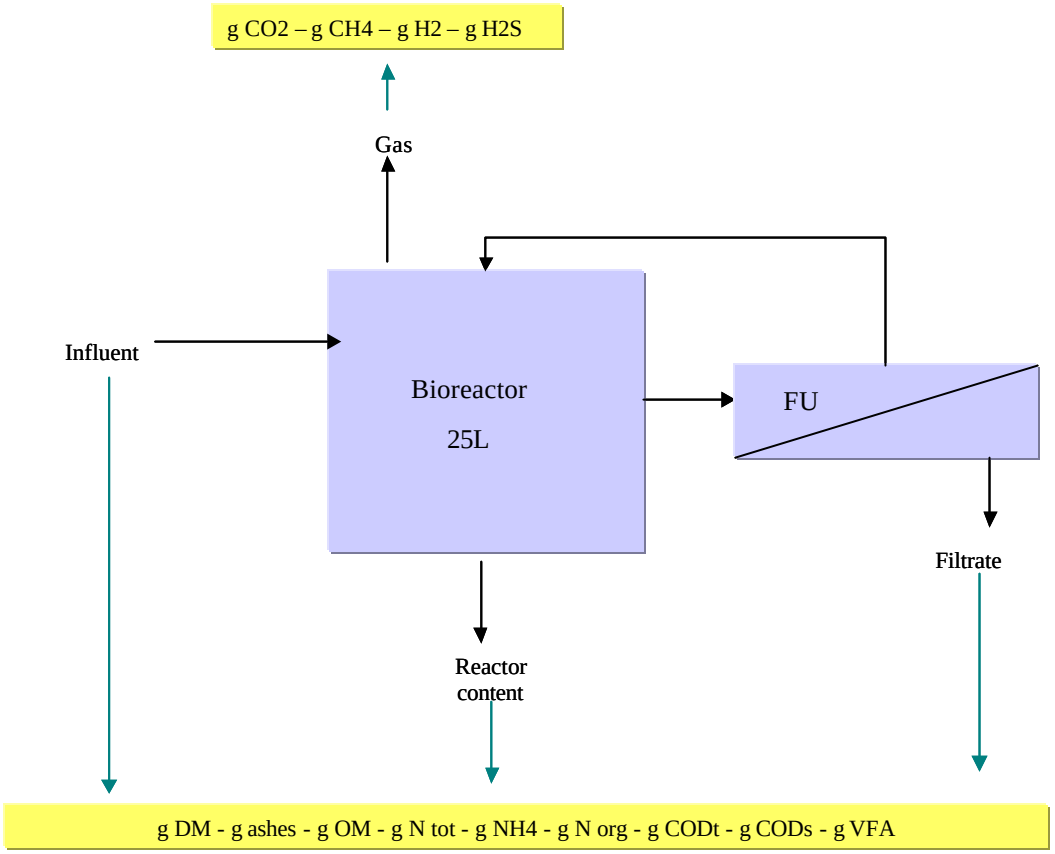


Figure 7. Mass evolution

3.1.1.1 - Masses of dissolved components S:

- $\boxed{m_S = [C_S] \cdot V}$

Where:

- m_S = mass of component introduced or taken from the system (mg)

- C_S = concentration of component in influent or effluent (mg/L)

- V = volume of influent introduced or effluent taken (L)

3.1.1.2 - Masses of gaseous components G:

Volume of biogas produced: $V_{gas} = 10^{-3} \cdot p \cdot r^2 \cdot l$

Where:

- V_{gas} = volume of biogas produced (L)

- r = radius of the gas column (cm)

- l = liquid level in column (cm)

Mass of CO_2 produced (mg): $$m_{CO2} = \frac{\%CO_2}{100} \cdot V_{gas} \cdot \frac{P \cdot M_{CO2}}{R \cdot T}$$

Where:

- m_{CO2} = mass of CO_2 produced (mg)

- $\%CO_2$ = percentage of CO_2 in gas phase (%)

- P = pressure (Pa)

- M_{CO2} = molar mass of CO_2 (44 g/mol)

- R = Gas constant (8.314 $J.K^{-1}.mol^{-1}$)

- T = Temperature (K)

Mass of CH_4 produced (mg): $$m_{CH4} = \frac{\%CH_4}{100} \cdot V_{gas} \cdot \frac{P \cdot M_{CH4}}{R \cdot T}$$

Where:

- m_{CH4} = mass of CH_4 produced (mg)

- $\%CH_4$ = percentage of CH_4 in gas phase (%)

- M_{CH4} = molar mass of CH_4 (16 g/mol)

3.1.2 Efficiencies

The degradation efficiencies are calculated with 2 different methods. One possibility takes into account the biological degradation and thus compares the degradation products with the fed substrates. The other one considers the efficiency of the global system, including the separation step realised by the filtration unit and the accumulation of matter in the bioreactor, and thus characterises biological and mechanical removal of particles by comparing the composition of the influent and the filtrate. The first efficiency is a bit under-estimated since it does not include the exhaustive list of degradation products.

3.1.2.1 OM biodegradation efficiencies

- Biological biodegradation efficiency based on degradation products

$$h_{OM1} = \frac{OM_{biod}}{OM_{\inf}} = \frac{(VFA_{eff} - VFA_{\inf}) + CO_2 + CH_4}{OM_{\inf}}$$

Where:

- h_{OM1} = OM biological degradation efficiency

- OM_{biod} = cumulative biodegraded OM mass (mg)

- VFA_{inf} = cumulative VFA mass in influent (mg)

- CO_2 = cumulative mass of CO2 produced (mg)

- CH_4 = cumulative mass of CH4 produced (mg)

- Biological degradation efficiency including OM accumulation:

$$h_{OM2} = \frac{OM_{\inf} - \Delta OM_c - OM_{eff}}{OM_{\inf}}$$

Where:

- h_{OM2} = OM biological degradation efficiency

- ΔOM_c = moving average of cumulative accumulation (positive or negative) of OM in the bioreactor ($accumulation(t) = OM_{reactor}(t) - OM_{reactor}(t-1)$)

- OM_{eff} = cumulative mass of organic matter in effluent (mg)

- $OM_{reactor}(t)$ = mass of OM contained in the bioreactor at time t

3.1.2.2 - Nitrogen biodegradation efficiency including nitrogen accumulation

The organic nitrogen degradation efficiency gives indications particularly on the degradation of proteins. The organic nitrogen is approximated by making the difference between total nitrogen and ammonium (which is the major part of non organic nitrogen in the waste compartment). The protein content is not

directly measured since techniques for protein analysis use very small sample volumes and are thus not accurate with a solution containing a lot of particles such as the reactor content.

The efficiency can be calculated as following:

$$h_N = \frac{Norg_{\inf} - \Delta Norg_c - Norg_{eff}}{Norg_{\inf}}$$

Where:

- h_N = nitrogen biodegradation efficiency

- $Norg_{inf}$ = cumulative organic nitrogen mass in influent (mg)

- $\Delta Norg_c$ = moving average of cumulative accumulation (positive or negative) of organic nitrogen in the bioreactor ($accumulation(t) = Norg_{reactor}(t) - Norg_{reactor}(t-1)$)

- $Norg_{eff}$ = cumulative organic nitrogen mass in effluent (mg)

- $Norg_{reactor}(t)$ = mass of organic nitrogen contained in the bioreactor at time t

3.1.2.3 - Fibres biodegradation efficiency

Some analysis of total fibres, cellulose, hemicellulose and lignin were performed on samples of influent and reactor content with the Van Soest method. Since the lignin appeared in the past as the component the most difficult to degrade in the first compartment, the evaluation of the results is focussed on degradation efficiencies of total fibres and lignin. Since the analysis showed that the composition in fibres is very constant in the time, a unique factor was used to estimate the fibres concentrations. The efficiencies calculations include the accumulation in the bioreactor. The results of fibres analysis are given in Appendix 1 : Results of fibres analysis (Van Soest).

3.1.2.3.1 Total fibres

$$h_{fibres} = \frac{Fibres_{\inf} - \Delta Fibres_c - Fibres_{eff}}{Fibres_{\inf}}$$

Where:

- h_{Fibres} = total fibres biodegradation efficiency

- $Fibres_{\inf}$ = cumulative total fibres mass in influent (mg)

- $\Delta Fibres_c$ = moving average of cumulative accumulation (positive or negative) of total fibres in the bioreactor ($accumulation(t) = Fibres_{reactor}(t) - Fibres_{reactor}(t-1)$)

- $Fibres_{eff}$ = cumulative total fibres mass in effluent (mg)

- $Fibres_{reactor}(t)$ = mass of total fibres contained in the bioreactor at time t

3.1.2.3.2 Lignin

$$h_{lignin} = \frac{Lignin_{\inf} - \Delta Lignin_{c} - Lignin_{eff}}{Lignin_{\inf}}$$

Where:

- h_{Lignin} = lignin biodegradation efficiency

- $Lignin_{\inf}$ = cumulative lignin mass in influent (mg)

- $\Delta Lignin_{c}$ = moving average of cumulative accumulation (positive or negative) of lignin in the bioreactor ($accumulation(t) = Lignin_{reactor}(t) - Lignin_{reactor}(t-1)$)

- $Lignin_{eff}$ = cumulative lignin mass in effluent (mg)

- $Lignin_{reactor}(t)$ = mass of lignin contained in the bioreactor at time t

3.2 Evaluation of the test period results

This section presents the graphs of the main parameters during the test period and their evaluation. On each graph, the two vertical lines correspond to the perturbations.

3.2.1 System operation and perturbation calendar

The aim of the test period was partly to obtain new data on the up-scaling of the process. One other objective was to gain knowledge on the inhibition processes of the first compartment in order to allow adaptations and further development of its model and control. For this purpose, a perturbation calendar was established by the Melissa partners in order to test the effect of different inhibitors on the prototype reactor.

The system was operated in the following conditions:

- Influent: Melissa mix as defined by the Melissa partners (human faecal material, lettuce, red beet, wheat straw)
- Feeding mode: semi-continuous with 2.5 L/d fed via an influent buffer tank
- Filtration unit: continuous running
- Filtrate: semi-continuous production of 2.5 L/d
- Liquid reactor volume: kept constant around 25L
- Hydraulic Residence Time (HRT): 10 days
- Daily follow-up

The perturbation calendar is presented in Figure 8.

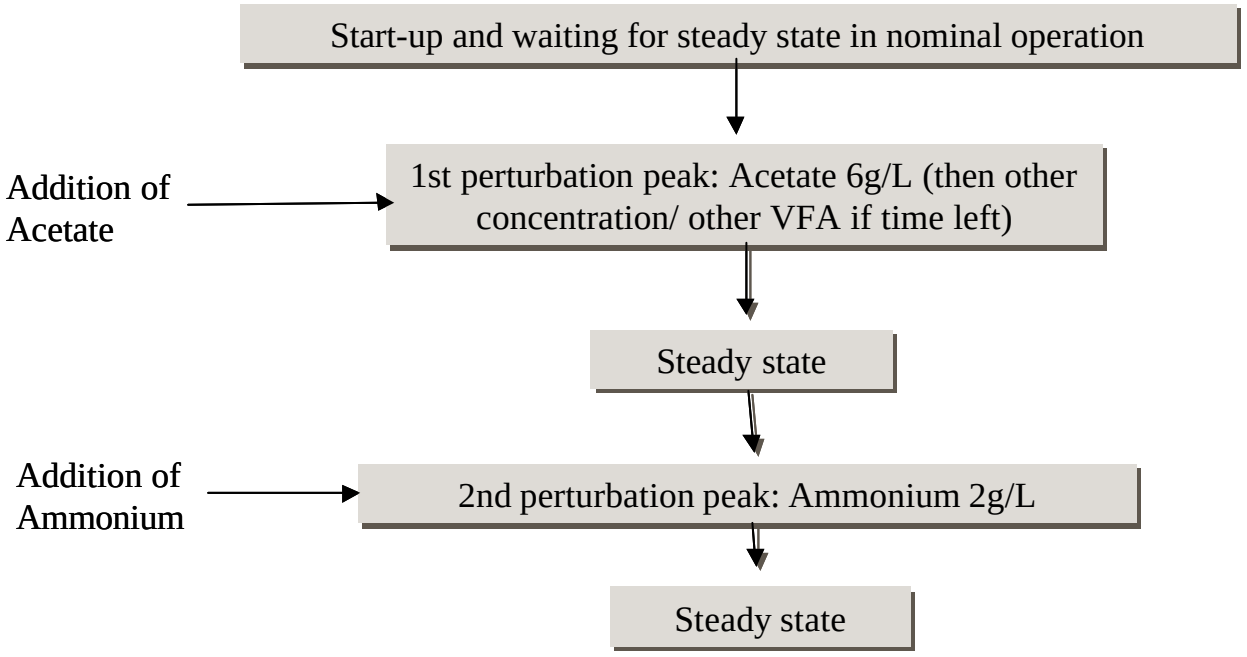


Figure 8. Perturbation calendar

3.2.2 General parameters

3.2.2.1 pH

The pH is regulated in the prototype reactor by addition of base or acid in the range 5.15 – 5.6 (see Figure 9). A low pH inhibits the methanogenic species and prevents methane production. As shown on the graph, the pH is relatively stable in the bioreactor. The pH of the filtrate is very similar. It fluctuates more since it is not controlled. The pH of the influent is more unstable due to the natural variations in the composition of plants and faecal material.

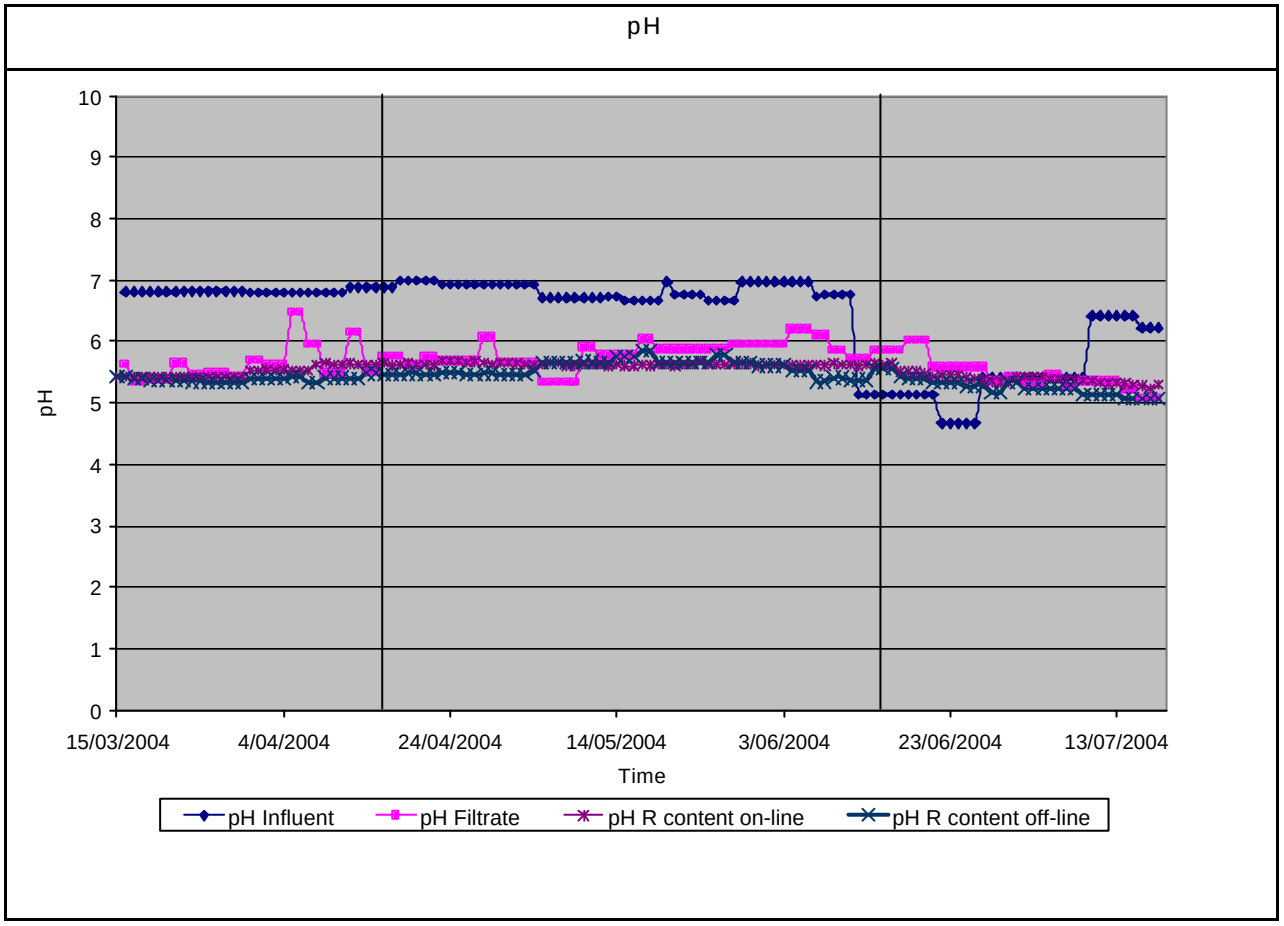


Figure 9. Evolution of pH

3.2.2.2 Electroconductivity

The electroconductivity (EC) reflects the salts content of a solution (see Figure 10). At certain concentrations, salts can have inhibitory and even lethal effects on the inoculum.

The EC is usually stable in the reactor. Indeed the salts are not retained by the filtration unit and are simply washed out. The two perturbations peaks induced an important increase of EC. This is due to the fact that both additions of acetate and ammonium caused an important change in the pH. The system regulated the pH back to its wanted value by adding respectively a base (NaOH 3N) for the acetate case, and an acid (HCl 3N) for the ammonium case. The added volumes of base or acid are relatively important as shown in the graph (up to 600 mL). Since base and acid contain salts (sodium and chlorine), this resulted in a consequent increase of the EC.

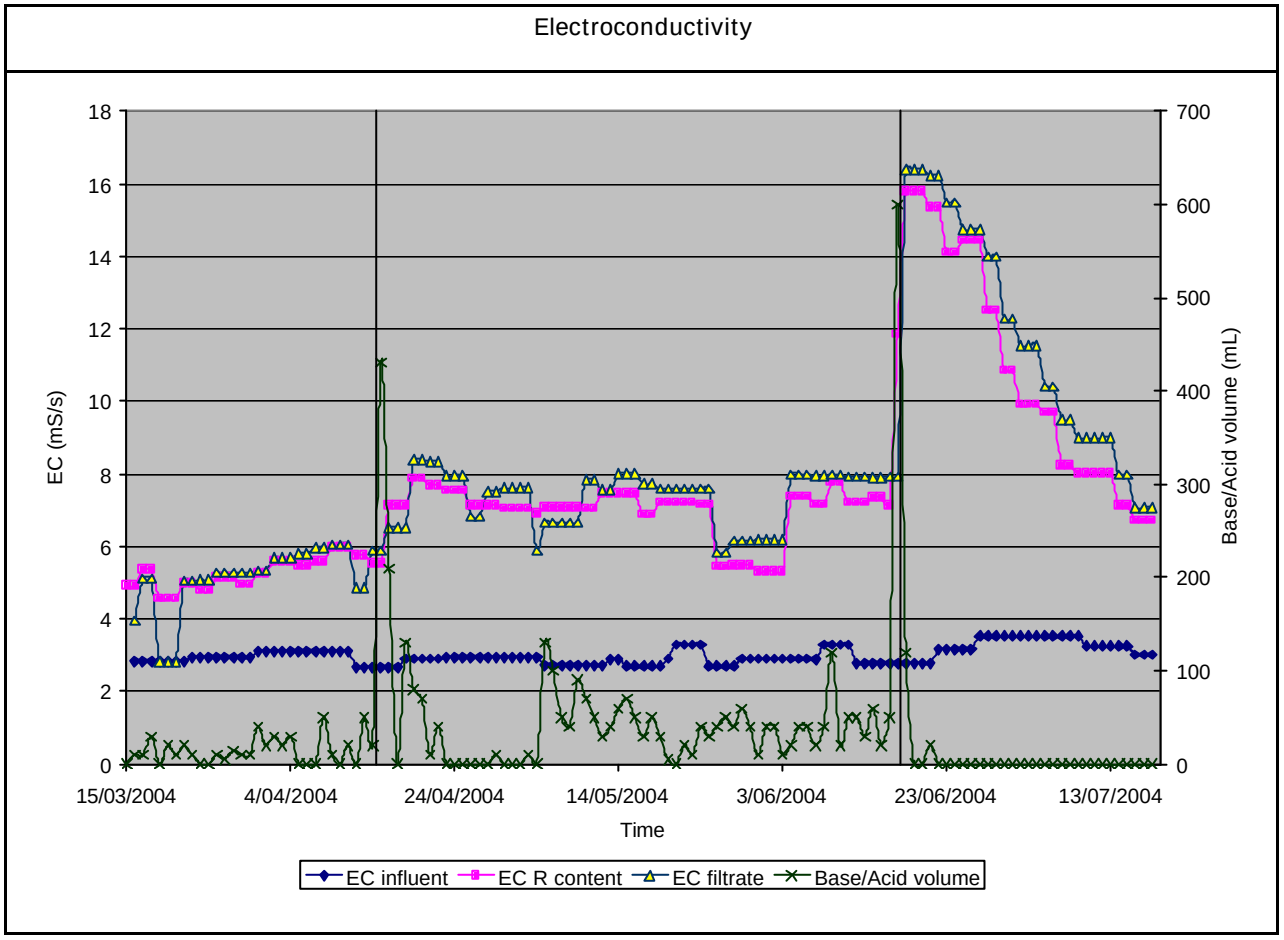


Figure 10. Evolution of electroconductivity (EC)

3.2.3 Degradation products

3.2.3.1 VFA

The anaerobic degradation occurring in the first compartment produces mainly VFA, ammonium and CO_2. The VFA concentration is a particularly good indicator of the degradation quality since VFA production is relatively sensitive to variations of operation conditions, influent composition… (see Figure 11 and Figure 23). In a nominal operation and after stabilization, a concentration around 5000 mg/L in the reactor can be expected. The concentration in the filtrate is very similar since the VFA are soluble compounds and flow through the filtration membrane.

In the first perturbation, acetate was added to the influent. It appeared that all the VFA were not found back in the bioreactor. This can be due to a loss of VFA in the gas phase of the influent tank, the VFA being very volatile. A delay occurred between the moment when the acetate was added and was found back in the reactor. This is due to the fact that the acetate was added on a period of 2 days together with the influent. Then it seems that the VFA are simply slowly washed out, without inhibiting their production in return. The concentration goes back to its nominal value around 5000 mg/L. The amount of acetate added seems to not have affect s the efficiency of the degradation process (see Figure 23).

The perturbations have also no significant effect on the VFA production rate, which fluctuates around an average of 300 mg/d and per gram of OM fed (Figure 12). The second perturbation corresponds to the addition of ammonium directly inside the bioreactor. In the days following the addition, a slight decrease in the VFA concentration can be noticed, but this is not shown by the VFA production rate, and is thus probably due to a fluctuation of volume or flow.

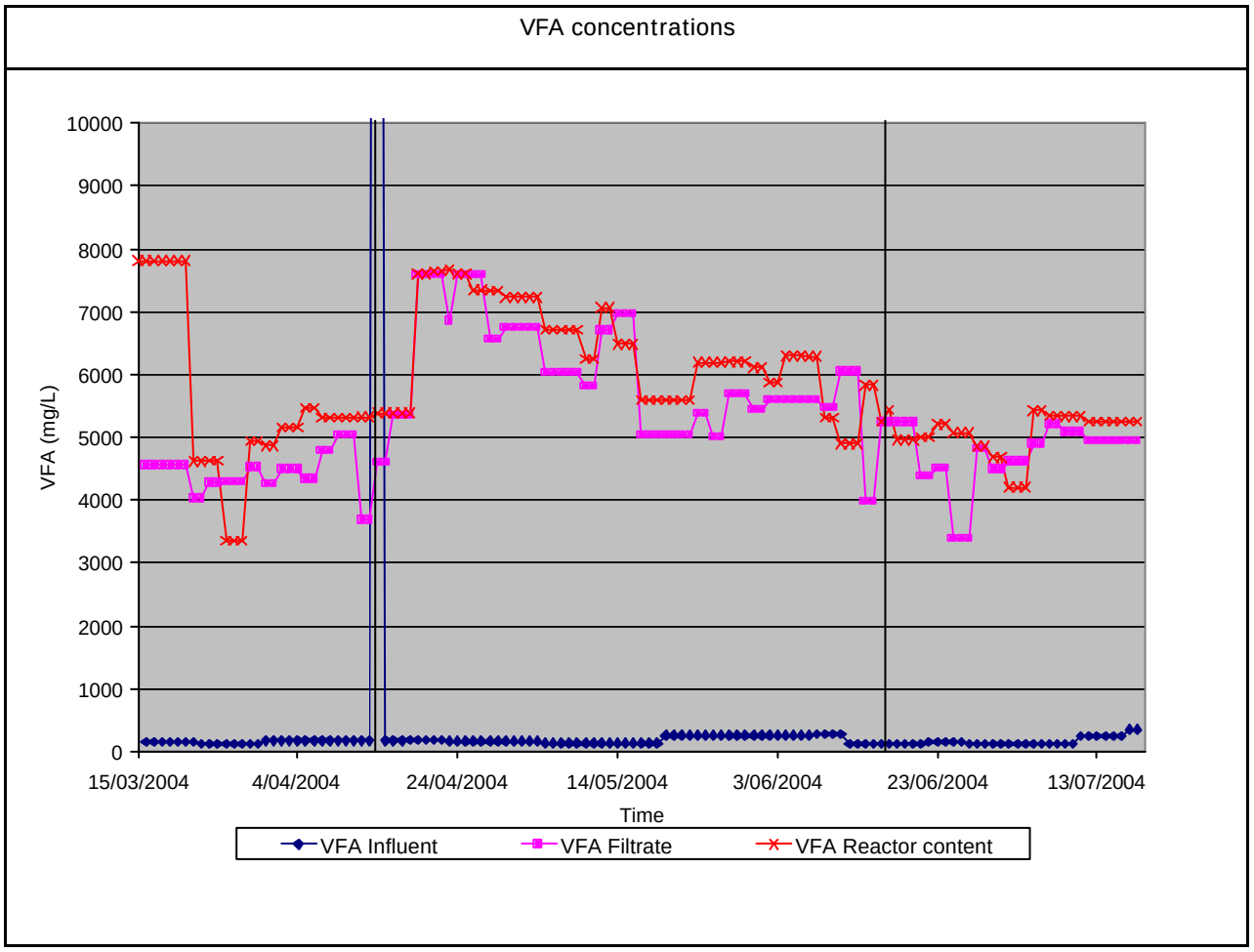


Figure 11. Evolution of VFA concentration

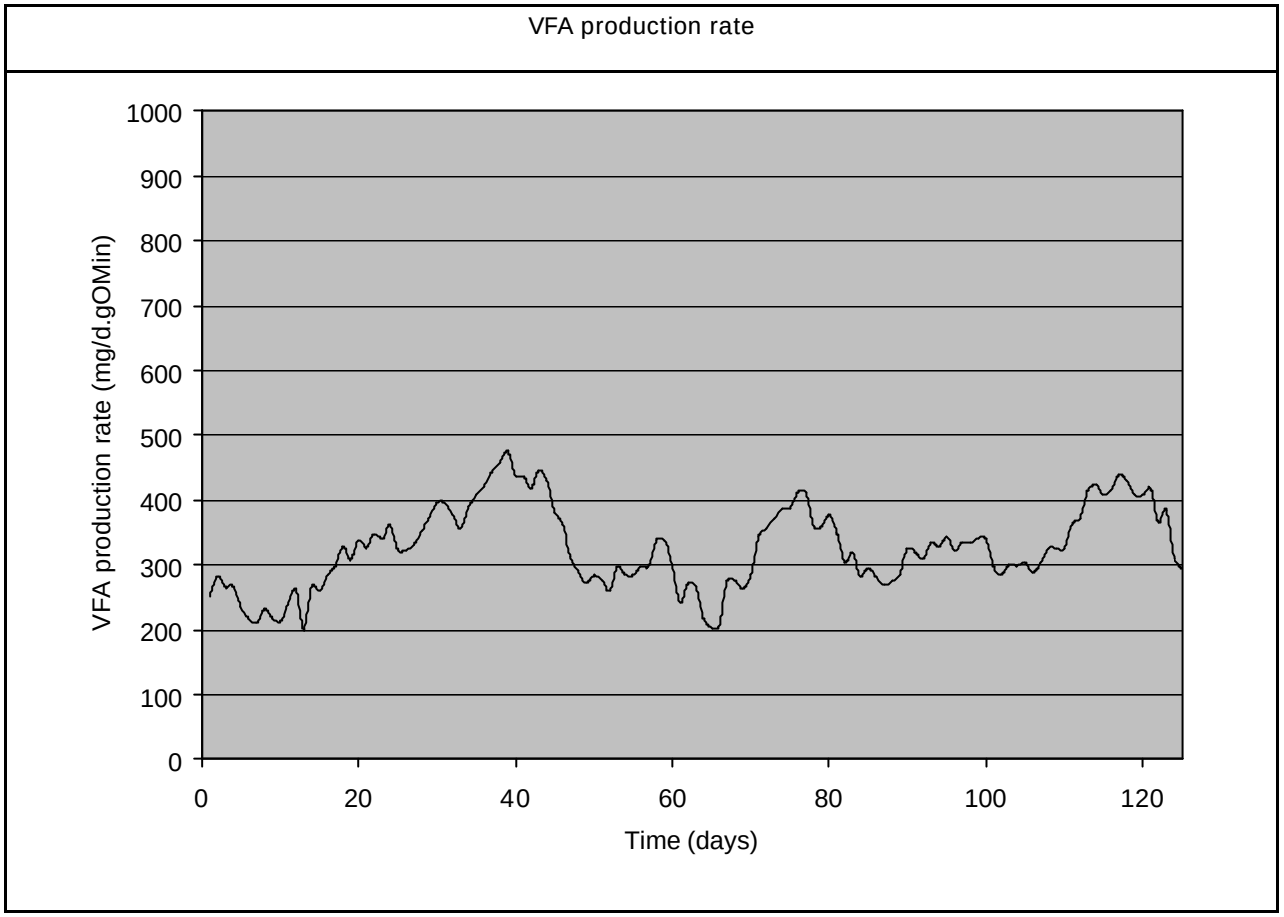


Figure 12. Evolution of VFA production rate

Figure 13 presents the repartition of the different VFA in the time. Acetate and butyrate appear to be the most present VFA. The first perturbation peak caused a change in the composition and an increase of the acetate proportion. This was firstly due to the addition of acetate that changed the total VFA composition. However it seems that the system did not return to its initial equilibrium. Indeed after 3 months meaning 9 hydraulic residence time, the proportion of acetate remains higher than before the acetate perturbation, which was not expected. This would mean that the perturbation disturbed the balance between the different species and / or metabolic ways, favoring the way producing acetate, and that this new equilibrium is stable and becomes the new nominal composition.

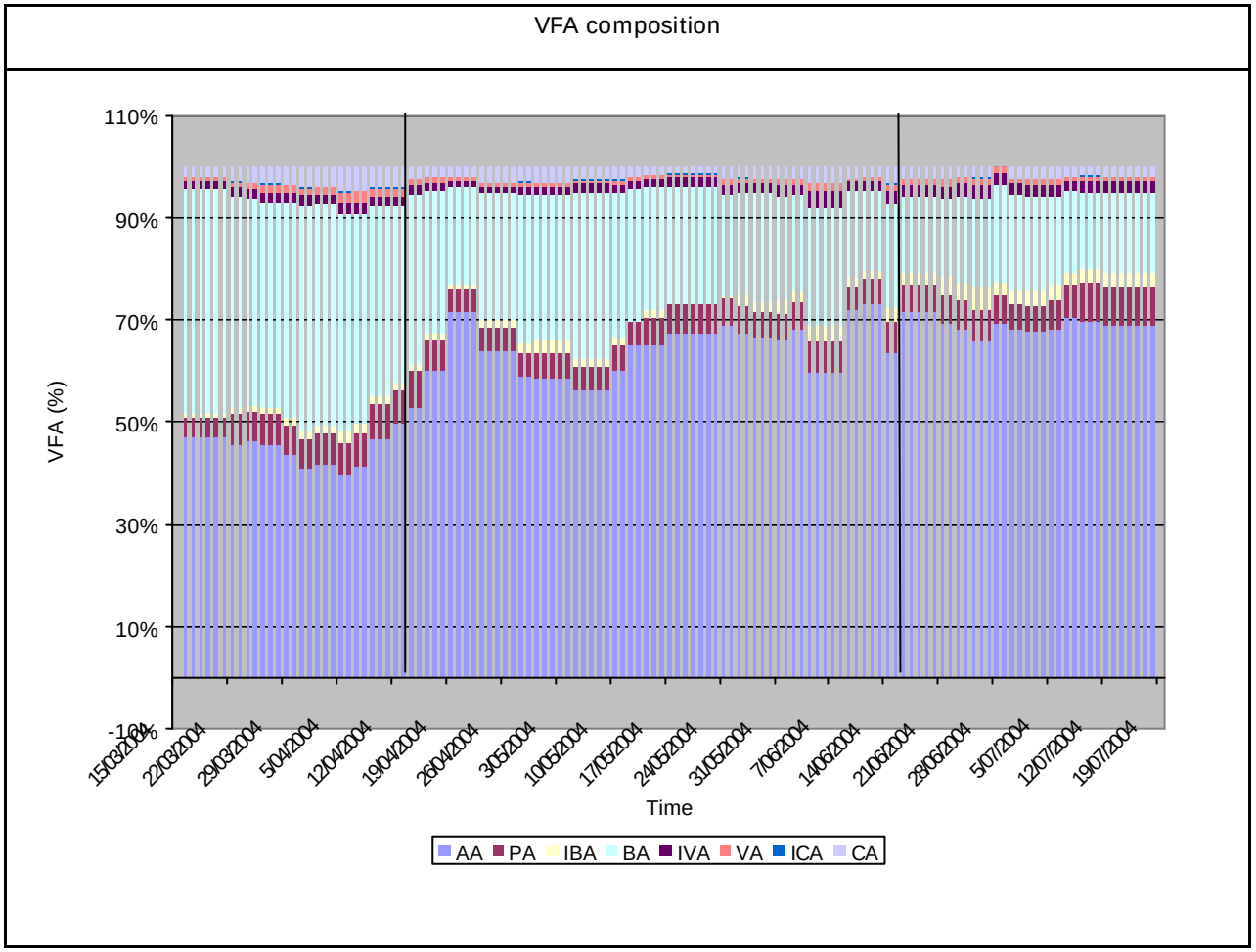


Figure 13. Evolution of the VFA composition

3.2.3.2 Ammonium

The nominal concentration of ammonium is around 250 mg/L in the bioreactor. The addition of acetate seemed to not disturb the ammonium production. After the addition of ammonium, it was immediately found in the bioreactor, due to the fact that it was added directly in it. Then the ammonium is slowly washed out, without showing a particular inhibitor effect (see also Figure 23).

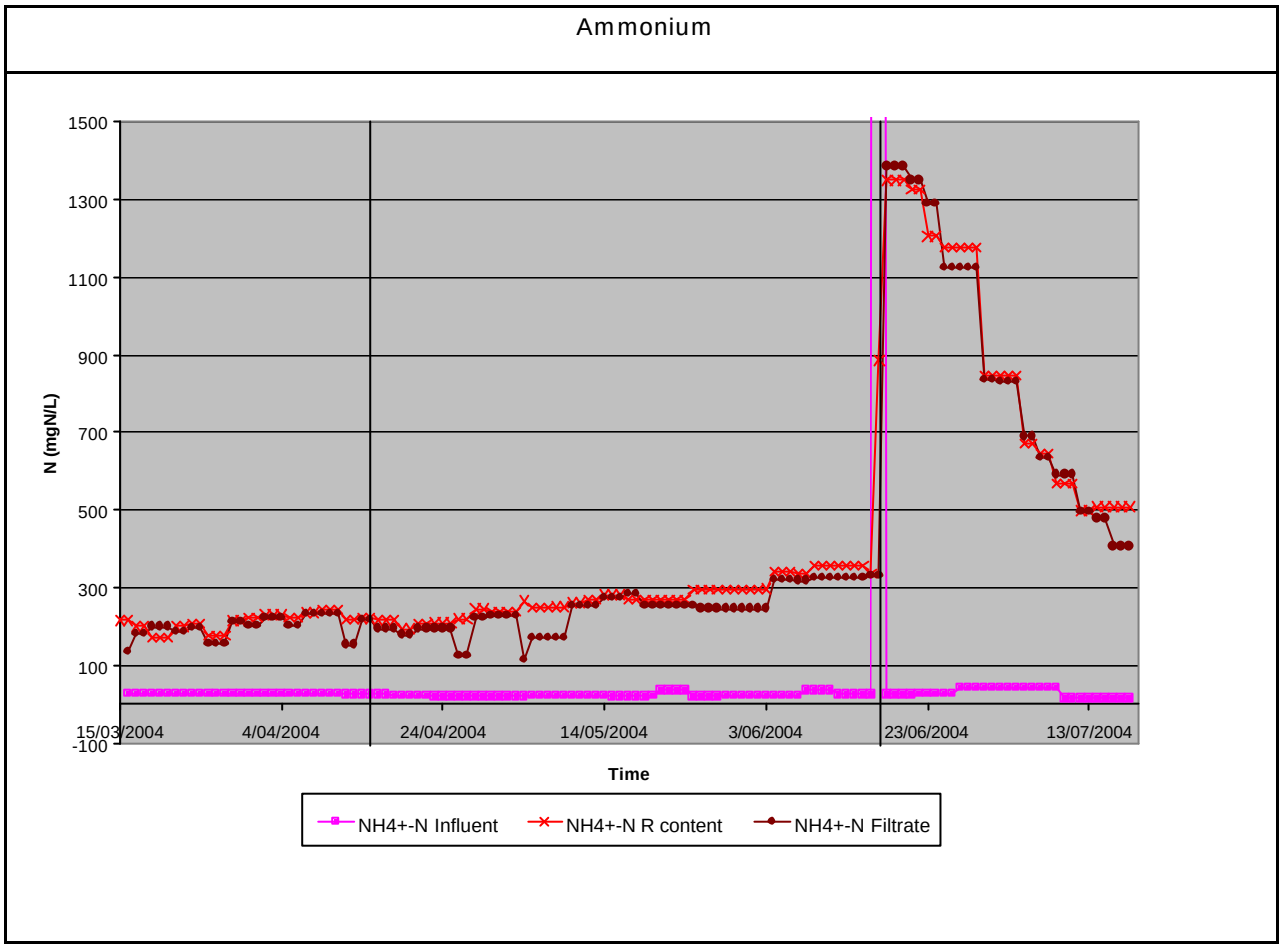


Figure 14. Evolution of ammonium concentration

3.2.3.3 Gas

In nominal mode, a production of around 1.7 g/d of gas can be expected by the prototype reactor (around 2 L/d). This parameter fluctuates on a short time scale, due to the fact that the gas produced is not released continuously in the gas columns but punctually when a certain pressure is reached in the bioreactor (see Figure 15).

On a longer time scale, it seems that the acetate perturbation induced an increase of the gas production, which is visible both in terms of volume (Figure 15) and of gas composition (Figure 16). On the contrary, the addition of ammonium seemed to induce a decrease of the gas production. This perturbation was also followed by an increase of the methane production as shown on Figure 16. The ammonium may have inhibited the acidogenic bacteria, giving an opportunity to the methanogenic bacteria to grow more in spite of a low pH (5.5). The shot of ammonium induced moreover a short increase of the pH up to 8.8 that was regulated within 2 hours by addition of acid. This may have played a role in the emergence of the methanogenic bacteria too. At the end of the test period, the methane was still produced up to 12%. The pH set point was reduced to 5.2 in order to inhibit the process. It resulted in a decrease of the methane proportion, that remained however higher than initially (around 5%) even 2 months after the end of the test period. There again, the perturbation seemed to induce a definitive change in the consortium equilibrium.

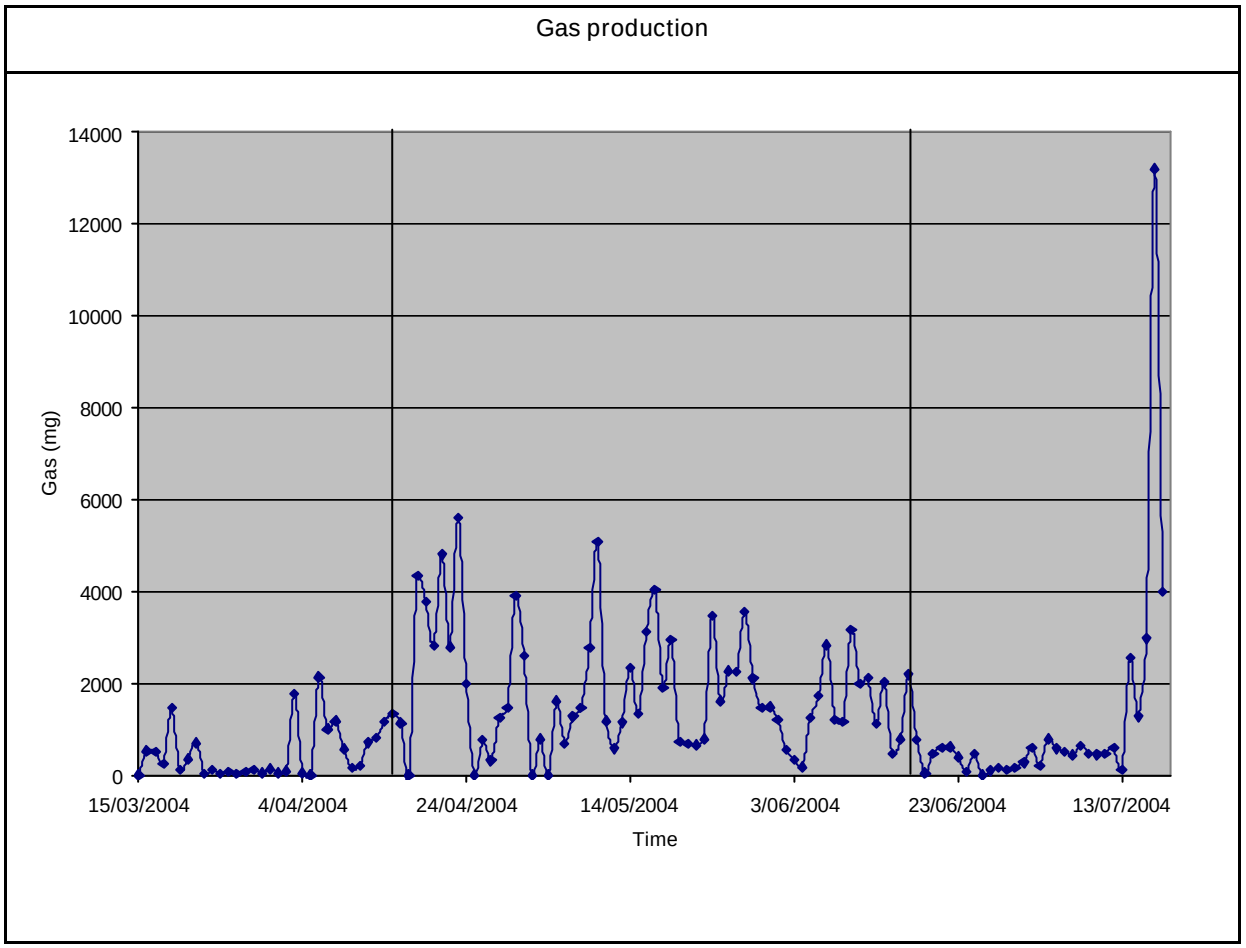


Figure 15. Evolution the mass gas production

CO_2 is the major component of the gas phase. Its concentration increases regularly after flushing the reactor up to 80%. In nominal operation, O_2 and CH_4 are found in very little concentrations. A concentration of about 200 ppm of H_2S can be expected. H_2 is produced in higher concentrations than expected. Its wide variation in the time poses however a problem in the precision of its measurement. Its concentration varies from 1000-2000 ppm up to 50 000 ppm. The H_2 production seems to be sensitive to the perturbations. Indeed, the shots of acetate and ammonium induced both a consequent increase of the H_2 concentration for a few days.

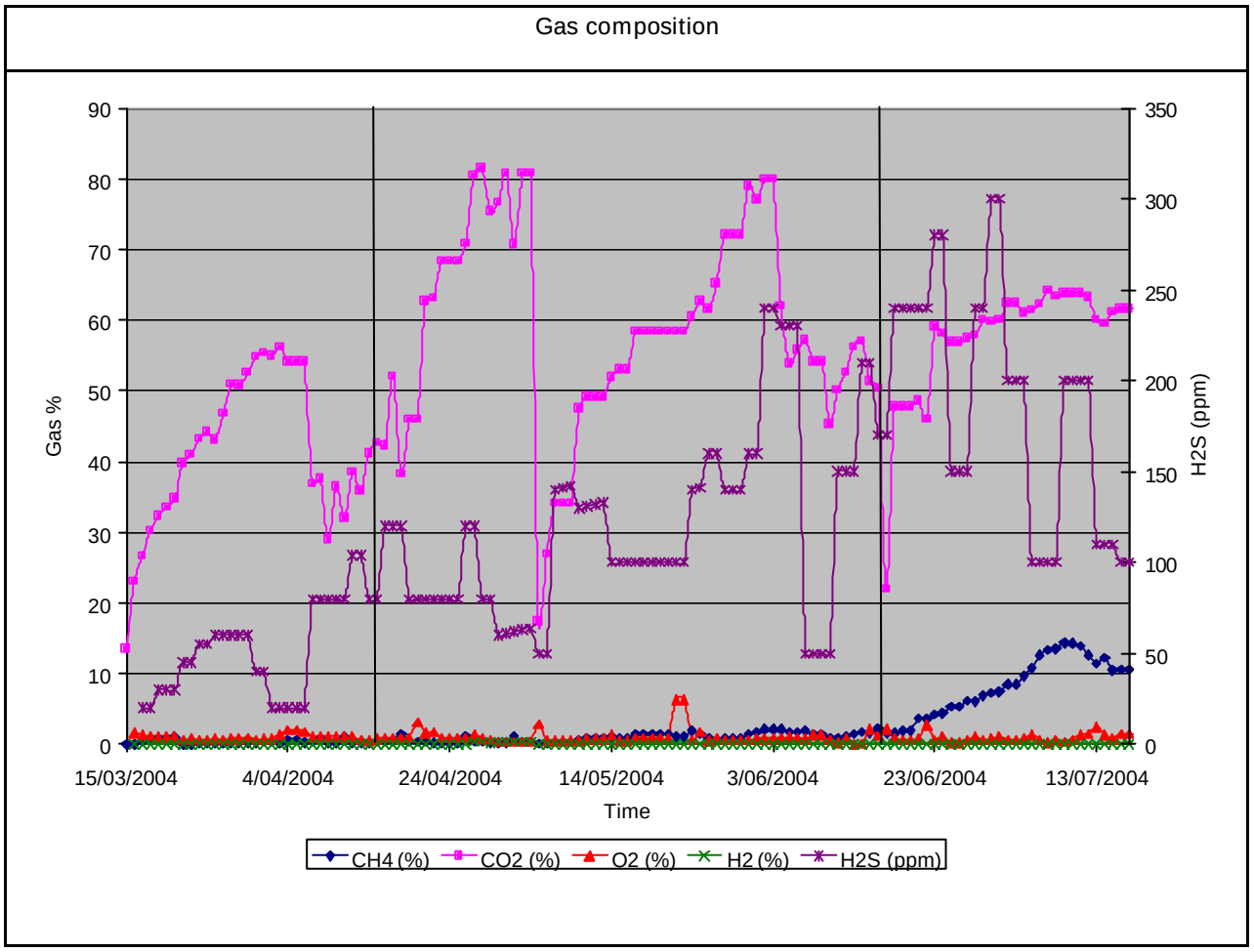


Figure 16. Evolution of the gas composition

3.2.4 Solids accumulation

3.2.4.1 Dry matter, ashes

The evaluation of the degradation process at prototype scale is of high importance, especially regarding drain issue and strategy. Indeed, because of the use of a filtration membrane, non-degraded solids are accumulating inside the bioreactor, resulting in an increase of the total dry matter in the time. To allow an optimal process both in terms of microbial degradation and of hardware operation, the dry matter content of the sludge needs to be limited and regulated under a maximal value, that was fixed to 50 g/d in the past (see technical note 71.1). The strategy proposed to regulate the dry matter content consists to drain part of the sludge in the bioreactor at regular intervals of time. Additional treatments of this drain are at present investigated, which could allow a further degradation of the solids and then recycling of the drain back to the bioreactor. Because the first compartment was tested at lab-scale before the construction of the prototype, the drain volume and frequency could not be established precisely due to the limited inoculum volume of the lab reactor. The prototype reactor showed a rapid accumulation of dry matter in the first weeks of tests, followed by a stable state around 40 g/L where the dry matter content increased very slowly. The total sludge drained from the reactor during the 4 months of test corresponds to 15L, meaning 0.5% of the reactor content per day (mainly samples). This is very few compared to what was expected, and indicates a better degradation, which is reflected in the biodegradation efficiencies (see Figure 23). The accumulation rate remained quite stable (Figure 18).

The vertical lines on some dry matter measurements indicate the range of variation of the measurements between 3 samples taken at the same moment (see Figure 17).

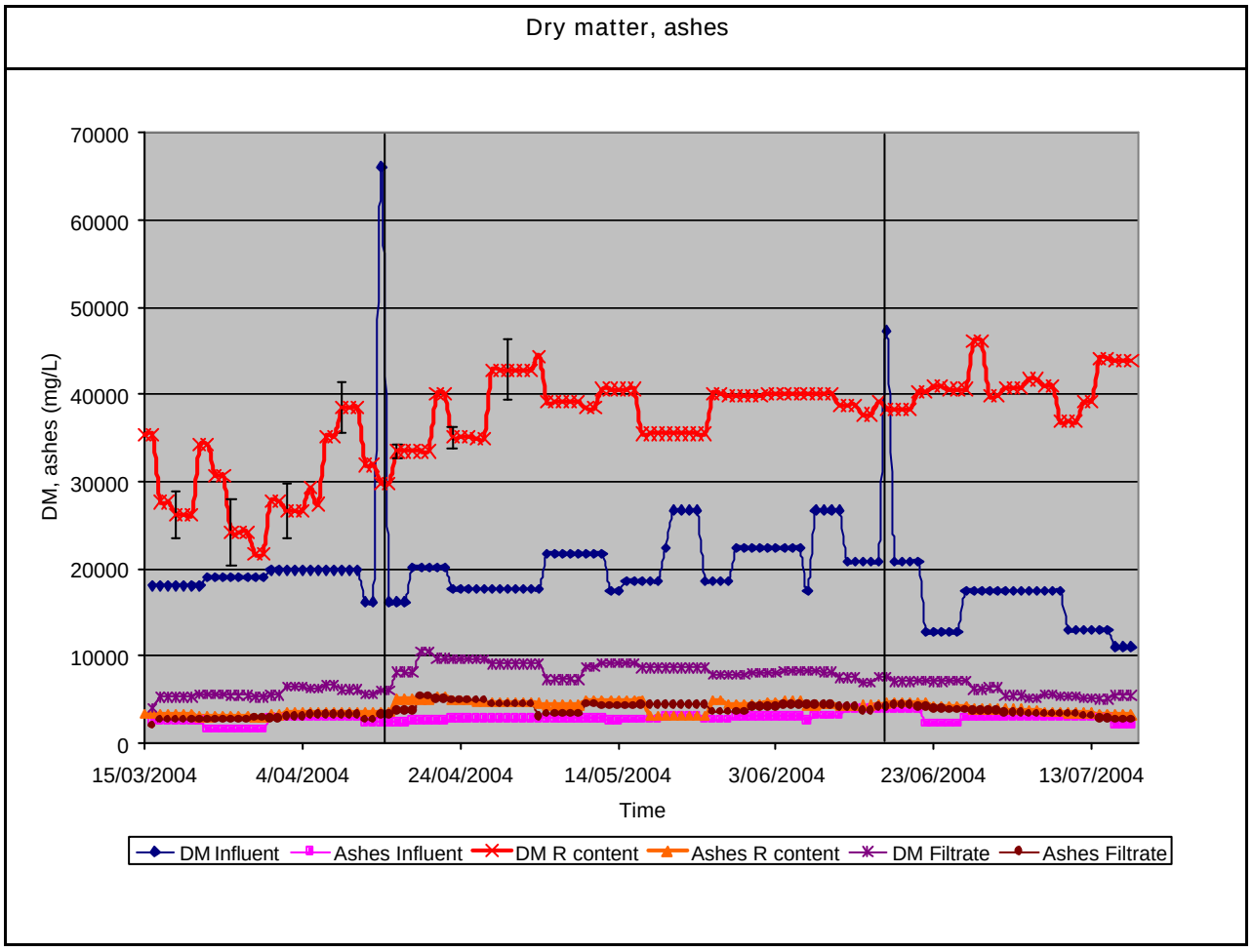


Figure 17. Evolution of dry matter and ashes

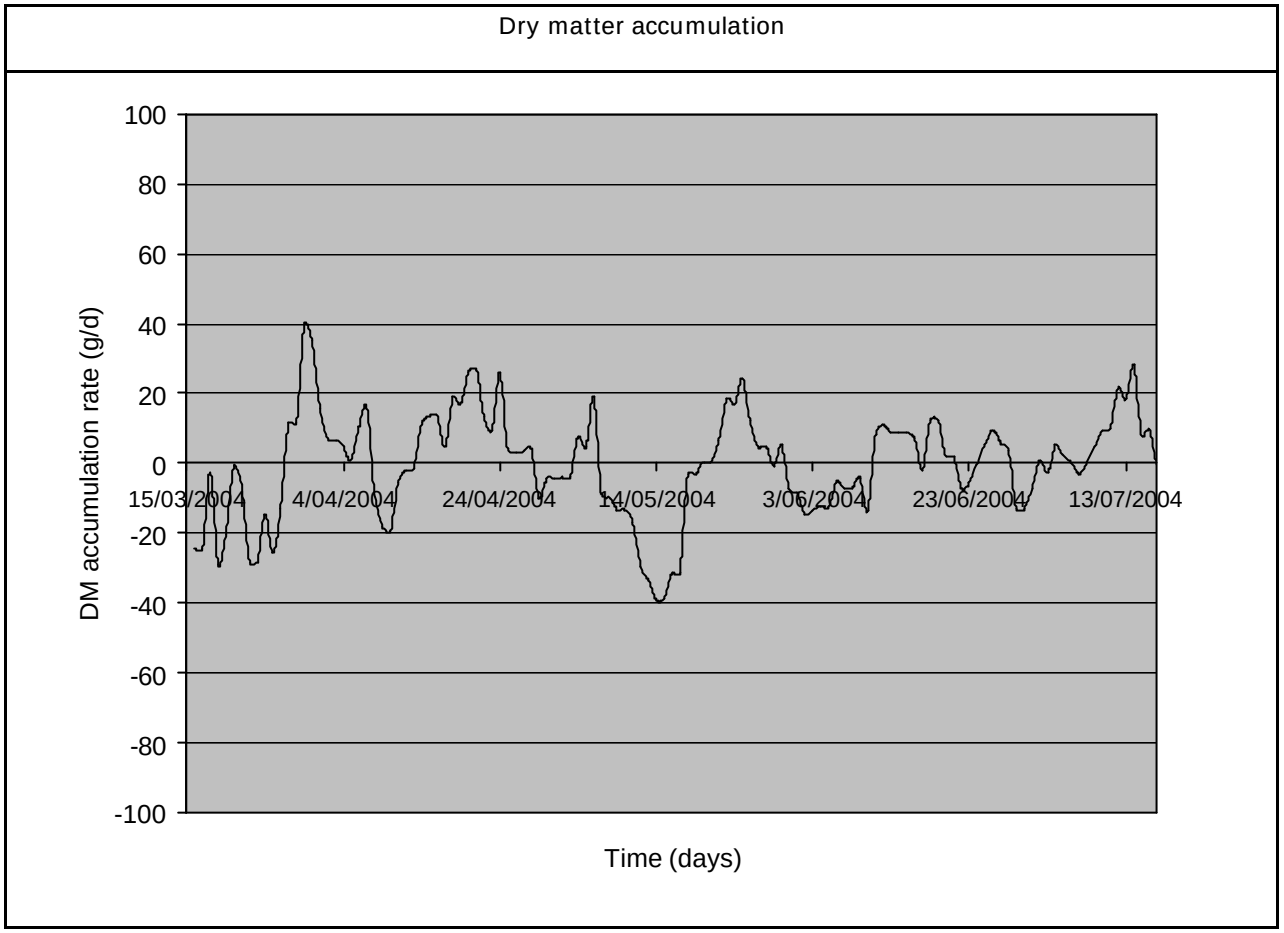


Figure 18. Dry matter accumulation rate

3.2.4.2 Total nitrogen

The total nitrogen shows the same profile of accumulation as the dry matter. The ammonium perturbation induced a peak in the total nitrogen concentration, which is washed out in a few weeks.

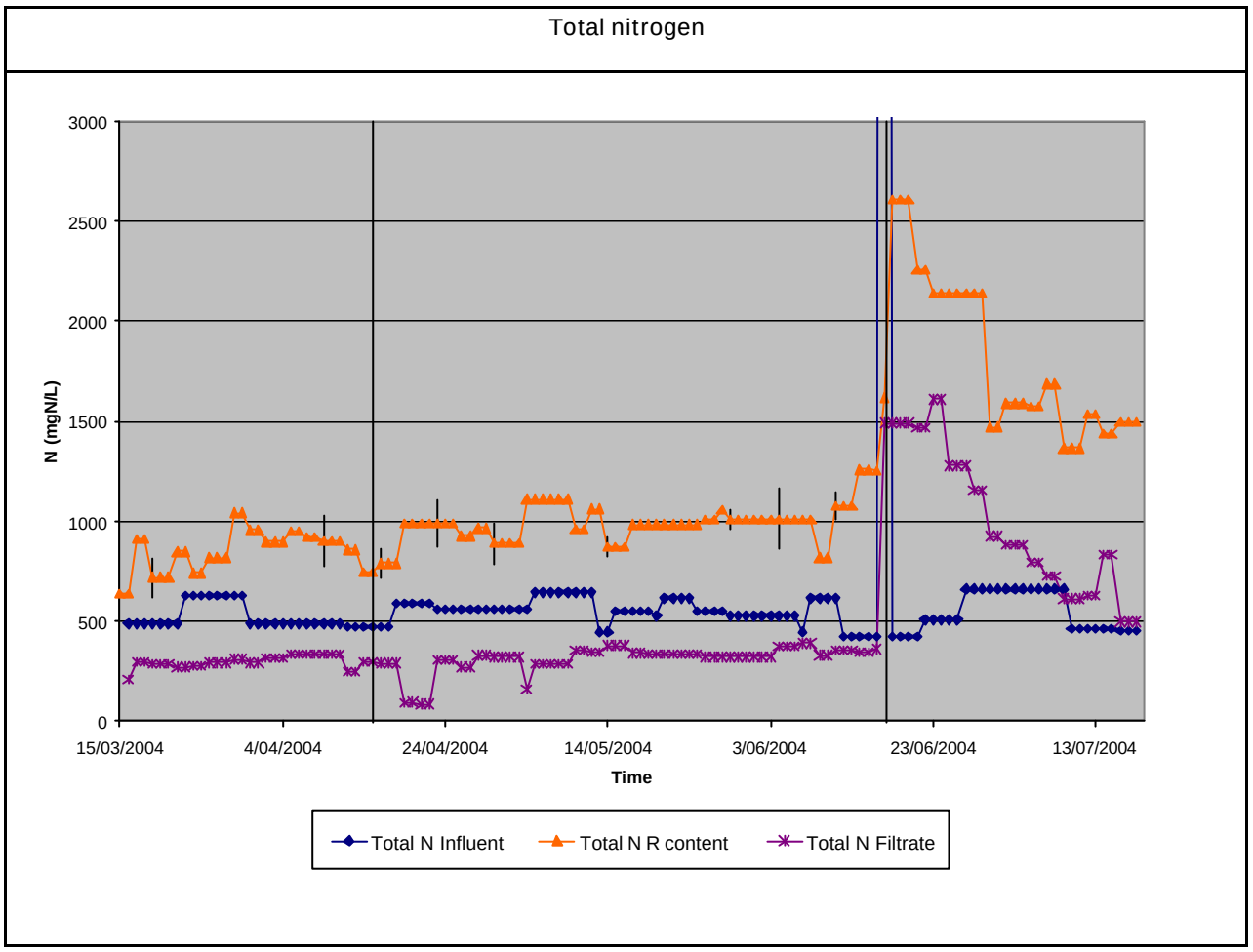


Figure 19. Evolution of total nitrogen

3.2.4.3 COD

The total COD is a parameter to interpret with precautions since the presence of particles in the sludge decreases the precision of the measurement. The vertical lines on some measurements indicate the range of variation of the COD measurement between 3 samples taken at the same moment. From a global approach, the results show the same evolution as for dry matter (see Figure 20).

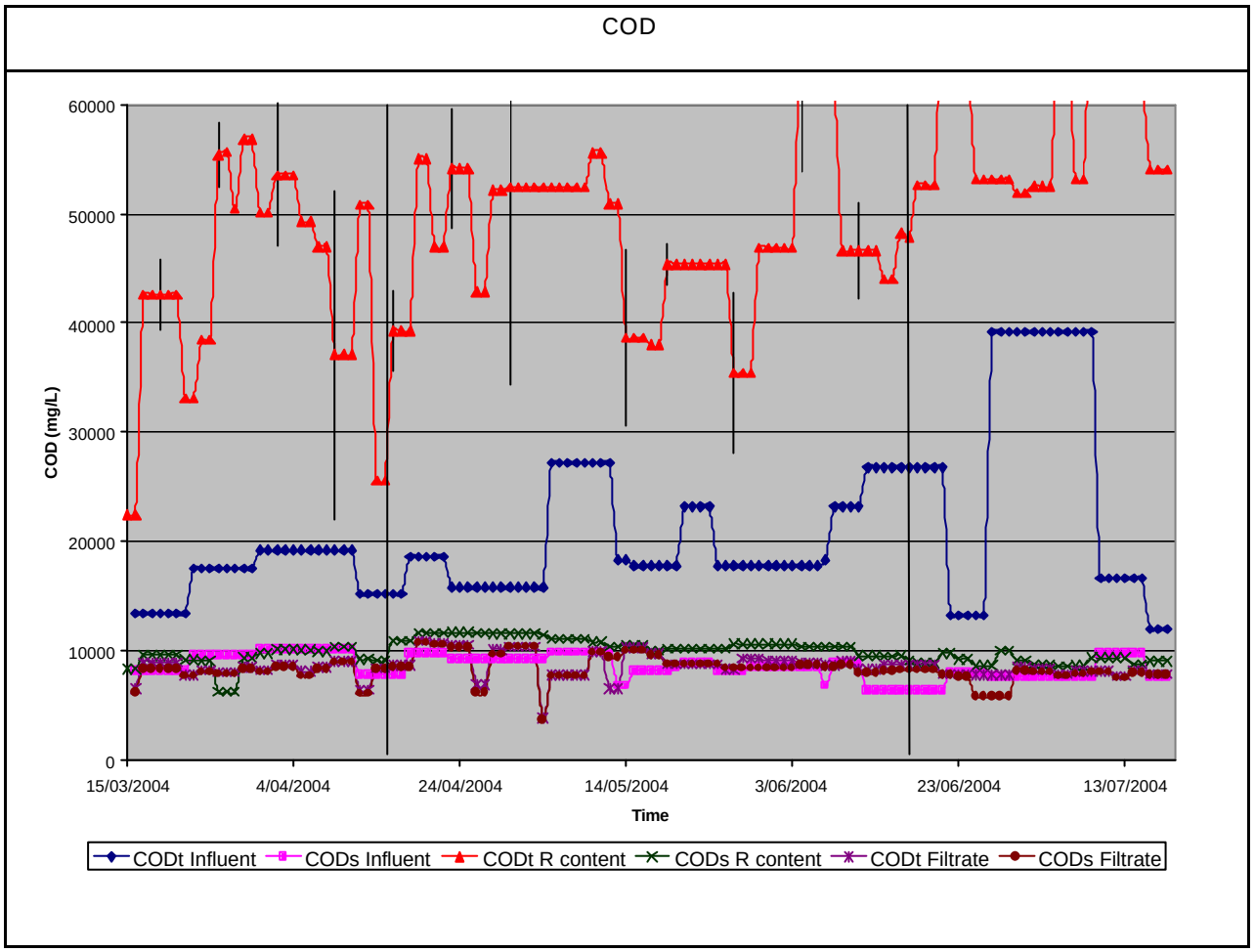


Figure 20. Evolution of COD

3.2.4.4 Fibres

Fibres represents a critical issue in the Melissa loop. Indeed, the influent of the first compartment, and particularly the plants contains a lot of fibres and especially lignin, which is hardly degradable by an anaerobic bacteria consortium. Several additional treatments of the drain of Compartment I are at present investigated in order to improve its global degradation efficiency.

Several fibres analysis including total fibres, hemicellulose, cellulose and lignin were performed on the prototype reactor content, at different moments of the test period. These measurements showed a stability in the proportions of the different fibres types. As a first estimation, these proportions were thus considered as constant in the time. This is however not completely exact since there is a slight accumulation of solids in the reactor, and particularly of the lignin, which is indeed hardly degraded and retained by the filtration unit. This means that the lignin concentration necessarily increases slowly in the time.

Figure 21 shows the evolution of the fibres concentrations. The concentrations in the reactor are higher than in the influent, due to the fact that fibres are insoluble and thus retained in the bioreactor by the filtration unit.

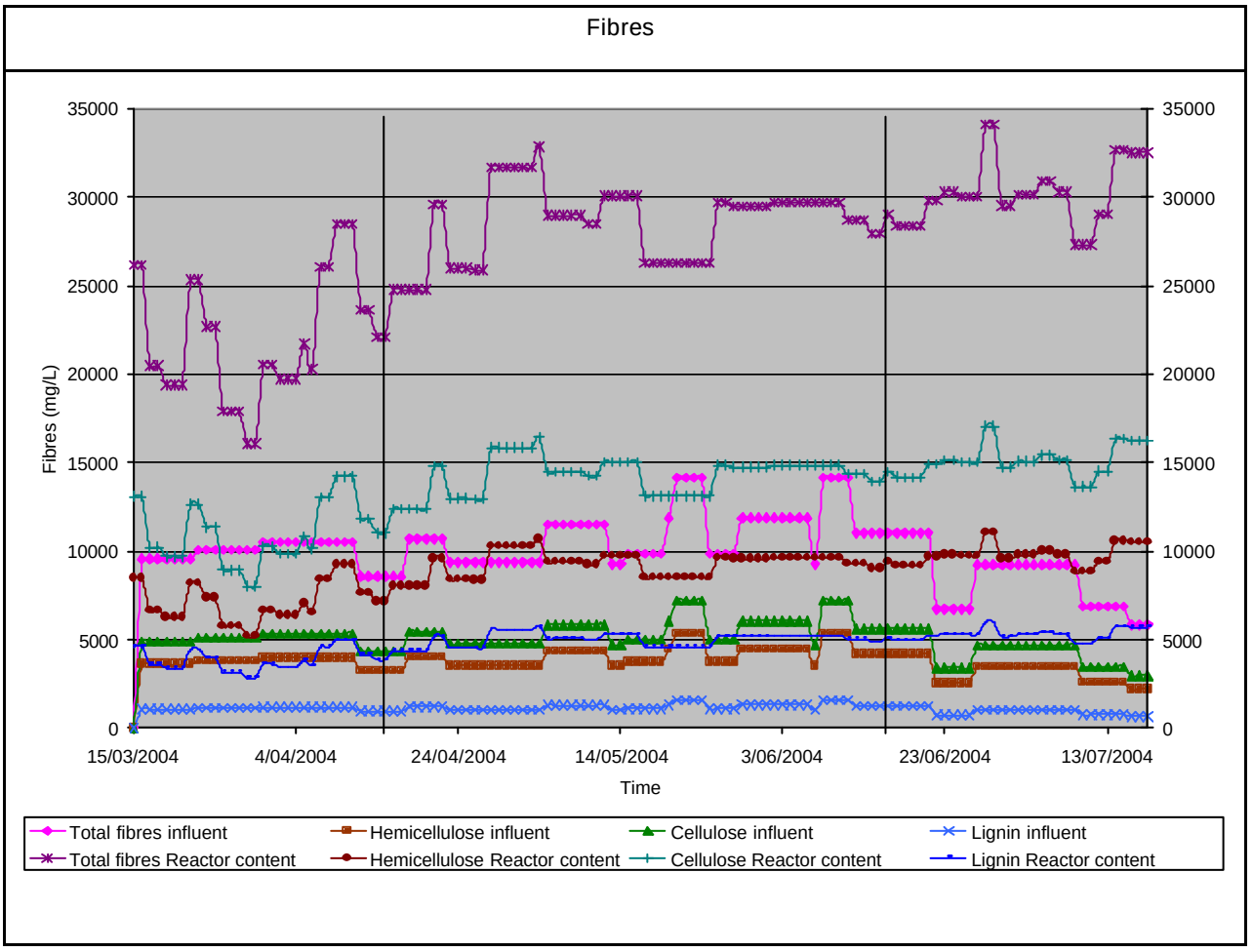


Figure 21. Evolution of fibres concentrations

Fibres can be considered as a good indicator of the global accumulation of matter in the bioreactor when considering the drain issues. Figure 22 shows the cumulative accumulation of fibres in the time. The two major drains operated in the test period are represented by two vertical lines. These drains caused a consequent increase in the fibres substracted from the bioreactor. At the end of the test period, the efficiency of total fibres degradation is stabilized. The degradation efficiency is reflected in the cumulative accumulation of fibres in the reactor that shows a very slow increase in the time.

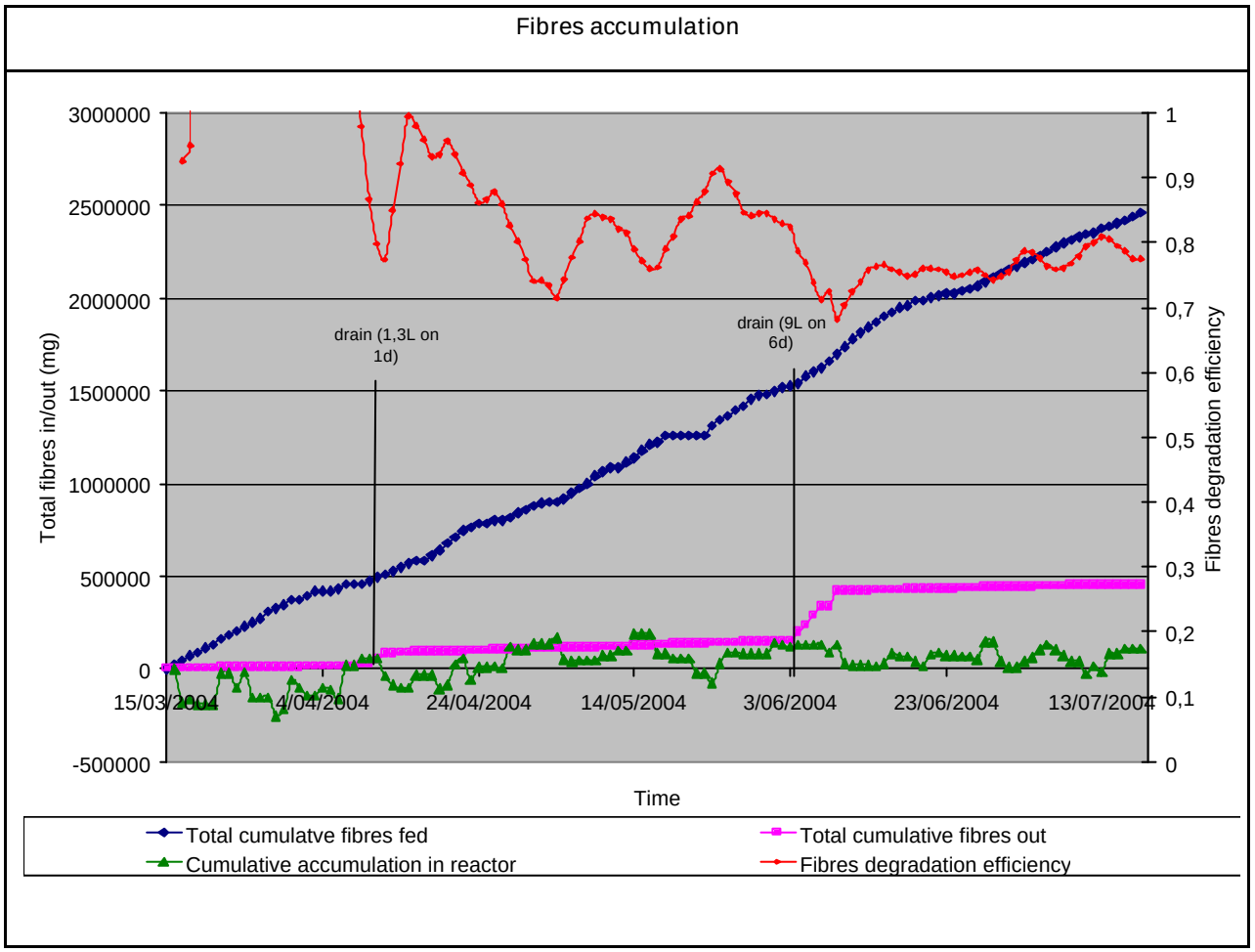


Figure 22. Fibres balance

3.2.5 Efficiencies and drain issues

As already discussed when considering the solids accumulation, the total dry matter and fibres are accumulated very slowly in the bioreactor in nominal and stabilized operation. This corresponds to a good global degradation efficiency, and is indeed reflected in the efficiencies calculations as shown in Figure 23.

The OM degradation efficiency reaches a nominal value of 47 to 65 % depending on the calculation method. It has to be noticed that the first value is deduced from the known degradation products, and is thus necessarily underestimated. The second value expresses the ratio between the total OM fed in the system and what is taken out of the system, including drains and positive or negative accumulation in the bioreactor itself (see section 3.1.2.1).

The organic nitrogen degradation efficiency (corresponding mainly to proteins degradation) and also the lignin degradation efficiency are estimated around 65%. The total fibres degradation efficiency reaches about 75%, due to the presence of cellulose and hemicellulose which are easily degradable. This result should however be lower than the global efficiency, and a continuous follow-up of the fibres should be performed at pilot scale on a longer period to refine this result.

Slight decreases can be visualized on the different efficiencies curves. They are due to the drains and the perturbation tests.

To conclude on the performances of the prototype regarding biological degradation, the up-scaling of the process seemed to be benefic. The degradation seems to reach an optimum after a concentration of the dry matter in the bioreactor up to around 40 g/L. At that concentration, the global OM efficiency reaches 65% and the solids are very well degraded and thus very slowly and slightly accumulated in the system. The drain that needs to be performed will thus be likely smaller than what was expected. However this should be further investigated with the pilot reactor in order to confirm and validate these assumptions and establish a complete drain strategy. Particularly the fibres degradation should be further studied with regular analysis.

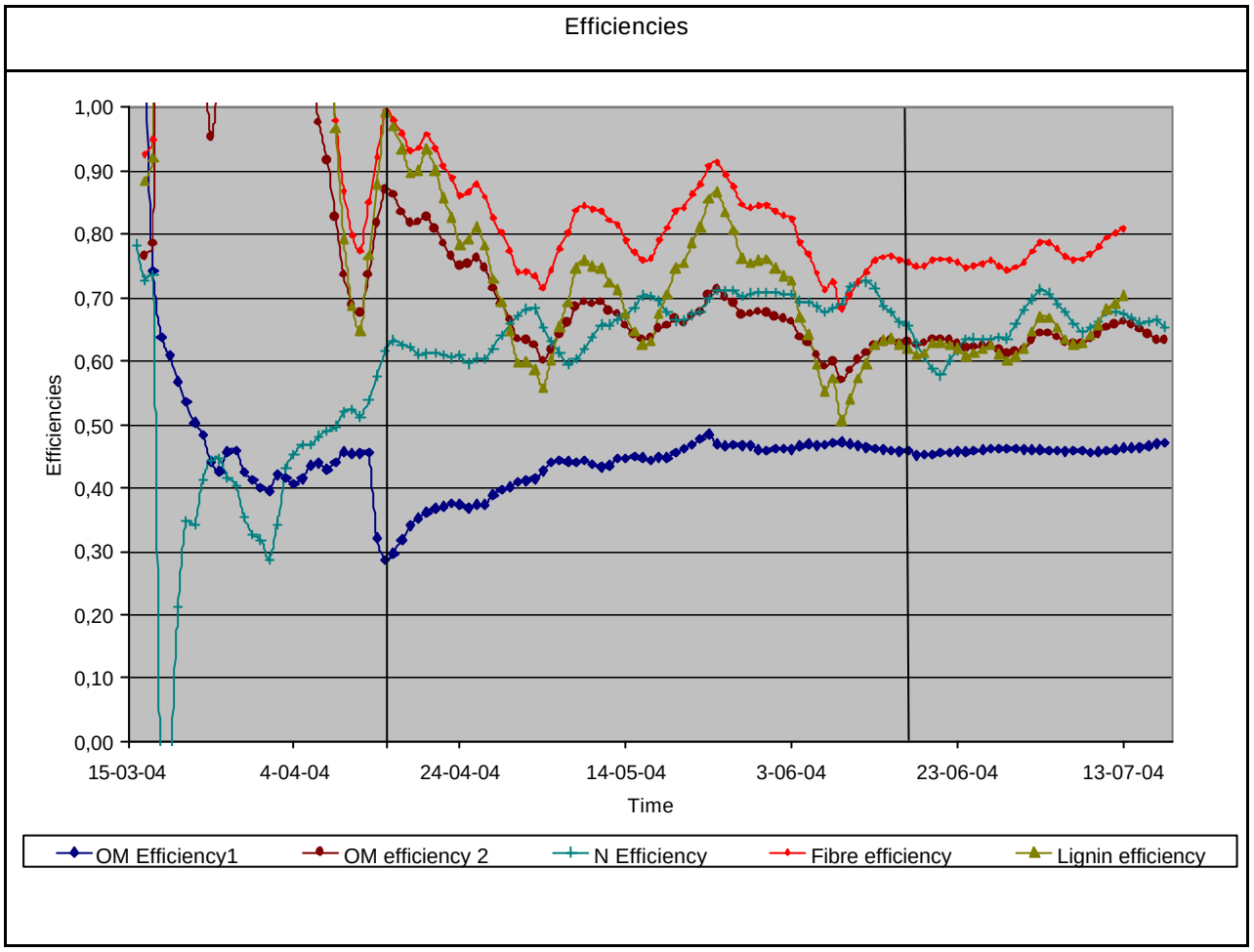


Figure 23. Degradation efficiencies

3.2.6 Microbial aspects

3.2.6.1 Materials and methods

Samples from influent, reactor content and membrane filtrate were taken in anaerobic conditions and shipped to Vito at 4°C and processed within 24 h. The samples were diluted in 10^{-2} M $MgSO_4$ and to 1 ml subsamples yeast extract agar was added for aerobic incubations or Schaedler sheep blood agar (Oxoid) for anaerobic incubations. Total aerobic counts were determined at 22, 37 and 44°C and cell numbers counted after 6 days, 2 days and 2 days respectively. Total anaerobic counts were only determined at 44°C after 2 days of incubation. Anaerocult A was added to obtain anaerobic conditions. For each incubation, two types of blanks were included:

- non-inoculated blanks
- inoculated blanks on agar plates without carbon source or medium

3.2.6.2 Results and discussion

For practical reasons it was not always possible to count the total cell number according to the ISO norm. Samples were shipped from EPAS to Vito and did not always reach Vito on the same day of the week.

As shown in Table 5, anaerobic incubations were only started form April 27th onwards. Initially, it was thought that a determination of the total aerobic cell count at different temperatures would suffice to get an indication of the log reduction over the membrane filtration unit. Because cell numbers in the reactor content were lower than expected, and because it was assumed that this was due to the aerobic incubation conditions for the cell counts, anaerobic cell counts were included as well. From the data, anaerobic and aerobic counts were mostly comparable for similar samples. This may be related to the fact that bacteria involved in acidification reactions as the ones occurring in the reactor, often are facultatively anaerobic and can grow both in aerobic and anaerobic conditions.

Total cell counts are typically only determined at 22 and 37°C. However, because the reactor was operated at higher temperatures and 44°C was expected to be more optimal for the thermophilic bacterial population, this temperature was included as well. However, Table 5 does not clearly demonstrate that this was the case. For the first sampling dates, cell counts were indeed lower at lower temperatures, but at later samplings, the differences between cell numbers at 22 and 44°C were low. Influent samples generally showed higher counts at lower incubation temperatures. This can be expected because bacteria on (frozen) vegetable material are adapted to environmental temperatures and fecal bacteria show optimal growth at 37°C.

The main aim of the determinations was however to evaluate the log reduction over the UF membrane. In spite of the fact that the 30 nm pores of the selected membrane should retain all bacterial cells (membrane sterilization in laboratory conditions is performed with membranes of 0.2 µm), high cell numbers were found in the membrane filtrate. We believe that this is due to the following factors:

- the UF unit was not designed to operate in sterile conditions and cannot be sterilized
- Permeate flow through in the membrane filtration is low: because the HRT in the bioreactor is high and the fluxes relatively low, the permeate stagnates in the unit and this allows for bacterial contamination and regrowth after filtration
- the effluent contains high levels of volatile fatty acids, an ideal growth substrate for a wide range of bacteria.

Table 5 Total aerobic and anaerobic cell counts (CFU.10^5/ml) at different temperatures for three types of samples: influent, reactor content or membrane permeate (effluent).
CFU: colony forming unit, /: not determined

date	sample	aerobic			anaerobic
		22°C	37°C	44°C	44°C
23/03/04	influent	12,60	0,03	/	/
	reactor	0,23	2,46	/	/
	effluent	0,09	0,06	/	/
30/03/04	influent	2910	2660	/	/
	reactor	12,95	20,05	/	/
	effluent	4,8	3,50	/	/
6/04/04	influent	680	720	/	/
	reactor	0,72	0,79	/	/
	effluent	160	165	/	/
14/04/04	influent	1375	1280	/	/
	reactor	8,20	9,70	/	/
	effluent	229	184	/	/
21/04/04	influent	252	310	0,8	/
	reactor	15,70	19	16,7	/
	effluent	23	62	12,8	/
27/04/04	influent	920	810	2,83	5
	reactor	1,92	27,10	21,80	7,70
	effluent	18,2	36	17,60	14,40
4/05/04	influent	1420	830**	0,01**	0,088
	reactor	13,2	15,4**	10,3**	9,3
	effluent	234	310**	10,6**	4,3
10/05/04	influent	1040*	900	950	12,3
	reactor	5,4*	4,5	5,8	11,2
	effluent	163*	157	4,6	1,04
18/05/04	influent	/	/	/	/
	reactor	2,72	9,4**	5,6**	6,7
	effluent	125	112**	3,9**	2,5

*counted after 3 days, **counted after 6 days of incubation

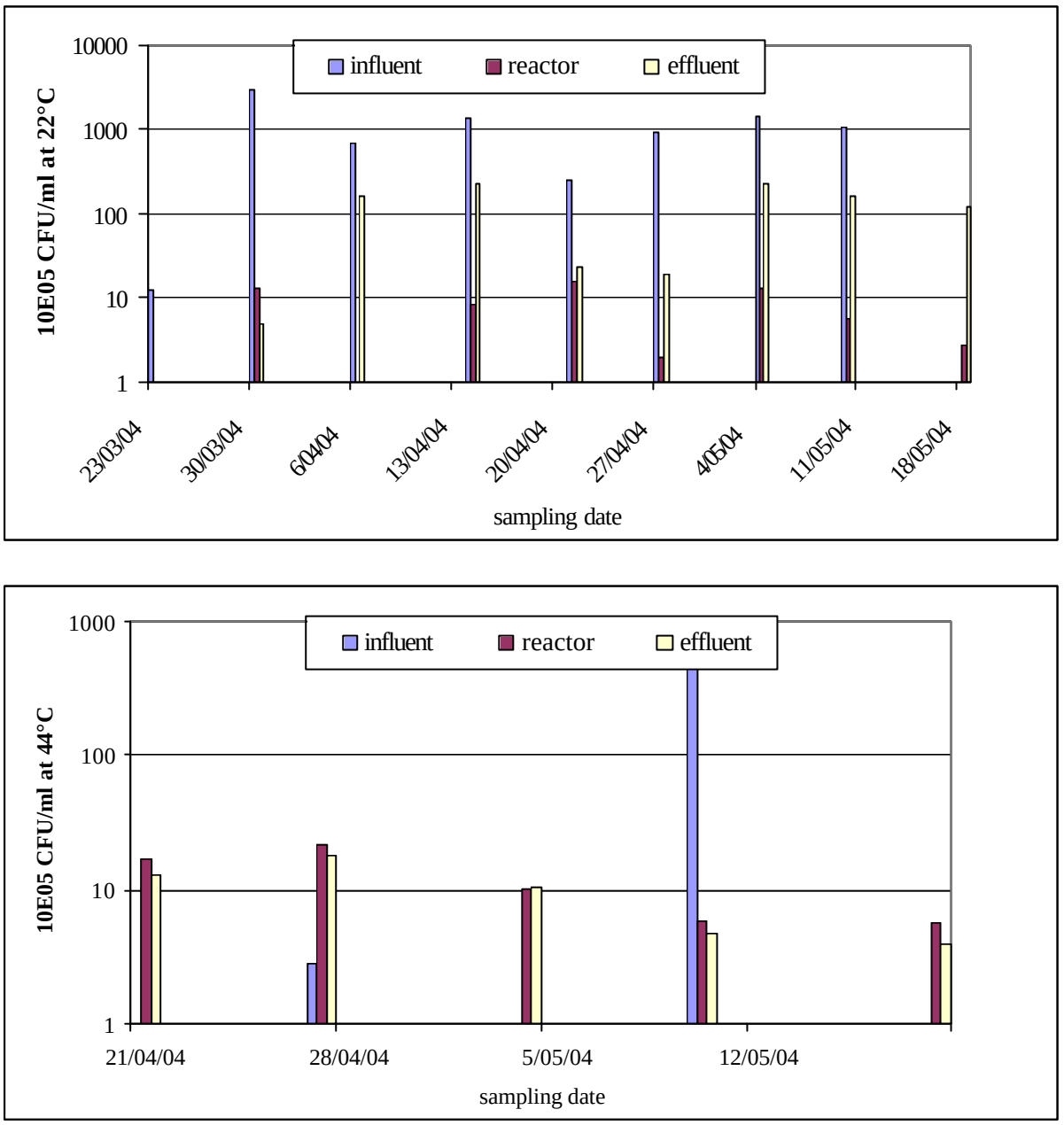


Figure 24 Aerobic cell numbers at 22°C (top) and 44°C (bottom).

In conclusion, the problem of high cell numbers in the membrane filtrate is not due to improper functioning of the membrane but rather to regrowth of bacteria on a rich substrate/medium in non-sterile operating conditions. This problem is also encountered in drinking water distribution systems where surplus chlorine addition is used to avoid regrowth of bacteria. Keeping in mind that drinking water contains hardly any growth substratescompared to the effluent of the first Compartment, the high cell number in the EWC effluent is not surprising. Even in drinking water applications, similarly high cell numbers have been detected.

Options to solve this problem are the following:

- provide frequent sterilization of the UF unit.
- adapt the FU design: reduce the dead volume of the filtration unit to avoid stagnation of liquid

- additional sterilization of the effluent after the filtration unit if the previous options are not sufficient. UV disinfection is well suited because the membrane permeate is devoid of particles which would reduce the efficiency of UV disinfection systems

Clearly, the problem of bacterial regrowth will have to be addressed in the design of the pilot reactor.

4 Evaluation of microbial population stability

4.1 Materials and methods

At regular time intervals, 5-ml samples were removed from the reactor centrifuged, resuspended in 2 ml of a solution containing 15% glycerol and 0.85% NaCl, and then stored frozen pending analysis. The stability of the microbial population was evaluated via molecular fingerprinting. This includes three steps:

- DNA extraction: Total DNA was isolated from the samples using the protocol reported previously by El Fantroussi *et al*. (1999) and modified by Dr. F. Faber, University of Groningen, The Netherlands (personal communication)

- PCR (polymerase chain reaction) amplification: A 455 bp eubacterial 16S rDNA from the extracted DNA was amplified by PCR as described by Marchesi *et al*. (1998) using the forward primer 63F (Marchesi *et al*. 1998) and the reverse primer 518R (Felske *et al*. 1996) in order to study the general bacterial community composition. For the methanogenic community, primers 915R and GC344F were used. The PCR conditions are given below:

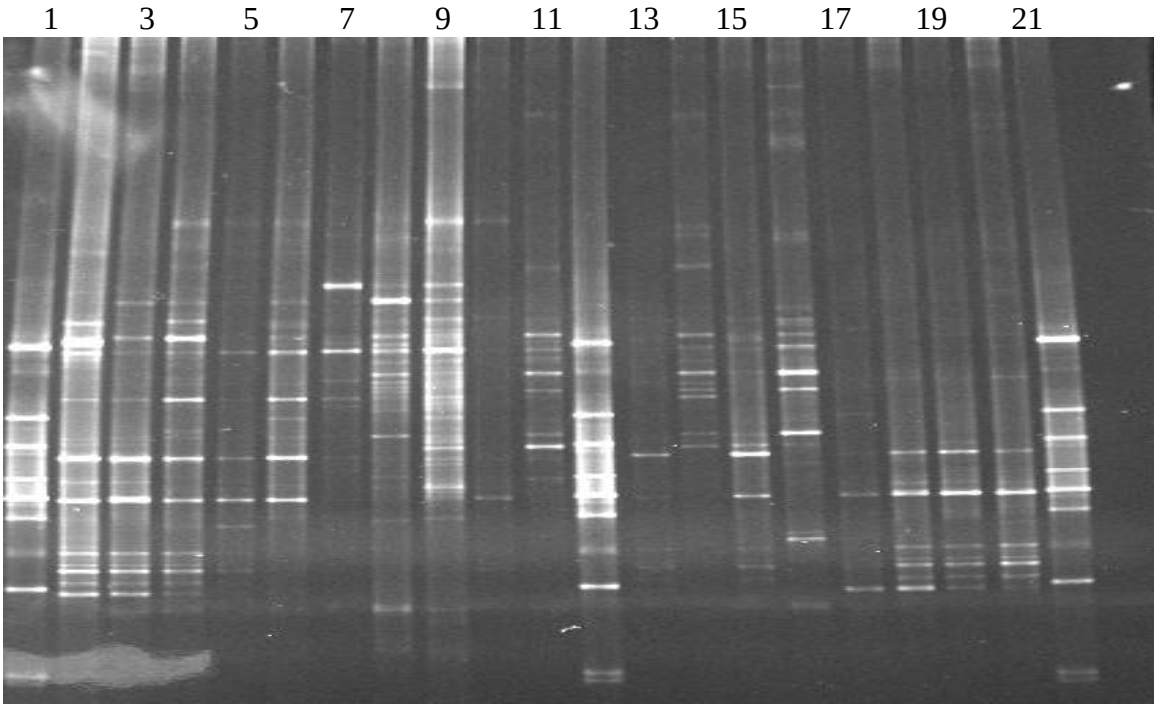
PCR conditions universal primers	PCR conditions methanogenic primers
- step 1 : 5 minutes 94°C - step 2 : 1 minute 94°C - step 3 : 1 minute 55°C - step 4 : 1 minute 65°C - repeat steps 2 to. 4 (40 cycles) - step 5 : 5 minutes 64°C - step 6 : 4°C	- step 1 : 5 minutes 95°C - step 2 : 1 minute 95°C - step 3 : 1 minute 61°C - step 4 : 1 minute 72°C - repeat steps 2 to 4 (35 cycles) - step 5 : 10 minutes 72°C - step 6 : 4°C

Proarchea primers were not used. They could not bring additional information since no changes were observed.

- DGGE analysis: The 16S rDNA amplicons were separated by denaturing gradient gel electrophoresis (DGGE) (Muyzer *et al*. 1993) with a 35 to 65% denaturant gradient on a 8% acrylamide gel. Resolved PCR products were visualised by UV transilluminatio n. The digitised images were analysed with Bionume rics software. Cluster analysis was performed with Jaccard. Diversity was calculated as the Shannon index in the Bionumerics software. Positive and negative controls were included. The negative control consisted of the PCR mixture without template. The positive control was superficial for the universal primer because it reacts with all bacteria by principle. For the Archaeal primer specific methanogenic bacteria were used as positive control. All controls and samples were loaded on an agarose gel to check for the presence of DNA. Only the samples were loaded on the DGGE gels.

4.2 Results and discussion

The DGGE fingerprints in Figure 25 visualize the general (acidogenic) bacterial community in the prototype reactor over a period of 27 weeks. The first samples were taken on October 22nd 2003 (week 78) and the last ones on April 29th 2004 (week 105). From visual observation of the fingerprints, it appears that the patterns of the 5 first samples (up till week 80) are similar. Likewise, the 4 last samples (from week 102 till week 105) show little variation and are similar to the 5 first samples in the sense that the lower bands correspond. .



lane	sample	lane	sample	lane	sample
1	DGGE ladder	8	week 89	15	week 101
2	04/395	9	week 92	16	week 102-1 (feed)
3	week 78	10	week 93	17	week 102-2
4	week 79	11	week 97 (feed)	18	week 103
5	week 80	12	DGGE ladder	19	week 104
6	week 85	13	week 99-1	20	week 105
7	week 86	14	week 99-2 (feed)	21	DGGE ladder

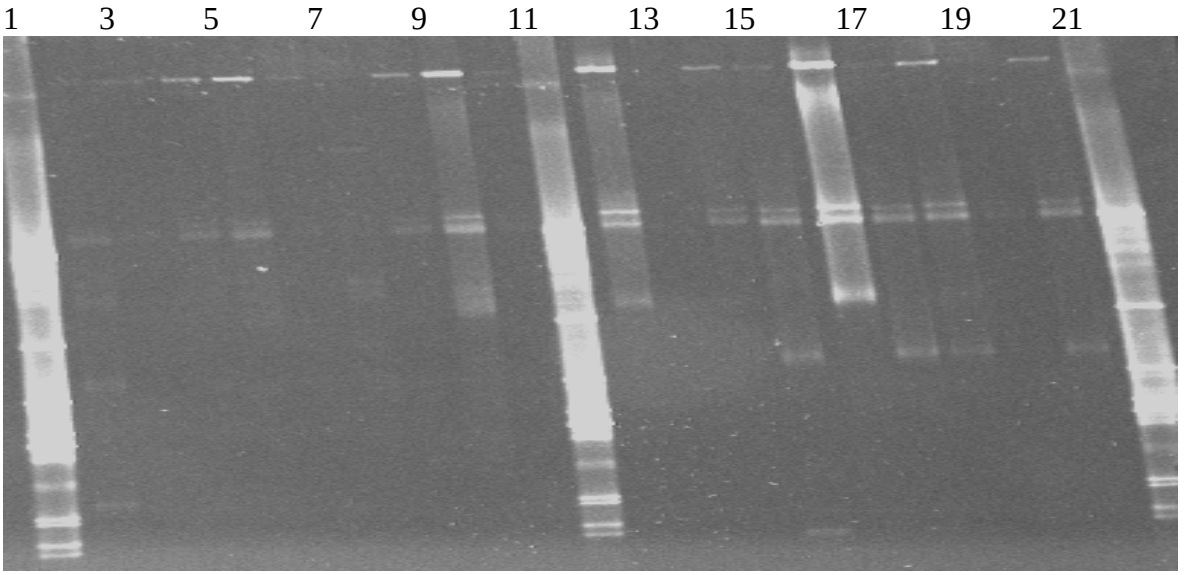
Figure 25 DGGE gel of samples taken from the prototype reactor after amplification with universal primer.

When interpreting and comparing the fingerprints, it is important to keep in mind that DGGE analysis only visualizes the most abundant species in the overall population and that these are not necessarily the most crucial ones for reactor operation. However, from the comparison of the fingerprints of the first and last samples, it appears that some populations were highly abundant at first, then their relative fraction in the total population decreased drastically and later on, they reappeared at high abundance. Probably, these dynamics are related to reactor operation. The prototype reactor was seeded with inoculum from laboratory-scale reactors which was gradually increased to the final 25-l volume without effluent removal. Around week 80, operation of the membrane filtration unit was started, which initially showed technical problems and did not operate continuously. It is evident that both the shear stress in the crossflow membrane filtration unit and the discontinuous operation resulted in a changing and unstable microbial population. From week 97 onwards, the membrane filtration unit worked continuously, but the population stabilized only a couple of weeks later. Whether this is related to the acetate pulse of week 103, can be argued. More than 50% of the VFA produced in the reactor is acetate. So, one would expect that particularly the acetate-producing acidogenic bacteria had already established at high abundances.

Lanes 11, 14 and 16 in Figure 25 are samples taken from the reactor feed rather than the reactor content. That explains why they have a different banding pattern than the other lanes. Comparison of the fingerprints also shows that bacteria from the reactor feed are not abundant in the reactor itself and cannot maintain themselves. The visualized reactor population clearly has a different composition.

Overall, it seems that the type of acidogenic bacteria dominating the reactor population, varies in time. But this does not seem to influence reactor operation and efficiency and VFA production rates.

Because the prototype reactor was inoculated with seed material from laboratory-scale reactors, samples from both types of reactors were compared. Fingerprints were different but had some bands in common. This may be related to a scale effect and to differences in operation of the membrane filtration units. At laboratory-scale, effluent was removed on a discontinuous basis which reduced the shear stress on the sludge flocs and affected the microbial population in a different way.



lane	sample	lane	sample	lane	sample
1	DGGE ladder	8	week 89	15	week 101
2	04/395	9	week 92	16	week 102-1 (feed)
3	week 78	10	week 93	17	week 102-2
4	week 79	11	DGGE ladder	18	week 103
5	week 80	12	week 97 (feed)	19	week 104
6	week 85	13	week 99-1	20	week 105
7	week 86	14	week 99-2 (feed)	21	DGGE ladder

Figure 26 DGGE gel of samples taken from the prototype reactor after amplification with archaebacteria/methanogenic primer.

Figure 26 show the fingerprints for the methanogenic populations. It should be noticed that this method does not allow a quantification of DNA. When the samples are loaded on the agarose gels to check for the presence of DNA, the intensity of the bands gives an indication of the relative amount of DNA present in the different samples. Even though the DNA amount loaded on the gels, was rather low and hence the fingerprints are less clear, it is evident that the banding pattern hardly changes over time. Lanes 12, 14 and 16 contain fingerprints of the reactor feed. These are similar to the ones of the reactor samples, except that the band intensity is higher in the feed samples. This indicates that the reactor feed provides a continuous supply of methanogens which are maintained in the reactor system.

The conclusions are therefore the following:

1. although methanogenic activity is inhibited in the prototype reactor, methanogens and/or other archaebacteria are still present in the system.

2. the methanogenic population hardly changes in time. This is not surprising because of their inactivity.

3. methanogens coming in the reactor with the feed material, can maintain themselves, probably because there is no competition from 'active' methanogenic species.

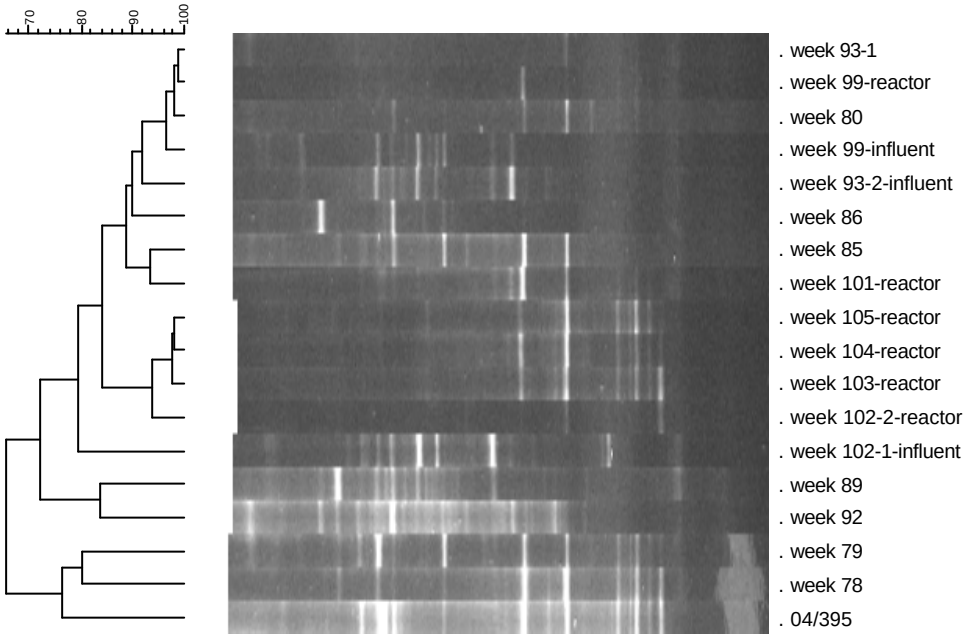


Figure 27 Dendrogram of the acidogenic population in the prototype reactor.

For a more objective evaluation of the DGGE data, the fingerprints were analysed with the Bionumerics software and clustered according to their similarity. The clustering is based on the calculation of Pearson correlation coefficients which are calculated between all fingerprints. These are then rearranged and clustered according to their similarity. The percentage can be read from the axis on the figure (e.g. for figure 27 between 70 and 100%). The similarity between two fingerprints is e.g. close to 98% for the two top ones, close to 78% for the samples of week 79 and 78 and close to 75% for the samples of week 78 and 04/395.

Figure 27 shows the result for the acidogenic populations. The following observations can be made:

1. two feed samples cluster together. The third one (week 102-1) does not. This was apparent from visual observation of the gels in Figure 25.

2. the feed samples are quite similar to the reactor samples and do not group in a separate cluster. Their similarity indicates that the bacteria entering the system with the feed, do affect the final population in the reactor somehow.

3. a big change in microbial population occurs between week 79 and week 80 (samples have a similarity of less than 70%). This is either due to a big difference in intensity of the gel lanes or it may be related to the start-up of the membrane filtration unit.

4. the earlier observation that microbial populations had somehow stabilised starting from week 102 is confirmed by the fact that this and all later samples show a similarity of over 90% and cluster in the same subgroup.

Here we need to bring to the attention that clustering itself largely depends on the relative intensity of the bands which tends to vary strongly even when running duplicate gels of the same DNA samples. These variations in relative intensity can be due to the fact that DNA is degraded during storage of the samples (e.g. enzymatically) and degradation may be different for DNA of different composition. Furthermore, the primers used in the PCR step in principle amplify all DNA present but may show some preference for amplifying specific DNA. This may result in different final amplification rates for the different types of DNA in the sample. Concerning the overall band intensity of a sample, the reason of variation is the following: after the PCR amplification, the total PCR product is put on an agarose gel to check whether DNA is present at all. If this is the case, the intensity of the band on agarose gives some indication about the DNA concentration. This is however not a quantitative procedure but is largely based on long-term experience of the technicians. The technicians choose a sample volume to be separated by DGGE based on the perceived intensity on the agarose gel. After DGGE separation, this quantity may however turn out to be too low. This can be improved by repeating the DGGE step with a larger sample volume. However, since no differences were visually observed in the fingerprints, it was not necessary to repeat the test. Indeed, repeating the test would not change the overall fingerprint, but only the band intensity.

For the methanogens/archaebacteria, the band intensity of the fingerprints (Figure 26) was too low to allow clustering analysis. Because no difference was visually observed for these fingerprints, no new gels were run.

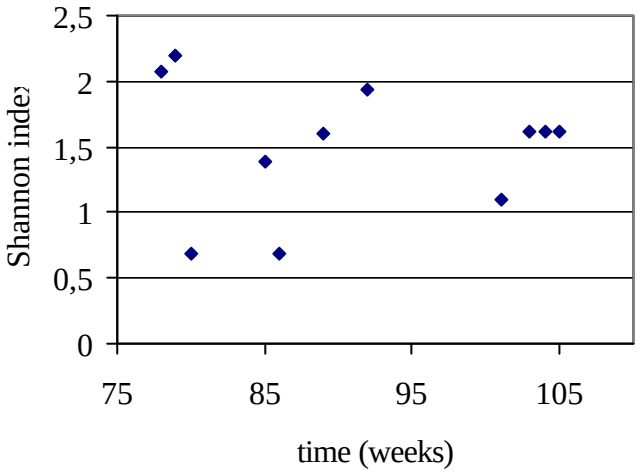


Figure 28 Evolution of Shannon index for the acidogenic population in the prototype reactor

Finally, the DGGE fingerprints were also used to assess the evolution in microbial diversity in the samples. This should ideally be fairly stable and low to allow for a more or less complete characterization of the microbial population in compartment I. Clearly, the assessment only takes into account those populations which are visualized by DGGE and these are only the most abundant ones. Less abundant groups of bacteria may also play a crucial role in reactor operation.

Figure 28 shows that the diversity index of the prototype samples fluctuates around 1.6 and more or less stabilizes for the final samples. However, this may also be due to the fact that these samples were taken in a shorter time period. It remains to be seen whether this stable and low diversity continues on the long run.

In this TN we only reported the data for the prototype reactor. More extensive work, including a comparison of an acidified and neutral compartment I, is described in Martens (2004). Several interesting bands from the DGGE fingerprints obtained in that work, were cloned and sequenced. The DNA sequences of 4 fragments were compared with 16S rDNA sequences in a database using the BLAST 2.0 software. Because the fragments are only partial 16S rDNA fragments, the identification is never

absolutely certain but it does give a strong indication on the genus. The analysis gave the following results:

- A first band showed highest similarity to an uncultured bacterium from an uranium mining waste pile. However, the sequence was also highly similar to a bacterium isolated from termite gut (90%), a *Bacteroidetes* endosymbiont of insects (90%), a *Bacteroidetes* isolate from an industrial biofilter for waste gas treatment in an animal rendering plant (88%), a novel anaerobic cellulolytic *Bacteroides* sp. 22C from a landfill leachate bioreactor (89%), a facultatively anaerobic *Dysgomonas mossii* from human clinical specimen (89%) and anaerobic *Bacteriodes* species present in swine feces and manure storage pits (89%). Schlegel (1986) mentions that *Bacteriodes* species belong to the dominant gram-negative flora of human feces (up to 10^{10}/g wet fecal material). They have a purely fermentative metabolism yielding succinate, acetate, formate, lactate and other acids.

- A second band showed high similarity to an uncultured human gut bacterium (95%), a *Eubacterium biforme* (94%) and two butyrate-producing bacteria from human feces (90%).

- A third band showed high similarity to two thermophilic cellulolytic *Clostridium* species (*C. straminisolvens* and *C.* JC3, 90 and 91%), *C. thermocellum (90%), C. aldrichii* (89%), and lower similarity to cellulolytic *Acetovibrio* species (90%).

- A final band was related to an unidentified strictly anaerobic phenol carboxylating bacterium (93%), another anaerobic bacterium transforming phenol and 4-hydroxybenzoate (92%), a *Pelotomaculum* species (92%) and an anaerobic phtalate-degrading organism from methanogenic sludge (91%).

This data seems quite plausible. Although these are only partial data referring to selected bands from the DGGE patterns, they seem to indicate that either bacteria from the feed maintain themselves in the reactor, that cellulolytic and/or fatty-acid-producing bacteria are present, and some other bacteria transforming phenolic compounds. It should however be noticed that for real identification of bacteria similarities over 95% are required.

5 Filtration Unit optimization

5.1 Action plan

One important requirement for the selected membrane is a long life time. The life time of the PVDF membranes tested was not sufficient. It is thus essential to study the fouling mechanisms and understand it in order to select the best suited membrane and to propose the most adapted strategy for cleaning and control.

The fouling mechanisms can be caused by several factors, such as concentration polarisation, adsorption, salt precipitation, biofilm development on membrane surface, pore blocking... Membrane properties (material nature, pore size distribution, porosity), module and system configurations, filtration mode, hydrodynamics but also biological conditions in the reactor also influence fouling phenomena. Fouling is difficult to control as the suspension is concentrated and contains a very large variety of compounds with different chemical properties and size. Whereas chemical cleaning is essential to remove compounds causing non reversible fouling by hydraulic means (adsorption of soluble compounds for example), hydraulic reversible fouling phenomena (polarisation, particle deposit) may be controlled by imposing tangential shear stresses close to membrane surface in association with back flushing regeneration.

Operational conditions in the reactor (temperature, pH, HRT, SRT, organic loading) condition biological kinetics rates but also membrane separation according to the suspension characteristics, the nature of compounds in solution (CO2, extrapolymeric substances EPS, soluble microbial products SMP...). On the other hand, the biological reaction may be modified by the filtration conditions notably the shear stresses that can damage the enzymatic activity, or the retention capacity that can cause some inhibitive compound concentration in the fermentor.

The membrane must play a role of barrier for the micro-organisms present in the first compartment. A filtrate free of contaminants must be sent to the second compartment since it contains an axenic culture. Tangential filtration such as the one coupled to the first compartment is not designed to provide a filtrate completely sterile. It must however reduce the bacterial population in an important way. Frontal filtration is usually use to sterilise liquids, in the pharmaceutical industry for instance. The design of the pilot filtration unit must integrate additional means to insure the microbial quality of the filtrate. Regular steam sterilisation of the membranes and of part of the unit, and a dead-end filter can be proposed. To validate a more precise design, it is essential to evaluate the log reduction that can be achieved by the Filtration Unit, when maximising the sterility in the initial conditions.

In any case, laboratory tests and experimental runs are necessary to characterise:

- the suspension properties corresponding to the optimal biological conditions (rheological properties, granulometric size distribution of compounds in solution and their physico-chemical properties)

- the evolution of the membrane permeability (runs are operated with the defined biological suspension in a specific filtration unit) to optimise the filtration conditions

- the functioning of the global system in optimal conditions

- the capacity of the membrane to reduce the bacterial contamination in optimal conditions

Based on these considerations, an action plan was defined (see Figure 29). Particularly, the mechanisms that lead to membrane fouling are important to study since they influence the control and the cleaning strategy of the unit. Another major point concerns the membrane type and the filtration control strategy.

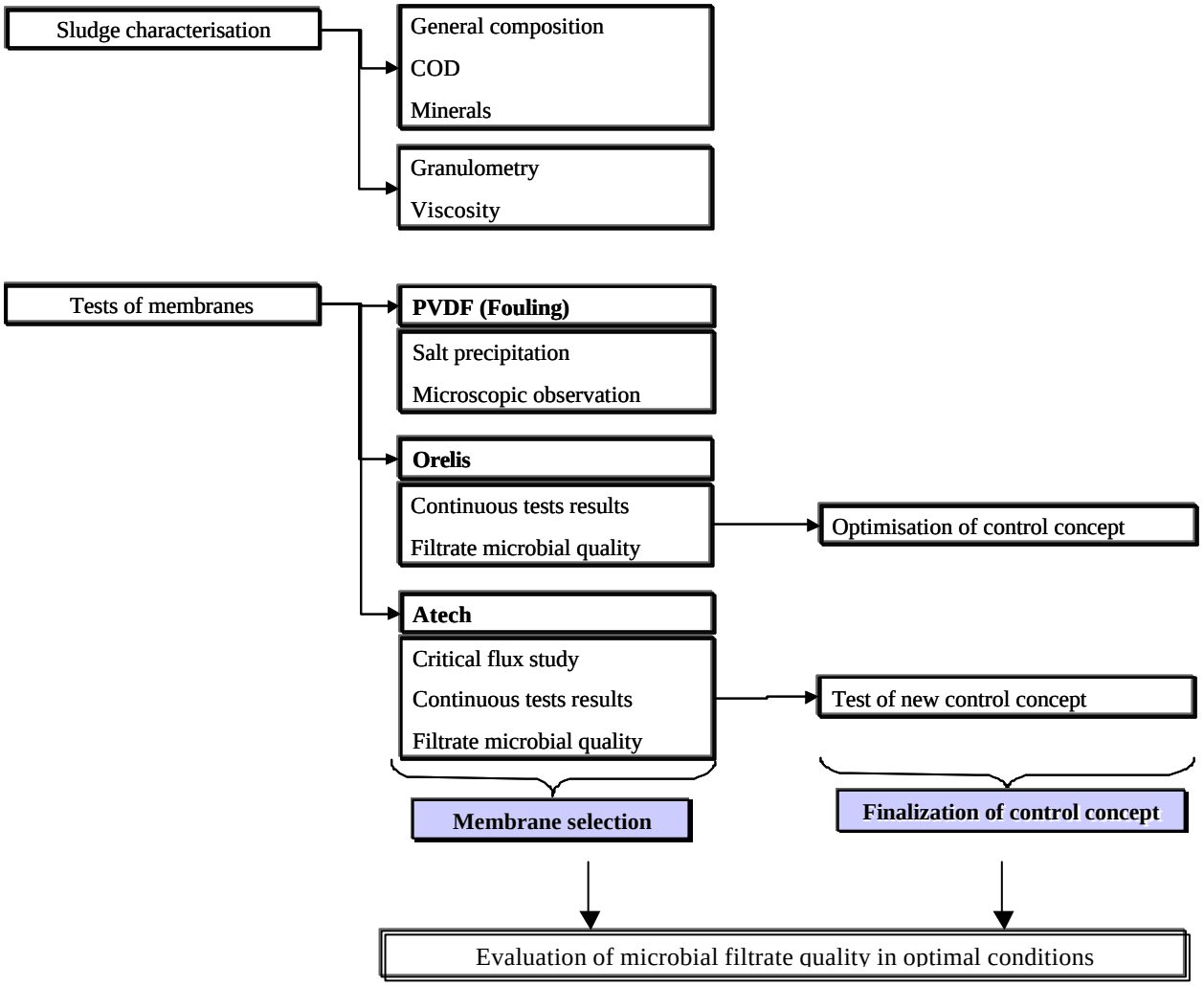


Figure 29. Additional tests

During the additional tests on filtration unit, the bioreactor was operated in the conditions described in Table 6.

Table 6. Operating conditions of the bioreactor during FU tests

Temperature	55 °C
pH	5 – 5.5
DM in reactor	30 –40 g/L
Liquid volume	25 L
HRT	10 d
SRT	6.6 months
Influent and filtrate flow	2.5 L/d
Organic load	48 gDM/d

The range of operation conditions of the Filtration Unit during the test are presented in Table 7.

Table 7. Operation conditions of the Filtration Unit

Filtrate flow	1.5 – 5 L/d
Cross-flow	2 – 4m/s
Flux	2 – 70 L/h.m^2
TMP	0.05 – 0.8 bar
Retentate flow	300 – 900 L/h
Retentate viscosity	10 – 20 cP (80 – 30 rpm)

Two control strategies were tested on the filtration unit:

- working at a constant TMP (Trans Membrane Pressure) of 0.4 bar monitored by the circulation flow of the retentate in the unit.

- working at a constant flux (maximum 5 L/h.m^2) monitored by a pump on the filtrate side.

Also, different types of membrane were tested. Their characteristics are presented in Table 8.

Table 8. Characteristics of the tested membranes

	Organic	Ceramic (1)	Ceramic (2)
Material	PVDF	ZrO2 TiO21	Alfa AI2O3 support with titanium oxide toplayer
Cut-off	30 nm	100 nm	50 nm
Length	1 m	40 cm	85 cm
Diameter	8 mm	6 mm	8 mm
Surface	0.0251 m^2	0.0075 m^2	0.0214 m^2

5.2 Study of fouling origin

5.2.1 Mechanisms inducing fouling

A lot of origins can be mentioned to explain such intensive fouling :

- A membrane degradation because of the relatively severe continuous functioning conditions (pH 5 and temperature rising 50°C). This phenomenon could explain the difficulty to regenerate the membrane properties. It could be interesting to compare structuring of new and used membrane (MEB…).

- Though hydrodynamics conditions a very tight biofilm forming can relatively quickly appear on the membrane surface because of the great biomass concentration in the fermentor. This biofilm development induces severe limitation in permeate flux even if TMP is increased. The membrane regeneration obliges to specific cleaning with oxidative reagents that can damage the organic membrane material (if the cleaning frequency is too important) but also modify the ecological anaerobic biosystem if a cleaning in place is practised (that can be avoided with a sidestream configuration).

- Despite working in acid conditions (pH 5), salt and complex precipitation may occur because of the presence of organic compounds and mineral ions in solution (Calcium, carbonate, sulfates, ammonium and phosphates).

- Because of the important CO2 production in the fermentor, a CO2 water saturation is probably obtained and a CO2 degassing can occur when permeate flows through the membrane material. Gas bubbles can then be blocked inside the pore and regeneration by back flushing is more efficient as the pore size is large.

- The liquefying role of the fermentor and the high biomass concentration developed in the system induce a large concentration of soluble by-products. Fouling may then be directly dependent of this soluble organic matter because of intensive sorption phenomena on membrane material or other interactions with salts that induce drastic drops of the membrane permeability. Membrane regeneration is then obtained by using chemical cleaning.

Of course all these phenomena may occur simultaneously and it is important to develop operational conditions that minimise fouling rates (biological conditions, membrane material and hydrodynamics) supposing that the system configuration is correct (by working with constant permeate flow rate).

5.2.2 Sludge characterization

5.2.2.1 General composition

The average composition of the filtered sludge during the tests of FU optimization is presented in Table 9. It can be noticed that dry matter content is particularly high.

Table 9. Average composition of the sludge

Dry matter (g/L)	33.5 (25 – 45)
Ashes (g/L)	3.5
Organic Matter (g/L)	30
Total COD (mgCOD/L)	50 000
Soluble COD (mgCOD/L)	8 500
Total Nitrogen (mg/L)	1 200
Ammonium (mg/L)	380
VFA (mg/L)	4 400
pH	5.2
EC (mS/s)	6.7

5.2.2.2 COD

Table 10 shows the composition of sludge and filtrate in terms of total and soluble COD. The particular COD is the difference between total and soluble COD, and represents thus the solids. It was shown that the 3 tested membranes with their 3 different cut-off allow an optimal retention of the solids since no particular COD was found back in the filtrate.

Table 10. COD composition of filtrate and sludge

	PVDF Membrane		**Ceramic membrane (Orelis)**		**Ceramic membrane (Altech)**	
Cut off	30 nm		100 nm		50 nm	
	Filtrate	Sludge	Filtrate	Sludge	Filtrate	Sludge
COD total (mg/L)	7000 – 9000	30000 – 60000	7000 – 9000	40000 – 60000	6000 - 12000	44000 - 54000
COD soluble (mg/L)	8000 – 9000	9000 – 10000	7200 - 8700	9000 – 10000	6000 - 12000	7500 - 8500
Particular COD (COD total – COD soluble) (mg/L)	0	21000 - 50000	0	31000 – 50000	0	36000-46000
VFA (mg/L)	4000 – 5200		4000 - 4700		3800 - 4600	

5.2.2.3 Organic and minerals precipitates

Depending on operational conditions such as pH and temperature, minerals and organic compound can precipitate and foul the membrane surface. High pH and low temperature favour the formation of these complexes (except for $CaCO_3$ precipitation that increases with temperature). The conditions of the prototype reactor (pH of 5.2 – 5.5 and temperature of 50°C) should thus prevent such precipitation. However it is interesting to measure the mineral composition of the sludge in order to evaluate whether these complexes can potentially be formed.

Table 11 shows the mineral composition of the sludge. It reveals important levels of phosphor, calcium, potassium, magnesium and sodium. Calcium and magnesium can precipitate in presence of carbonate in the form of $CaMgCO_3$. Magnesium can also be complexified with ammonium and phosphate to form struvite $MgNH_4PO_3$. Also other minerals can precipitate with organic molecules such as proteins.

Table 11. Sludge mineral composition

Element	**Concentration (µg/L)**
Total phosphor P	87 000
Aluminium Al	2800
Antimony Sb	<10

Arsenic As	19
Barium Ba	430
Beryllium Be	<15
Cadmium Cd	14
Calcium Ca	250000
Chromium Cr	5200
Cobalt Co	21
Iron Fe	70000
Potassium K	570000
Copper Cu	2200
Mercury Hg	2.7
Palladium Pb	73
Magnesium Mg	50000
Manganese Mn	1300
Molybdenum Mo	720
Natrium Na	120000
Nickel Ni	1900
Selenium Se	10
Silicon Si	32000
Strontium Sr	690
Tin Sn	160
Titanium Ti	150
Vanadium V	21
Tungsten W	<10
Zinc Zn	8300

5.2.2.4 Granulometry

Figure 30 shows the repartition of particles in function of their size. It should be mentioned that 33% of the particles are smaller than 2 µm. Particles with size in the range of the membrane cut off can induce mechanical blocking of the pores and possibly physical degradation of the internal surface of an inorganic membrane.

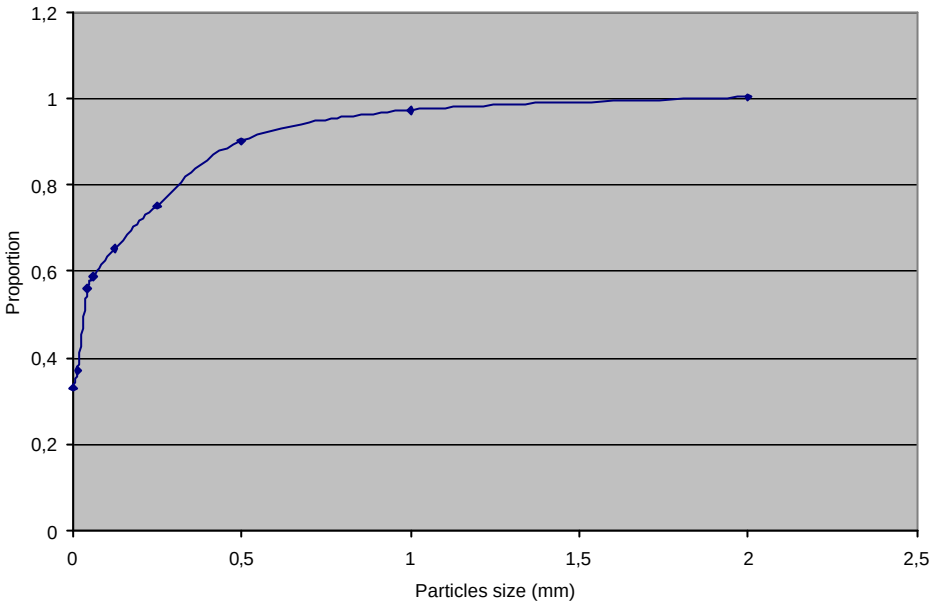


Figure 30. Size repartition of particles in the sludge

5.2.2.5 Conclusions

The suspension characterisation shows a very complex mixing of organic and mineral compounds present in soluble or particular form with a very large granulometric distribution.

So it can be supposed that the control of the membrane separation step will be difficult.

The challenge is then to choose :

- the optimal membrane characteristics to minimise interactions with compounds in suspension and facilitate membrane regeneration
- the optimal hydrodynamics conditions to minimise the fouling rates
- the optimal cleaning conditions

Some experiments must be carried on to identify the main fouling origins according to filtration conditions.

5.2.3 Investigation of the fouling origin of a PVDF membrane

5.2.3.1 Test of salt precipitation

As previously mentioned, salt precipitation can be partially responsible of the membrane fouling. These complexes can be dissolved and thus removed of the membrane surface by an acid cleaning. Therefore the acid cleaning of a fouled membrane can give useful information about the relative importance of salts in the fouling mechanism.

A PVDF membrane already in use for 3 weeks in the unit was removed and rinsed with water. Then a solution of nitric (50%) and phosphoric (50%) acids with a pH of 2.5 was circulated through the membrane during 1h45. Tap water was then circulated during 2h30.

Figure 31 shows the evolution of the flux just before and after the acid cleaning (cleaning is represented at time 0).

The TMP during the test was set at 0.4 bar. With a new PVDF membrane, a flux of around 35 –45 L/h.m^2 can be expected.

Even a permeate flux increasing is observed; it is clear that the acid cleaning procedure is not sufficient to allow the membrane regeneration (according to the initial membrane permeability),. So the membrane fouling has probably an other origin.

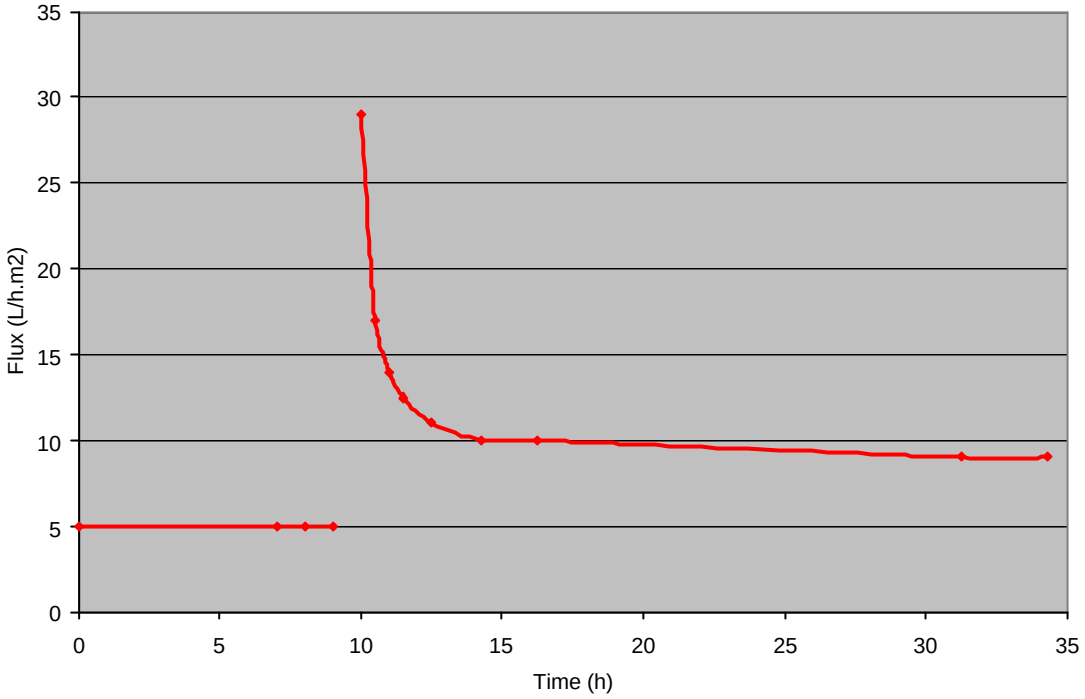


Figure 31. Flux evolution of a PVDF membrane cleaned with acid

5.2.3.2 Microscopic observation

A fouled PVDF membrane was used for microscopic observation. One extremity of the membrane was colored in yellow by liquid and solid residues. The other side was colored in black. Samples were taken on the three sectors as defined in Figure 32 : black side, center and yellow side.

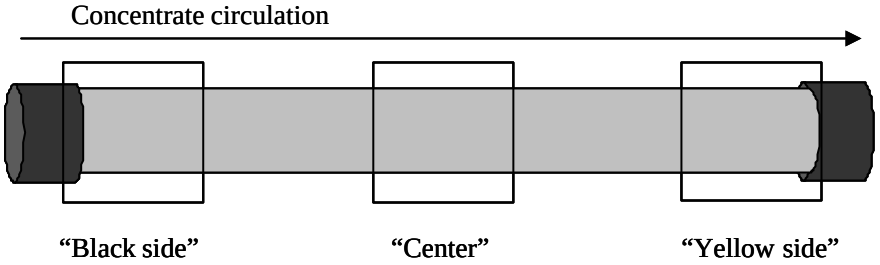
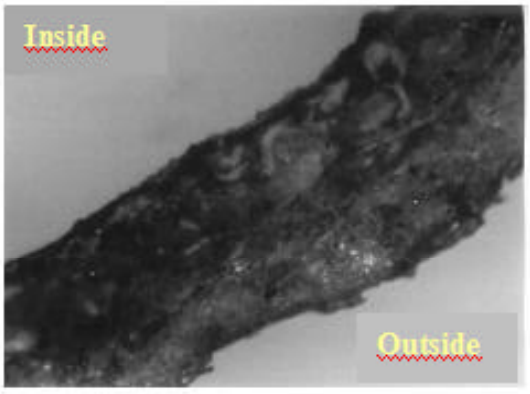


Figure 32. Schematic representation of a fouled PVDF membrane

Samples cut was done with immersing the membrane in liquid nitrogen. They were then coated with an epoxy resin and polished with SiC paper (120 to 1200). The different pictures taken with the photonic microscope show the presence of black residues in the internal part of the membrane, with a gloss and crystalline aspect. The same residues can be observed after coating and polishing.

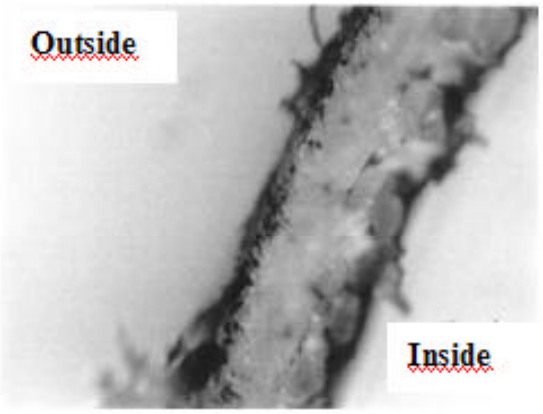
This preliminary observation will be completed with an observation of a new PVDF membrane for comparison. Moreover, observation with a MEB coupled with a spectrometer will be performed in order to analyze the composition of the membrane and the residues.

Figure 33 shows the pictures obtained with the first observation.

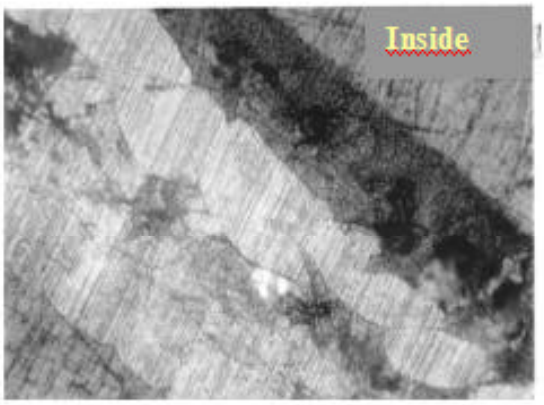


Black side : G*50
Liquid nitrogen cut

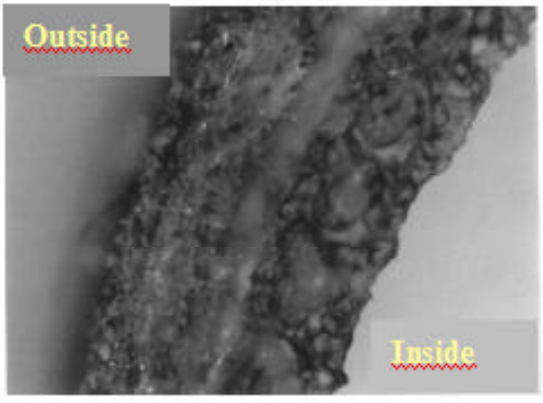
Black side : G*100
Paper polishing SiC 1200



Center : G*50
Liquid nitrogen cut



Centrer: G*100
Paper polishing SiC 1200



Yellow side : G*50
Liquid nitrogen cut

Yellow side : G*100
Paper polishing SiC 1200

Figure 33. Microscopic observation of a fouled PVDF membrane (cross sections)

5.2.3.3 Conclusions

The material seems to contain compounds in its whole depth. Because of the colour difference, this fouling would be more important at the membrane entrance (because of greater transmembrane pressure in this area).

The problem is to identify the blocked compounds:

- Are their coming from the suspension (SMP or VFA) but, because of their small size they should not be blocked inside the material except if interactions with membrane material are important (according to the high solute concentration in the permeate)

- Could they correspond to a biological development (pictures show deposit with filamentous shape on both sides of the material). In this case the biological contamination can only have a downstream origin but it should be more homogeneous along the membrane.

5.3 Process operation:

5.3.1 Tests with ceramic membrane Rhodia Orelis

From the conclusions of the tests with organic membranes, it was decided to test ceramic membranes. The Kerasep membranes from Rhodia Orelis were selected for a simple continuous test of 1 month, in the same conditions as for the PVDF membranes, in order to evaluate the difference in terms of fouling and behaviour.

The membrane used was a Kerasep KERMBMM Ceramic made of ZrO_2 and TiO_2, with a cut off of 0.1 µm. The membrane consisted in a monochannel of 6 mm diameter and 40 cm length (surface: 0.0075 m^2). It was inserted in an industrial stainless steel module, especially designed to reduce the risk of filtrate contamination (good tightness of the membrane at the extremities of the module, reduced dead volume for filtrate).

5.3.1.1 Unit configuration

The configuration used for this test was the same as used with the PVDF membrane, in order to compare their performances in the same conditions (see Figure 34). This means a control of the process based on TMP control (fixed at 0.6 in a first period, then at 0.4 bar). This TMP is regulated by the flow of sludge through the unit and thus the pump speed. The buffer tank of the unit is used, thus the concentrate and the reactor content are not continuously homogenized, causing variations of temperature and dry matter content in the sludge filtered inside the unit. The use of the buffer tank is not optimal since these variations may interfere with the filtration process and should be avoided.

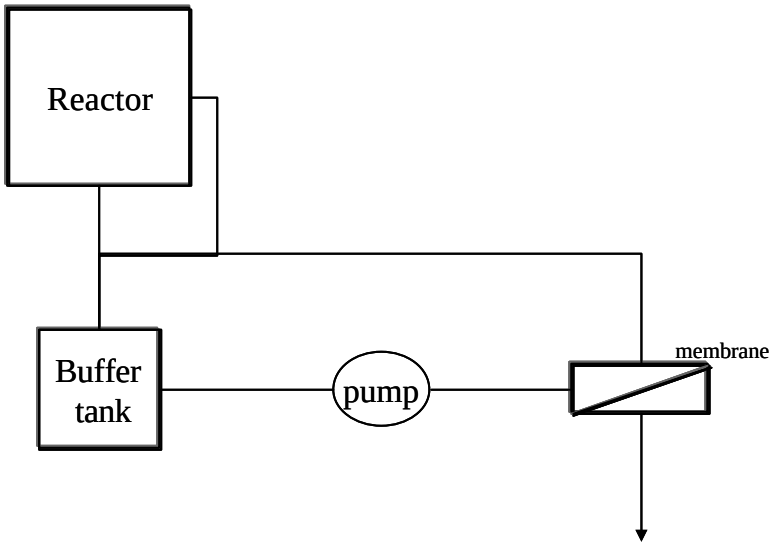


Figure 34. Schematic representation of unit configuration (Orelis membrane)

5.3.1.2 Results in continuous operation

Figure 35, Figure 36 and Figure 37 show respectively the evolution of flux, pressures and cross flow during one month of test.

The flux shows high fluctuations around an average of about 30 L/m^2.h, as a consequence of the pressures variations. This average seems not to decrease for a period of at least 25 days, which is significantly better than with a PVDF membrane. After 25 days, a decrease of flux was measured. This is correlated with an increase of the pressure at entrance of the membrane module and a decrease of the cross flow velocity. This is likely due to a beginning of clogging of the membrane channel more than to fouling.

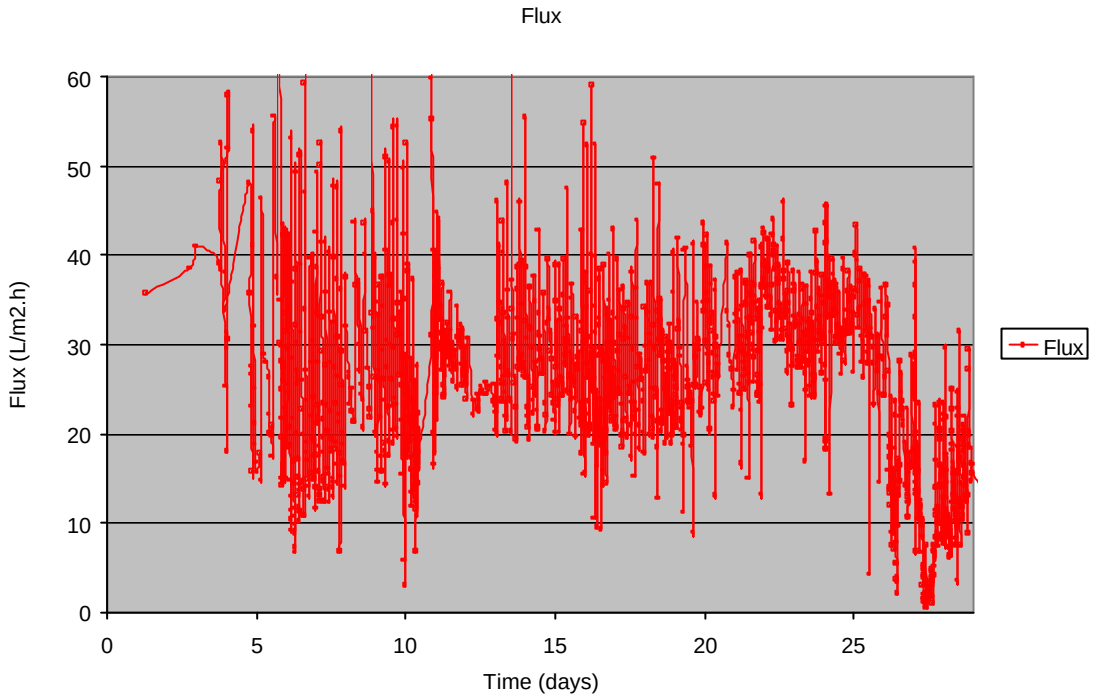


Figure 35. Flux evolution (Test of Orelis membrane)

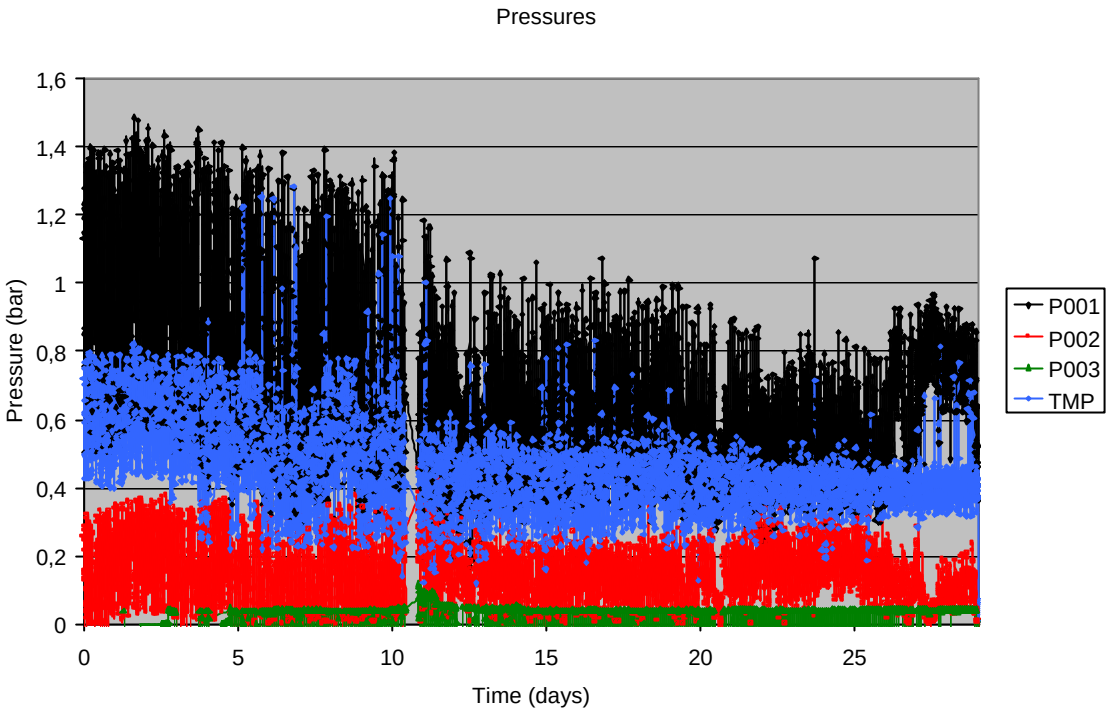


Figure 36. Pressures evolution (Test of Orelis membrane)

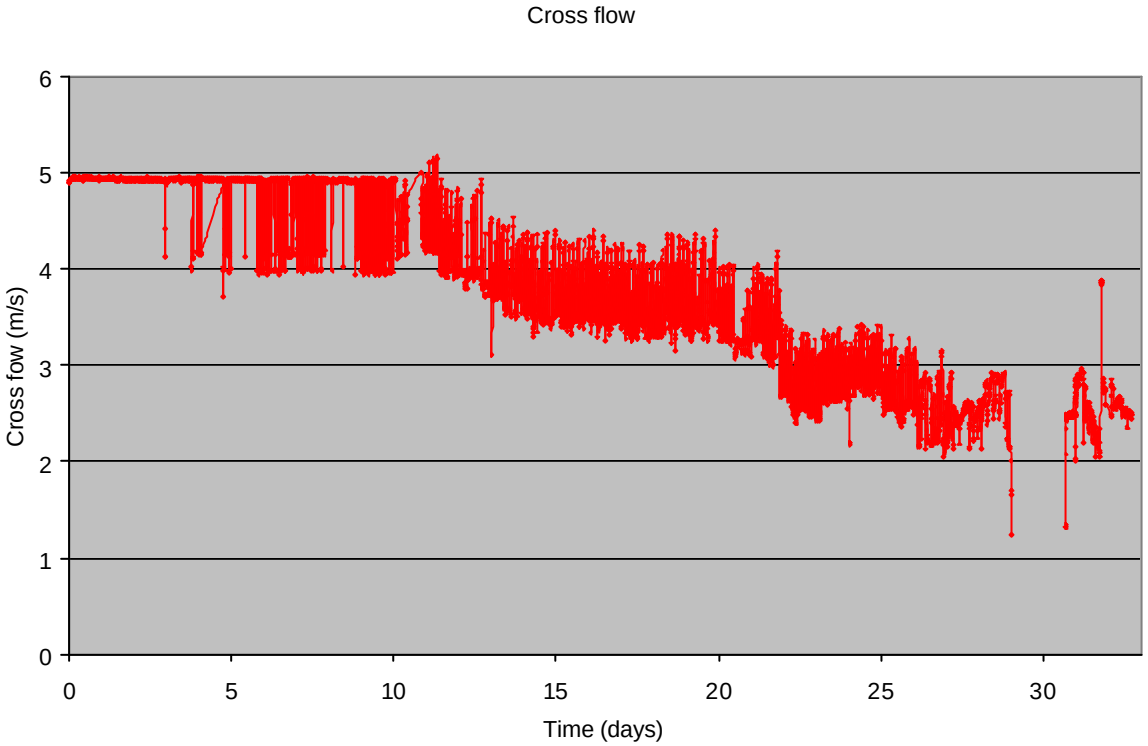


Figure 37. Cross flow evolution (Test of Orelis membrane)

5.3.1.3 Conclusions: TMP control versus flux control

From these results, it can be conclude that a ceramic membrane shows less drastic decrease of the flux when it is not controlled (see comparison in Figure 38). It is thus interesting to continue to test ceramic membranes, while optimizing the unit configuration and filtration concept. The results showed that TMP control induces variations of cross flow, which can accelerate the fouling. Important variations of pressure must also be avoided. Also, the variations of temperature and dry matter content of the concentrate induced by the use of the buffer must be avoided. The cross-flow evolution also points out clogging phenomenon and the importance to avoid any particle flocculation in the suspension.

Additionally, a preliminary aerobic count was done on one sample of filtrate during the test of the Orelis membrane. No contaminant was found.

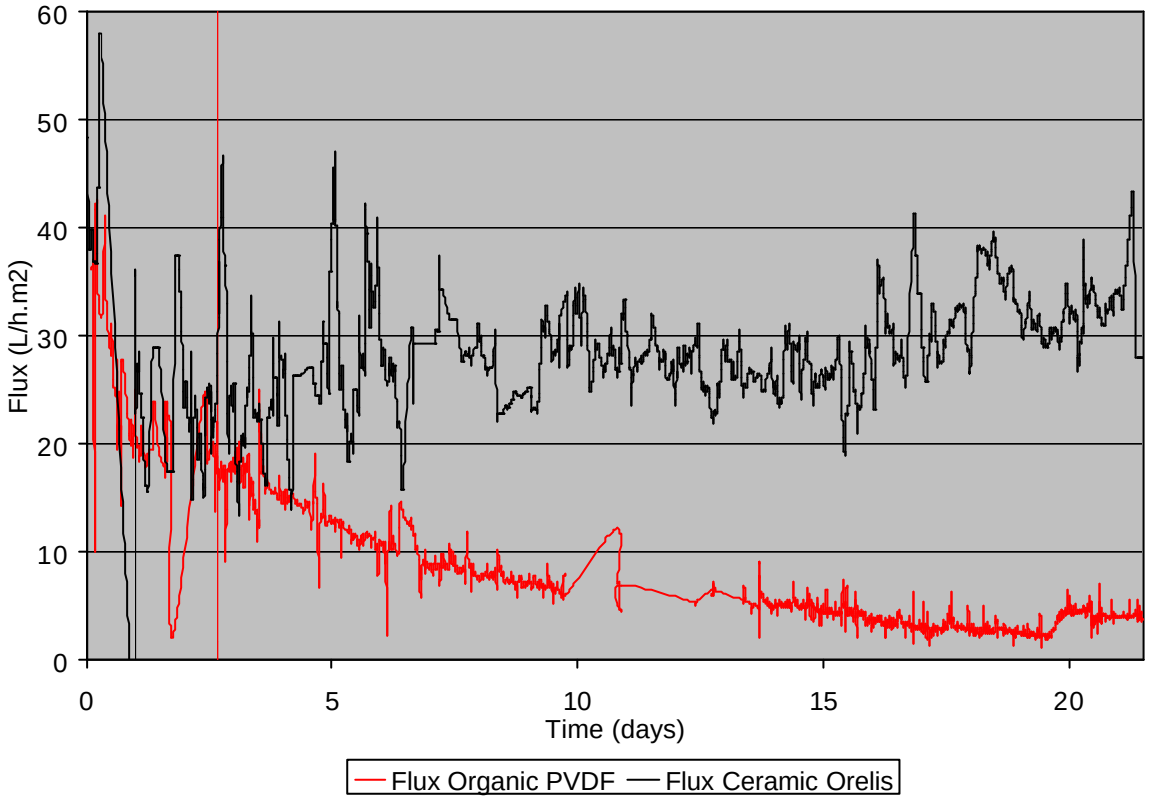


Figure 38. Comparison of flux evolution of 2 membrane types (organic and ceramic) under constant TMP

5.3.2 Tests with ceramic membrane Atech

For further tests, another type of ceramic membrane from Atech was tested. The Alpha membrane was selected, with a support of Al_2O_3 and a top layer of titanium oxide. The membrane is a monochannel of 8 mm diameter, 85 cm length (surface: 0.0214 m^2) and a cut off of 50 nm (200 – 500 kD).

5.3.2.1 Critical flux study

5.3.2.1.1 Principle

This test must be carried on with a new membrane. It consists in increasing the permeate flux J step by step (5, 10, 15, 20 ...$lh^{-1}m^{-2}$) during short periods (20 to 30 minutes) and measuring TMP variations. The TMP evolutions vs J have then to be compared to its evolution when filtering pure water.

This test allows the identification of:

- a critical flux Jc (above which linearity between TMP and J disappears and an external hydraulic reversible fouling appears)

- the presence of an internal fouling (difference between straight line slopes observed between water and suspension filtration at the beginning of each graph, see Figure 39).

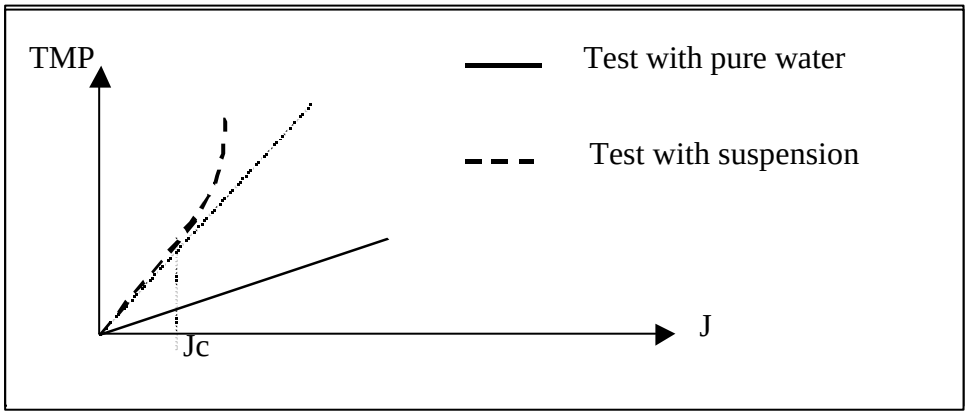


Figure 39. Principle of a critical flux study

A very important internal fouling exists if no straight line appears on the suspension curve, Jc can not be identified. Fouling has then to be identified by cleaning procedure before being able to optimise the system by changing adequate operational conditions (membrane material, pore size, biological, physical conditions...).

If Jc is clearly identifed and its value acceptable on a technico-economical point of view, two strategies can be developed:

- If internal fouling does not appear important on short period (straight parts are superposed), working can be done under the Jc value and non reversible fouling studied on longer periods.

- Internal fouling is important and obliges to frequent chemical cleaning: in this case working can be developed but imposing an external deposit (J>Jc) to block fouling material inside the deposit and not inside the membrane material. A control of the deposit development can be ensured by periodical back-flushing. This procedure necessitates a good knowledge of the system characteristics. If internal fouling can not be controlled by this way, only modification of operational conditions can solve the problem.

5.3.2.1.2 Results

Figure 40 shows the curves obtained with water and sludge on a new ceramic membrane. It can be concluded that external fouling occurs only for very high fluxes on short periods of time. Furthermore, the significant difference between the lines slopes indicates an important internal fouling.

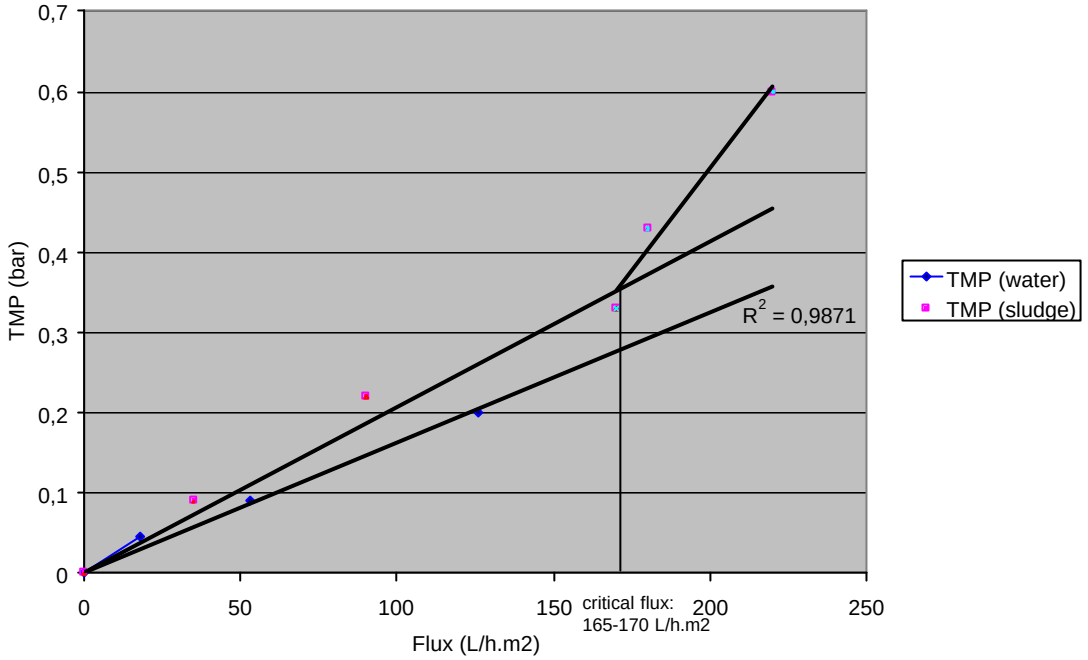


Figure 40. Critical flux study

5.3.2.2 Unit configuration and process optimisation

After the critical flux study, the ceramic membrane was used in continuous operation to provide 2.5 L/d of filtrate. The configuration used was adapted in order to optimize the process (Figure 41):

- the buffer tank was skipped: the unit was directly connected to the bioreactor, inducing a continuous homogenization of the sludge in the whole system and preventing variations of temperature and dry matter content in the sludge. The effect on temperature is shown on Figure 43.

- An expansion consisting in a vessel containing compressed gas regulated by a membrane was inserted in the unit to soften the pressure variations. Figure 42 shows the effect of the expansion on the pressure. Pressure fluctuations were reduced to 10– 20 mbar and can still be lowered by optimization of the settings.

- Control strategy was switched to flux regulation by using a small pump on filtrate side.

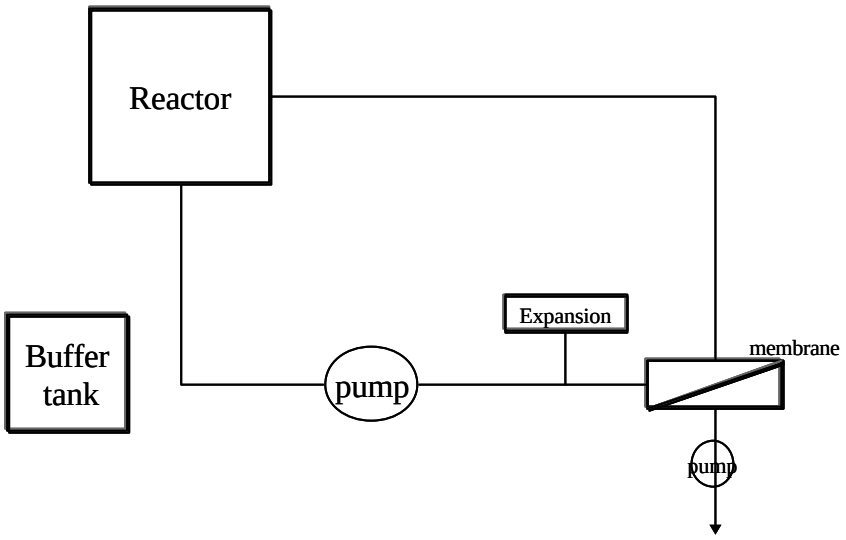


Figure 41. Schematic representation of unit configuration (Atech membrane)

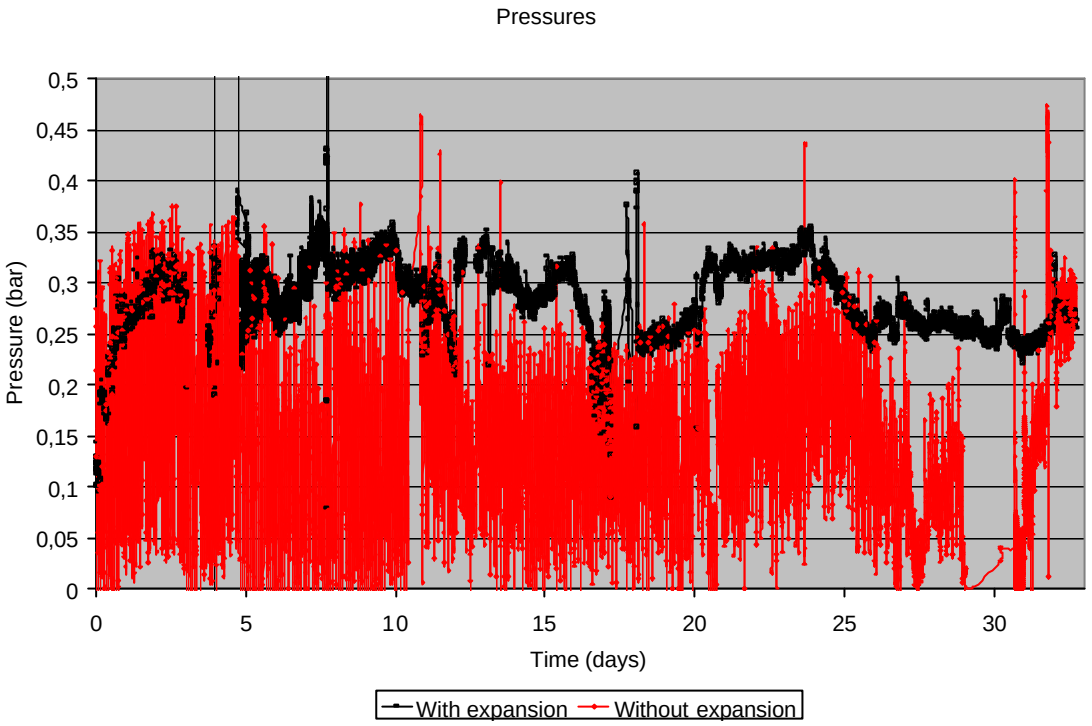


Figure 42. Pressures fluctuations with / without expansion

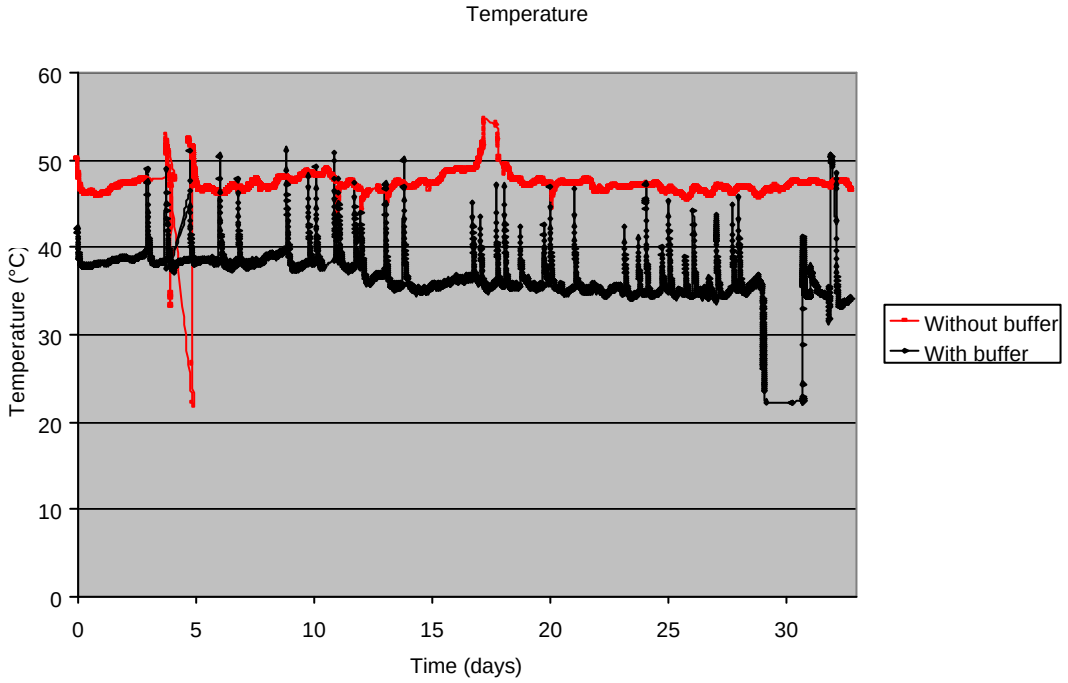


Figure 43. Temperature variations with / without using buffer tank

5.3.2.3 Results of continuous operation

Figure 44, Figure 45 and Figure 46 show respectively the evolution of flux, pressures and cross flow during the test. The total period of test was 125 days. From 80 days, higher fluctuations of pressures appeared and punctual clogging of the membranes. This was due to the concentration of solids in the reactor: the dry matter concentration was of 32 g/L at day 0, 52 g/L at day 80 and 55 g/L at day 12. (accumulation for drain study). This result confirms that the dry matter content should be kept under 50 g/L to prevent clogging.

The flux fluctuates a bit around the set point due to the type of pump used on the filtrate side. The test was indeed realized with a centrifugal pump which could create slight variations of the flux. This could be avoided on the pilot unit by using a volumetric pump. Two set points were used in this period, 5 L/h.m^2 during about 17 d and 2 L/h.m^2 in a second period, during the first 70 days.

The pressure graph (Figure 45) indicates slight punctually increases of the pressures and TMP likely due to clogging of the channel (because of the increase in Dry Matter content in the reactor) rather than to a fouling of the membrane pores. On a long time scale, the TMP seemed to stay relatively stable, until the increase of the dry matter content above the limit of 50 g/L. Therefore the fouling during the 39 d of test seems very minor. The cross-flow was stable (2.5 m/s) due to a stable flow through the unit (fixed to 450 L/h).

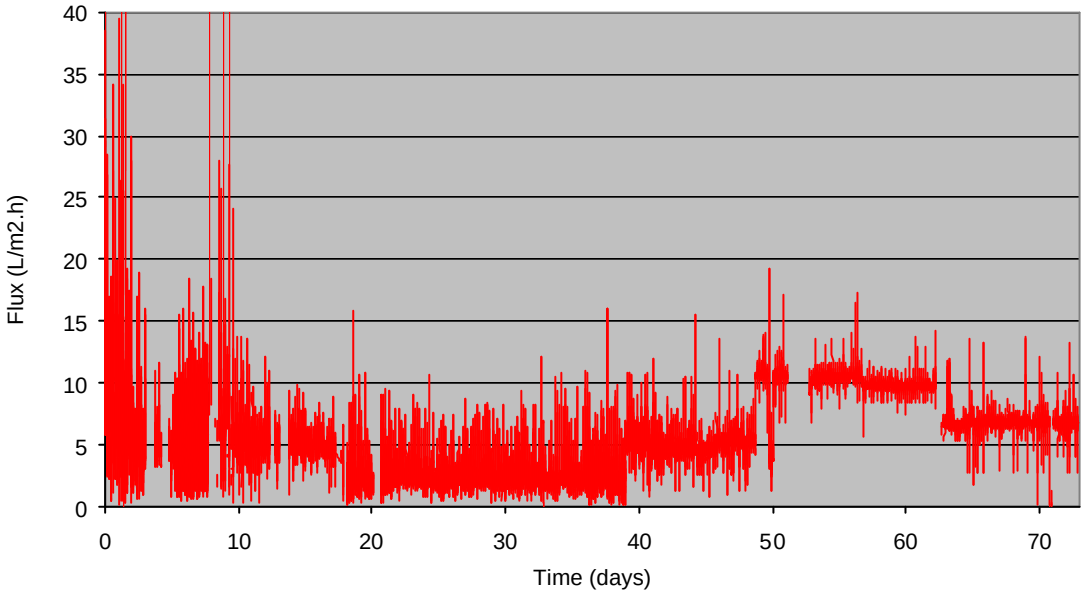


Figure 44. Flux evolution (test of Atech membrane)

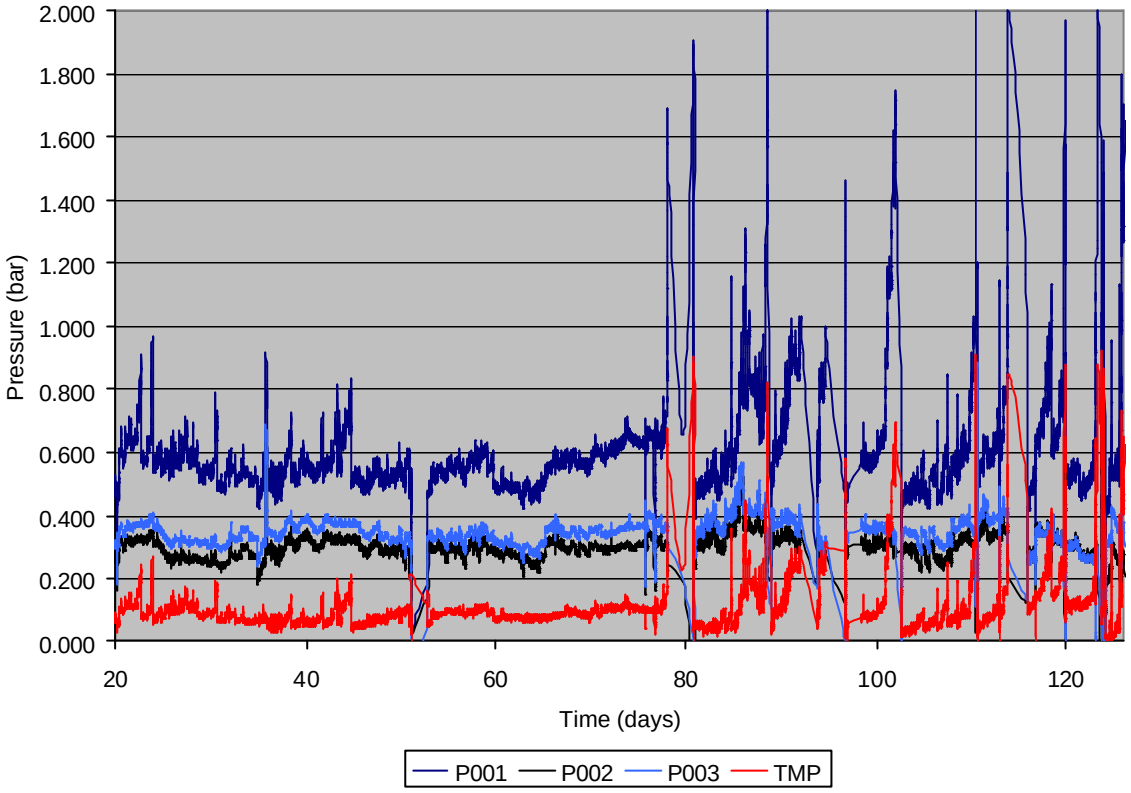


Figure 45. Pressures evolution (Test of Atech membrane)

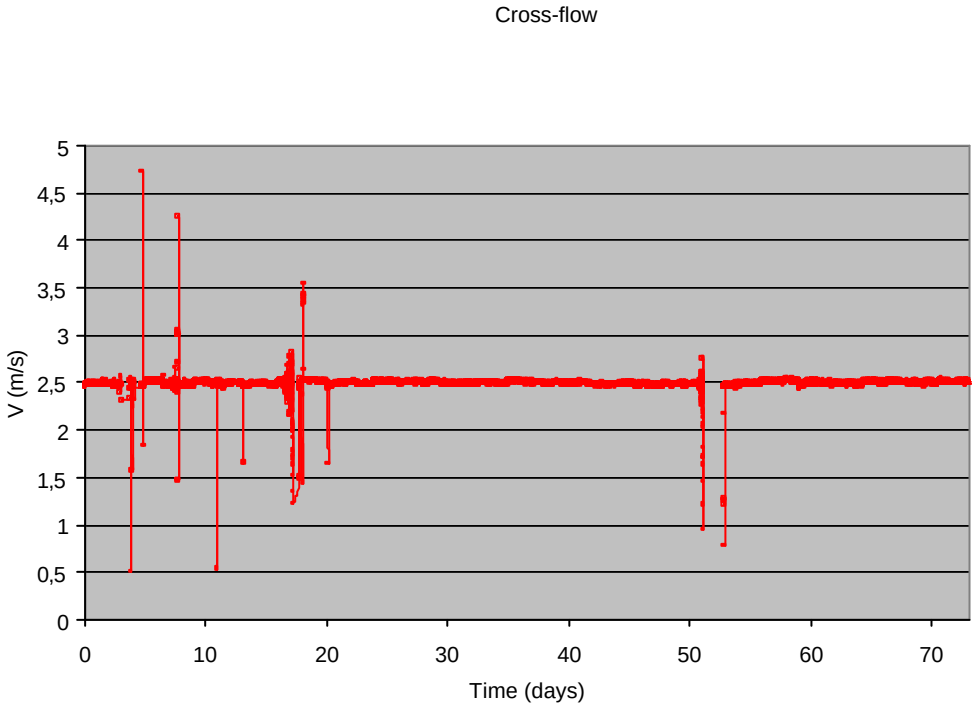


Figure 46. Cross-flow evolution (Test of Atech membrane)

5.3.2.4 Filtrate microbial quality

5.3.2.4.1 Test 1

Samples from reactor content and membrane filtrate were taken. Total aerobic counts were determined at 25°C on a medium for total aerobic count (APHA), total anaerobic counts were determined at 44°C on Schaedler sheep blood agar. Figure 47 presents the results of the count. It can be seen that still high level of contamination is obtained in the filtrate, even though dead volume of module was reduced. The prototype filtration unit was not designed to be sterilizable, therefore on-line sterilization is not possible and increase the difficulty to work in sterile conditions.

It can be noticed that the populations (both aerobic and anaerobic) seem to follow in proportion the population of the reactor content samples when it fluctuates. This could mean that the contaminants come directly from the filtered sludge, and may pass on the filtrate side on the extremities of the membrane where it is fitted in the module. However, this level of contamination is too important and adaptations of the prototype unit to allow sterilization should allow to decrease in an important way the bacterial community present in the filtrate.

date	sample	aerobic	anaerobic
		25°C	44°C
05/10/04	reactor	$1 . 10^6$	$5 . 10^5$
	Filtrate	$3 . 10^4$	$4 . 10^6$
11/10/04	reactor	$5 . 10^4$	$7 . 10^4$
	Filtrate	$5 . 10^3$	$6 . 10^3$
18/10/04	reactor	$3 . 10^6$	$2 . 10^7$
	Filtrate	$2 . 10^4$	$2 . 10^6$

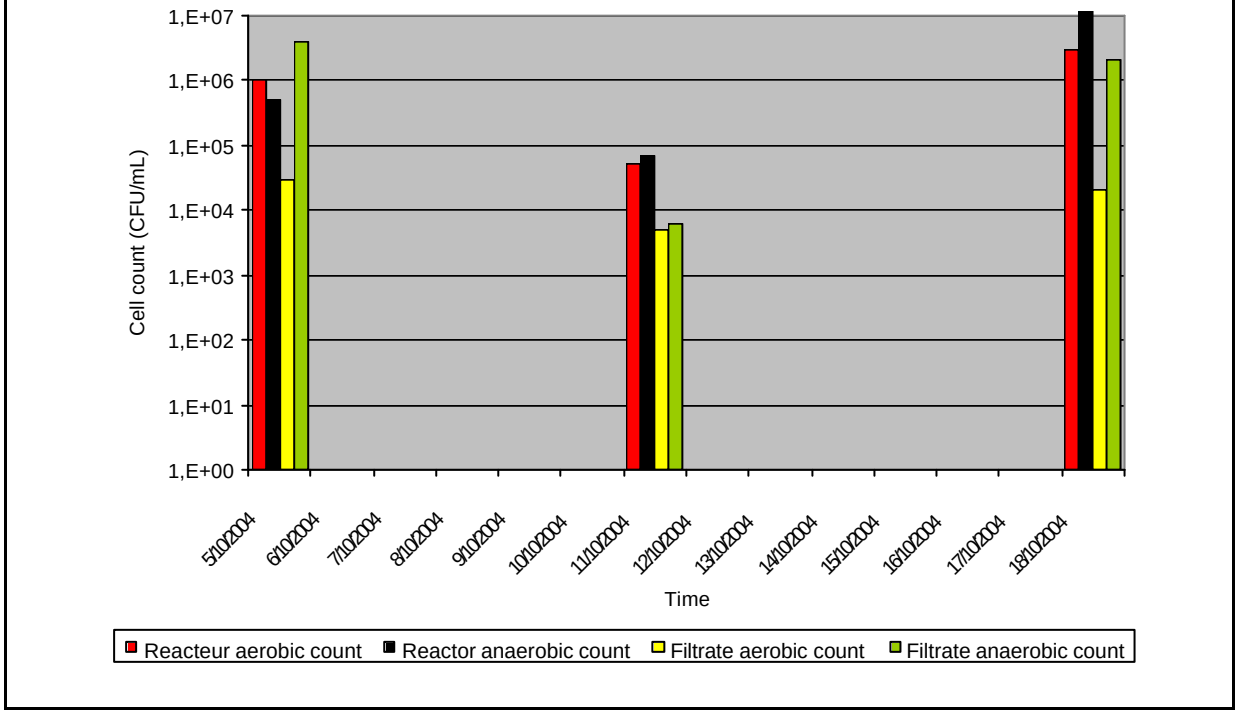


Figure 47. Aerobic and anaerobic count (Test of Atech membrane)

5.3.2.4.2 Test 2

For this test, the configuration of the unit was adapted to produce a filtrate as sterile as possible (see Figure 48). The membrane was introduced in an industrial stainless steel module also from Atech. New Teflon tubes were installed, and sterilisable Swagelok valves and pump tubes. The filtrate tank used to evaluate the flux was replaced by a sterilisable Schott bottle. A dead-end filter was added on the line, in order to prevent backward contamination. Special sterile bags were used for sampling (Figure 49).

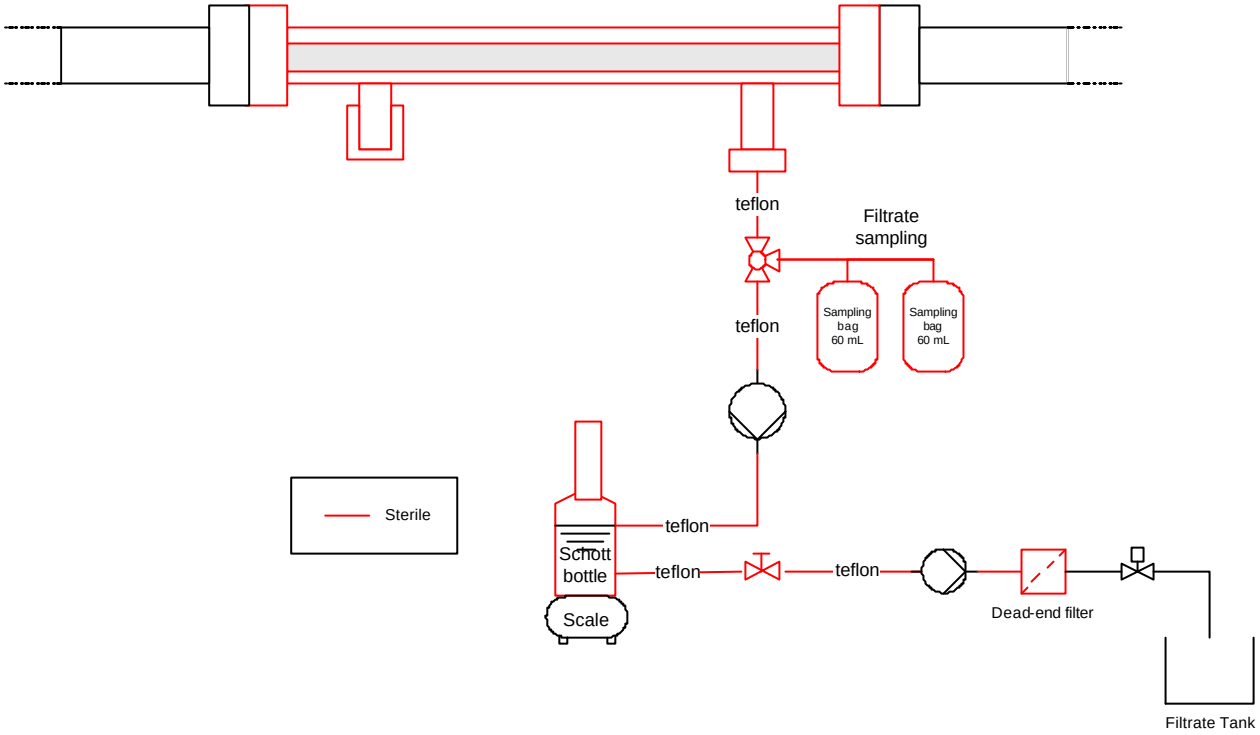


Figure 48. Configuration of the prototype filtrate line

Figure 49. Sterile bags for filtrate sampling

On forehand, the new membrane was mounted in its module and its retention capacity was tested (Figure 50). The curve shows a good retention, in concordance with the specifications given by the constructor. This indicates that the membrane is tight for bacteria as in theory, which is of high importance to interpret the results of the test.

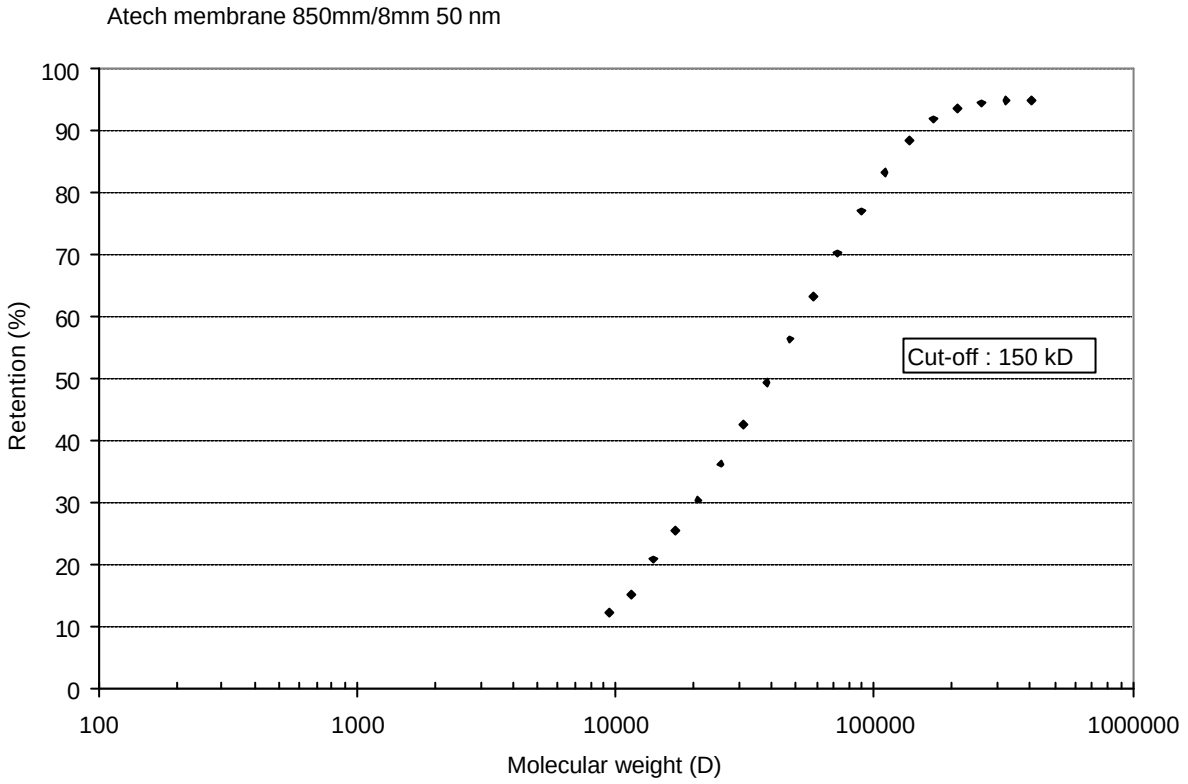


Figure 50. Retention test on the new membrane

Samples from reactor content and membrane filtrate were taken. Total aerobic counts were determined at 25°C on a medium for total aerobic count (APHA), total anaerobic counts were determined at 44°C on Schaedler sheep blood agar.

Figure 51 presents the results of the count. In this case, no contaminations were found in the filtrate during a period of test of 14 days for the aerobic bacteria, but a contamination was found from day 3 and then increasing.

Because anaerobic cell counts increased in later samples, some bacteria could have been already present in the first permeate samples, but in too low numbers to be detected by plating. To check this, the first permeate samples were incubated at 44°C for 4 days and then plated again. Again, no anaerobic colonies were found. This implies that the first samples indeed did not contain any cells.

Because at a later stage only anaerobic bacteria and no aerobic bacteria were found in the permeate, some bacteria must have managed to grow through the membranes or pass through them after a while. Indeed, contamination of the permeate by air or the surroundings would lead to aerobic counts whereas bacteria from the reactor content would rather yield anaerobic contamination.

A new retention test was performed with the membrane after the test (see Figure 52), in order to check if a damage could be responsible of the contamination. It was shown that the retention was not in the expected range anymore, which let suppose that the membrane had been damaged. Damage may have occurred during transportation or sterilization, even if not likely. A bubble test was performed in order to

possibly visually localize the damage: the membrane was immersed in water and flushed with compressed air (Figure 53). This test allowed to show that one side of the membrane was damaged.

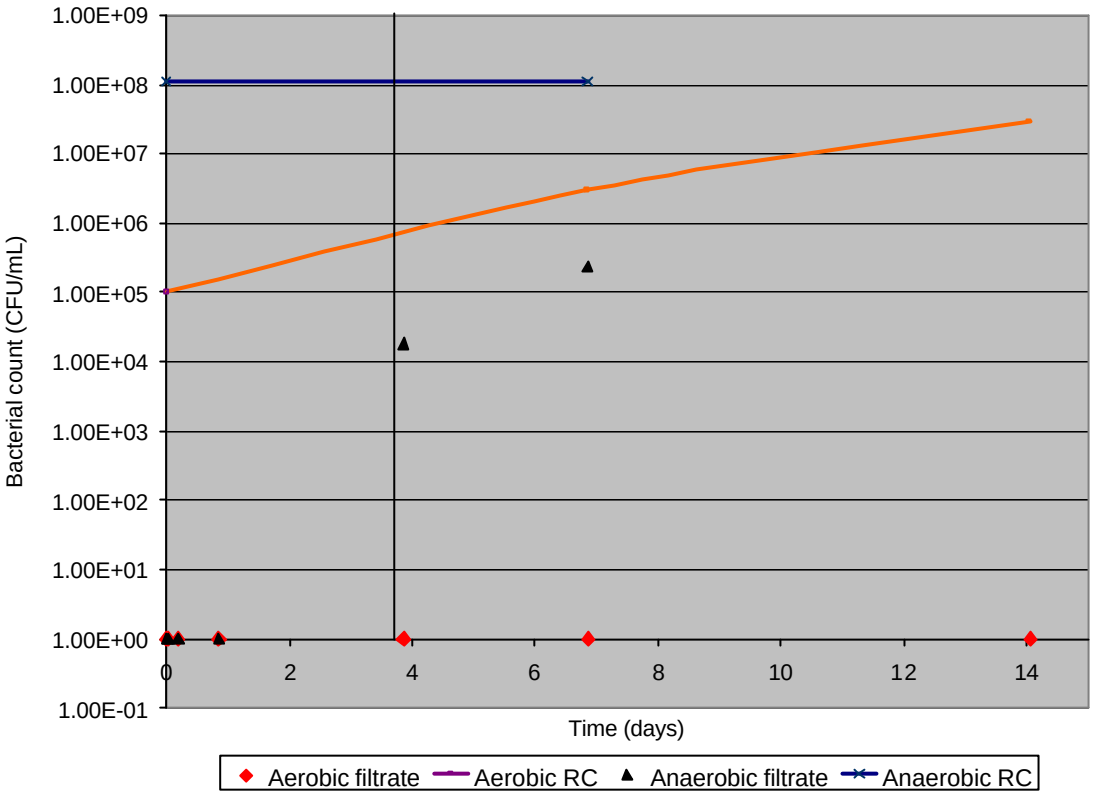


Figure 51. Aerobic and anaerobic count of filtrate

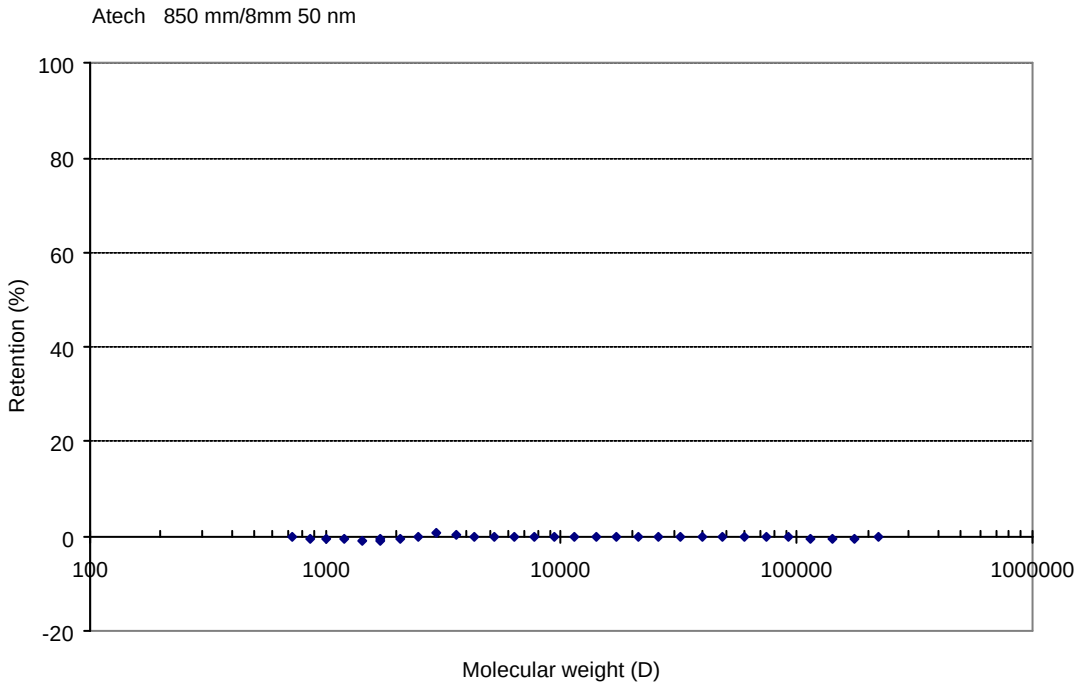


Figure 52. Retention test o the membrane after test

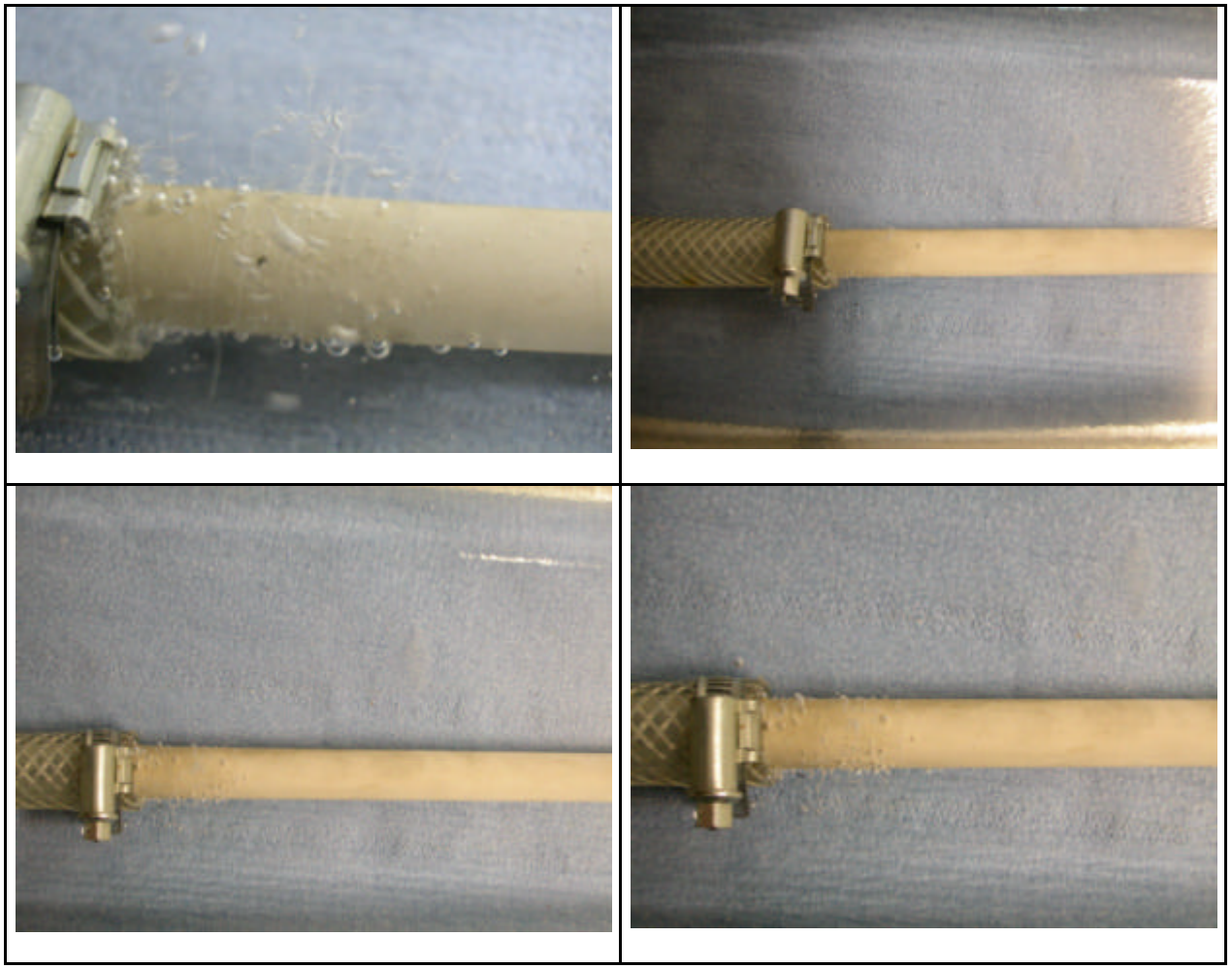


Figure 53. Pictures of the damaged Atech membrane during bubble test

5.3.2.5 Conclusions

These results confirm that a ceramic membrane is well adapted to the sludge to be filtered. Filtration with flux control seems also to be an adapted strategy.

Regarding bacterial contamination of the filtrate, the use of an industrial module with tight sealing allowed to produce a filtrate totally free of bacteria for a limited period. The membrane achieved therefore a log reduction of about 10^8 for anaerobic bacteria and 10^5 to 10^7 for aerobic bacteria. A contamination of anaerobic bacteria occurred after 3 days of test. The frequency of steam sterilization on the pilot unit will be adapted consequently in the future pilot characterization. The presence of a dead-end filter prevents that the possible contaminations reach the final filtrate.

Therefore it is proposed to use steam sterilization for regular hardware sterilization, and the Atech ceramic membrane and module coupled with a dead-end filter to insure the production of a non-contaminated filtrate

5.3.3 Test of a PVDF membrane under flux control

A PVDF membrane was tested on the prototype filtration unit under flux control in order the compare the performance and fouling of the two types of membranes (organic and ceramic) under the same control mode. The TMP evolution (Figure 54) shows that the fouling seems to not occur faster than with a ceramic membrane with the same control conditions. It is stabilized around 0.08 – 0.1 bar and shows no drastic differences between the 2 membrane types on a period of 50 days. This result allows to conclude that the filtration process control is the key in controlling the fouling process rather than the membrane material.

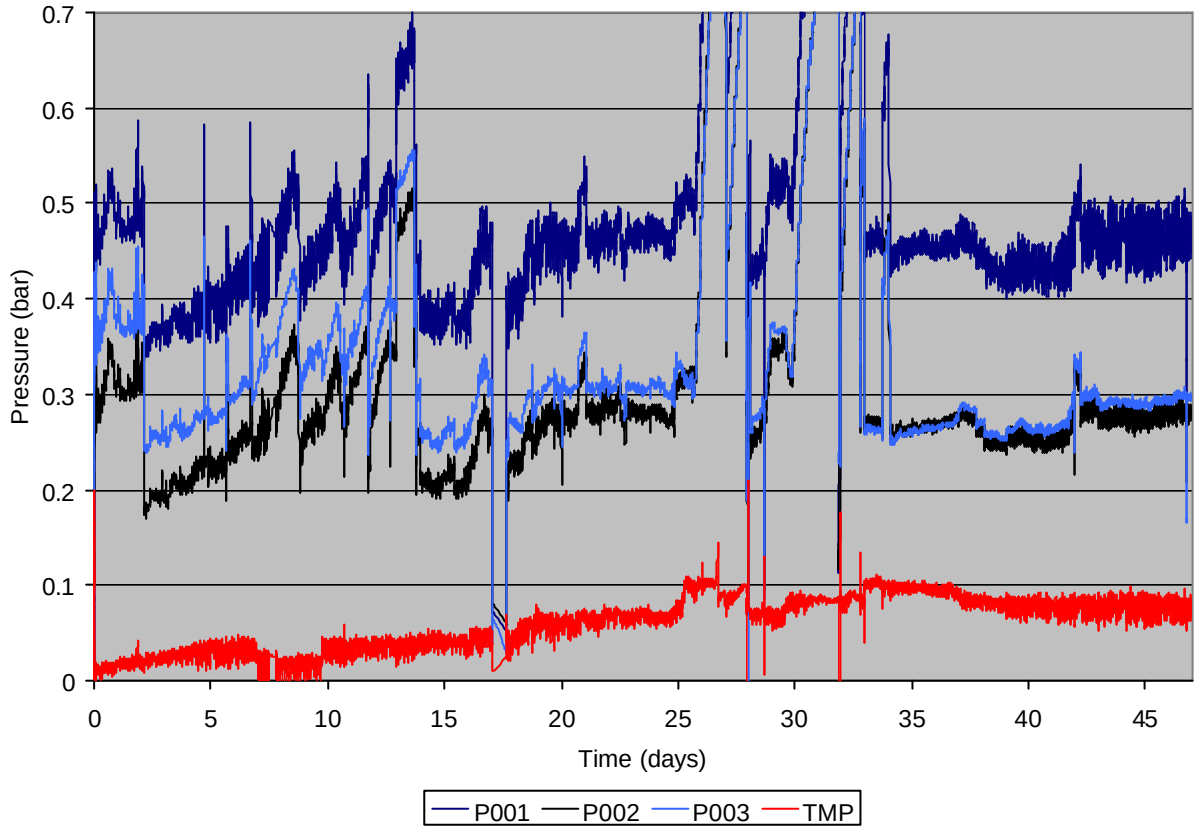


Figure 54. Evolution of pressures (PVDF membrane, flux control)

The comparison with the ceramic membrane (Figure 55) showed no drastic differences and allowed to conclude that the control rather than the membrane material is the critical issue for the optimisation of process stability and membrane life time.

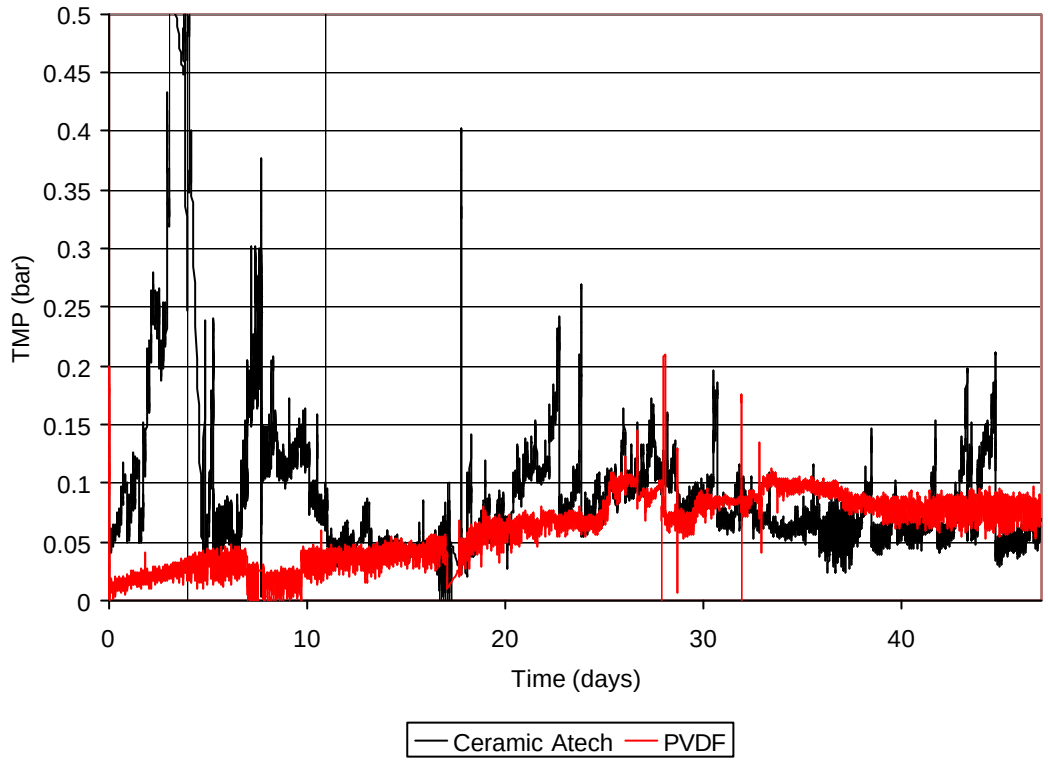


Figure 55. Comparison of TMP evolution when testing ceramic or organic membrane under flux control

5.3.4 Cleaning procedures

One of major problems in the operation of membrane process is membrane fouling. Membrane fouling is referred to the flux decline of a membrane filter caused by the accumulation of certain constituents in the feed water on the surface of the membrane or in membrane matrix. Certain fouling materials can be removed by hydraulic means such as filter backwash or scrubbing; most can be removed by chemical means such as Cleaning-In-Place (CIP), or chemical cleaning. Chemical cleaning is an integral part of membrane process operation that has a profound impact on the performance and economics of membrane processes.

Different types of cleaning agents can be used to clean membranes (Table 12). Caustic can increase the solubility of solutes by hydrolysis and solubilization. Oxidants such as free chlorine are able to oxidise natural organic matter and increase hydrophilicity by increasing the amount of molecules containing oxygen (e.g. carboxyl, phenolic groups). Acids are effective cleaners for some compounds and metal oxides using solubilization and chelating. Surfactants perform more complicated and multiple functions.

Table 12. Cleaning agents

Category	Major Functions	Typical Chemicals
Caustic	Hydrolysis, solubilization	NaOH
Oxidants/Disinfectants	Oxidation, disinfection	NaOCl, H_2O_2, peroxyacetic acid
Acids	Solubilization	Citric acid, nitric acid
Chelating Agents	Chelation	Citric acid, EDTA
Surfactants	Emulsifying, dispersion, surface conditioning	Surfactants, detergents

These cleaning agents can act specifically on different types of fouling (Table 13).

Table 13. Effect of operating strategies on membrane fouling

Type of Fouling	Effects of Operating Strategy			
	Hydraulic Cleaning	Feed Chlorination	Feed Acidification	Chemical Cleaning
Inorganic	-	-	++	++
Particulate	++	-	-	++
Microbial	+	++	+*	++
Organic	-	+	-	++

Note: - No effects or have negative effects. + Some positive effects, ++ Positive effects
* in conjunction of feed chlorination.

Concentration, cleaning time, temperature and hydrodynamic conditions during the cleaning are important factors affecting cleaning efficiency. Mass transfer barriers within the fouling layer are likely a limiting factor. Temperature has a significant impact on both the efficiency and rate of membrane cleaning (Liu *et al*, 2001).

Based on the results of the tests that were performed on both membrane types, additional cleaning tests will be performed at pilot scale, starting from the recommendations of the membrane manufacturer in order to find the optimal cleaning procedure and frequency.

6 General conclusions on the prototype tests: final design of the Pilot Compartment I

The test of the prototype reactor over a representative period allowed to evaluate and validate its hardware selection and design. Except for the filtration unit where major problems were encountered, the global design tested on the prototype will be used for the pilot compartment. Adaptations of the design were made based on the evaluation that was realized on the prototype reactor.

Regarding biological degradation, the results obtained showed a stabilization of the system at a certain dry matter concentration which allows an optimal bacterial degradation up to 65 % of the total OM. This behavior had never been shown at lab scale. A dry matter concentration of 40 g/L is thus chosen as the target optimal value for future tests. As a consequence, the drain necessity was estimated to only 0.5% of the reactor content per day. However, future tests on the pilot reactor will allow to check these preliminary conclusions, especially regarding fibres and lignin degradation.

The perturbations induced in the prototype (addition of acetate and ammonium) showed very slight answer of the system, proving the stability and robustness of the bacterial consortium. It was shown that H_2 was produced in very higher amounts after these perturbations. Both perturbations seemed moreover to induce changes in the composition of the bacterial consortium or in the weight of the different pathways. Indeed, after addition of acetate, the equilibrium between the different VFA produced was modified on long term. After addition of ammonium, methane started also to be produced in higher amount on long term. However, these changes did not affect the global degradation efficiencies. The DGGE analysis showed corroborating results: the populations vary slightly in the time but this does not seem to affect the degradation efficiencies.

The prototype test period also allowed to refine the strategy for control of the process. The results showed indeed that the degradation process is very well reflected by the VFA concentration, rather than ammonium production which seems to be less sensitive. The on-line ammonium analyzer appears thus not crucial for the first pilot tests. On the other hand, the H_2 production seems to be very sensitive to perturbations, and its follow-up is thus of high interest. It is thus proposed to install an on-line H_2 analyzer on the pilot reactor instead of an ammonium analyzer.

The filtration unit will include two stainless steel modules in parallel, allowing to clean and sterilize one membrane without stopping the filtration process. The sterilization of the unit will be done on-line with steam. The frequency and procedure of chemical cleaning and steam sterilization of the filtration unit will be tested and optimized at pilot scale.

The final design proposed for the pilot reactor, based on the prototype test period results is presented in Figure 56.

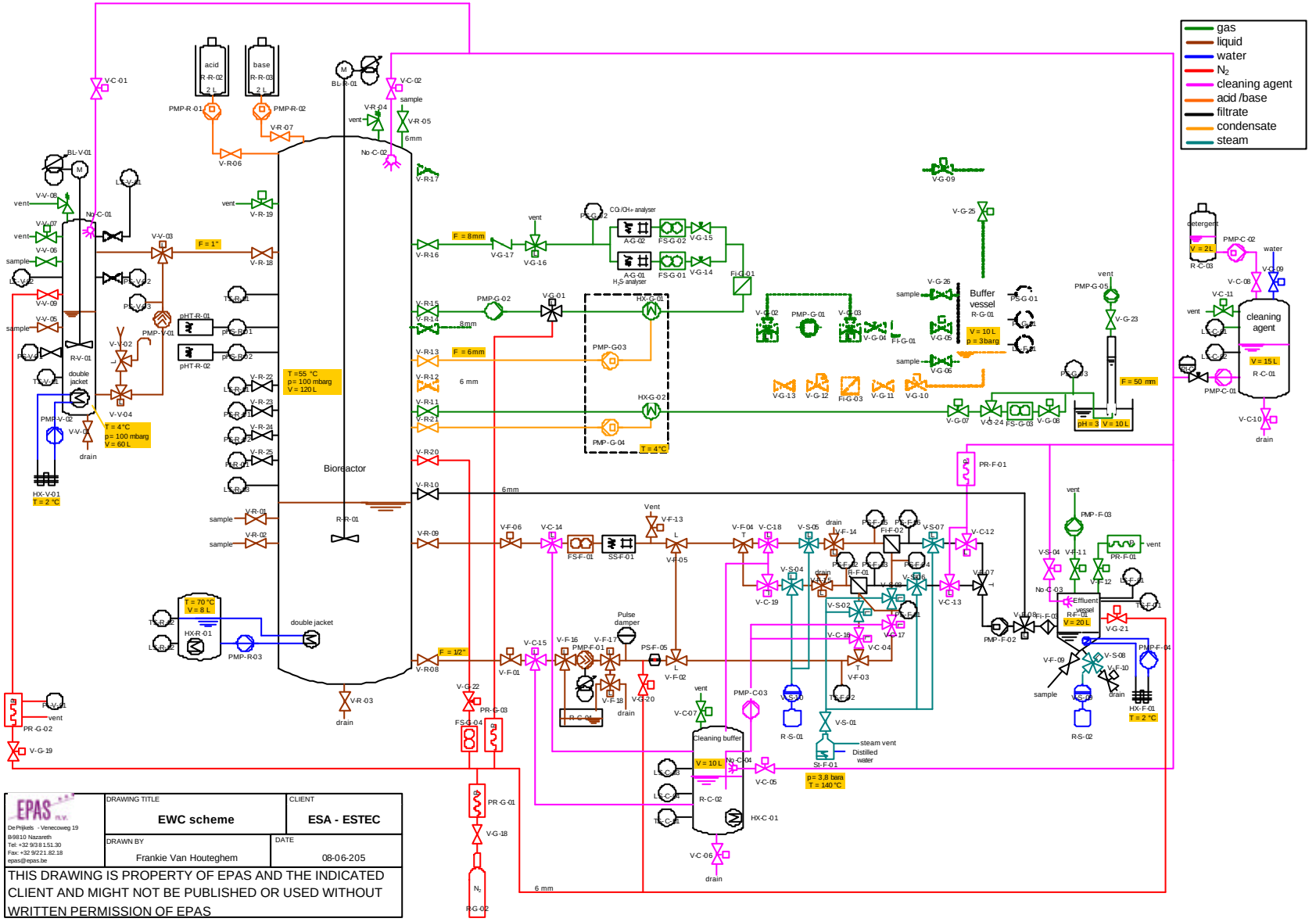


Figure 56. Final design of the pilot Compartment I

7 References

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Appendix 1 : Results of fibres analysis (Van Soest)

Reagents :

NDS : neutral detergent 1 L

- SDS 30 g
- EDTA 18,61 g
- sodium decahydrate borate 6,81 g
- disodium hydrogen phosphate anhydre 4,56 g
- 2 ethoxyl ethanol 10 mL
- Adjust to 1 L with distilled water
- pH 6,9-7,1

ADS : détergent acide 1 L

- Cethyltrimethylammonium bromide 20 g
- Adjust with sulfuric acid 0,5 mol/L

Sulfuric acid to 72 %

Protocol :

Samples preparation :

- Dry and grind samples with ultraturax
- Weight 1 g with precision E = **S**
- Weight a fritted from size 3 (40-90 µm) = F

Separation NDF : to eliminate components that are not fibres

- 100 mL of NDS in a cooled condenseur + 1 g of sample
- Boil softly during 1 h
- Filtrate
- Wash with boiling water
- Dry twice with acetone
- Dry for at least 8 h at 110°C
- Weight = **w1** (W1 = w1-F)

Separation ADF : to eliminate hemicellulose

- idem but 100 mL of ADS
- Weight after drying = **w2** (W2 = w2-F)

Separation of lignin : to eliminate cellulose

- Immerse the fritted containing the residues during 3 h in sulfuric acid 72%
- Filtrate
- Wash with boiling water
- Dry for at least 8 h at 110°C
- Weight = **w3** (W3 = w3-F)

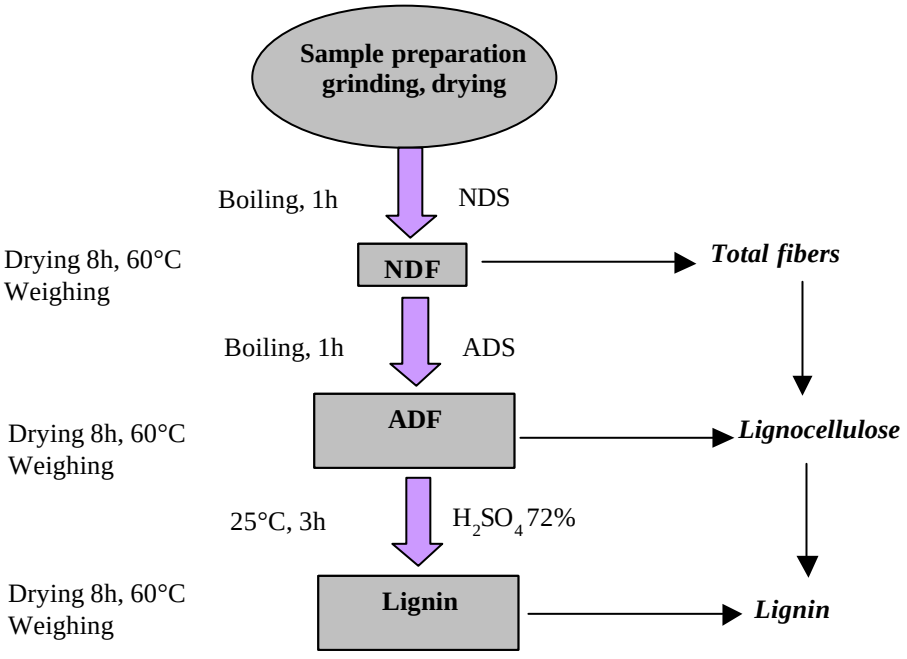
Estimation

Total fibres = [1-[(S –W1)/S]]*100

Hemicellulose = [(W1 –W2)/S]*100

Cellulose = [(W2 –W3)/S]*100

Lignin = (W3/S)*100



Measured values :

Samples	Masses measured in g					
	Date	Samples	Fritted	w1	w2	w3
RC	18.05.04	1	39,957	40,653	40,404	40,069
RC	18.05.04	0,968	38,242	39,003	38,756	38,378
RC	28.05.04	1,001	38,914	39,645	39,409	39,049
RC	04.06.04	1,003	38,678	39,391	39,165	38,798
RC	04.06.04	1	38,506	39,224	38,987	38,623
RC	02.07.04	0,999	36,353	37,166	36,922	36,533
I1	04.05.04	1,002	35,836	36,427	36,2	35,91
I2	04.05.04	0,719	38,905	39,285	39,131	38,939
I3	04.05.04	0,684	38,431	38,778	38,661	38,482
I		0,628	37,56	37,869	37,757	37,599

RC = Reactor content

I1, I2, I3, I = Influents

Calculated values :

Formula			E	w1-F	w2-F	w3-F	1-((S-W1)/S) *100	(W1-W2)/S *100	(W2-W3)/S *100	W3/S *100
Samples			Masses calculated in g				Proportion % of dry weight			
		Date	S	W1	W2	W3	Total Fibres	Hemi cellulose	Cellulose	Lignin
RC		18.05.04	1	0,696	0,447	0,112	69,60	24,90	33,50	11,20
RC		18.05.04	0,968	0,761	0,514	0,136	78,62	25,52	39,05	14,05
RC		28.05.04	1,001	0,731	0,495	0,135	73,03	23,58	35,96	13,49
RC		04.06.04	1,003	0,713	0,487	0,12	71,09	22,53	36,59	11,96
RC		04.06.04	1	0,718	0,481	0,117	71,80	23,70	36,40	11,70
RC		02.07.04	0,999	0,813	0,569	0,18	81,38	24,42	38,94	18,02
I1		04.05.04	1,002	0,591	0,364	0,074	58,98	22,65	28,94	7,39
I2		04.05.04	0,719	0,38	0,226	0,034	52,85	21,42	26,70	4,73
I3		04.05.04	0,684	0,347	0,23	0,051	50,73	17,11	26,17	7,46
I			0,628	0,309	0,197	0,039	49,20	17,83	25,16	6,21