EPAS n.v.

Eco Process Assistance

De Prijkels • Venecoweg 19 • B-9810 Nazareth
Tel. +32 9 381.51.30
Fax +32 9 221.82.18
www.epas.be • epas@epas.be

ENGINEERING OF THE WASTE COMPARTMENT

ESA contract 15689/01/NL/ND

TECHNICAL NOTE 71.4

Liquid Loop Design of the waste compartment

Version : 2
Issue : 1

	Name	Signature
Prepared by:	Farida Doulami	
	Henk Vanhooren	
	Veronik Hermans	
Approved by:	Dries Demey	

18 July 2003

DOCUMENT CHANGE LOG

Version	Issue	Date	Observation
1	0	20 August 2002	Draft
2	1	18 July 2003	Final

DISTRIBUTION LIST

Quantity	Company/Department	Name
4	ESA	C. Lasseur
1	EPAS	D. Demey
		H. Vanhooren
		F. Doulami
		V. Hermans
1	ADERSA	J. Richalet
1	UAB	J.Albiol
		F. Godia
1	UBP	C.G. Dussap
		L. Poughon
1	VITO	H. De wever
1	LabMET	W. Verstraete
1	U. Guelph	M.A. Dixon
1	SCK	M. Mergeay

CONTENT

LIST OF FIGURES

LIST OF TABLES

1. Introduction

The effluent resulting from the biodegradation of the human faecal material and the other wastes generated by the crew (non edible parts of plant material, urine, paper,…) flows through the ultrafiltration unit. The collected filtrate should be analysed for its content in minerals (ammonium) and Volatile Fatty Acids (VFA). Bacteria are normally retained by the ultrafiltration unit. However, a diagnosis should be done on the filtrate to verify the absence of micro-organisms and pathogens. The latter should not enter the second Melissa reactor (the Photoheterotrophic compartment) in order to avoid its contamination. For safety, an extra disinfection step of the filtrate stream might be necessary. Molecular techniques including FISH (Fluorescent In Situ Hybridisation) and DGGE (Denaturing Gradient Gel Electrophoresis) can be used to confirm the absence of pathogenic germs at the outlet of the liquid loop and to identify them if present.

This technical note is focussing on the general concept of the liquid loop including the necessary on-line instrumentation and the safety issues. Figure 1, shows a general scheme of the waste compartment specifying the position of the liquid loop, the solid loop and the gas loop.

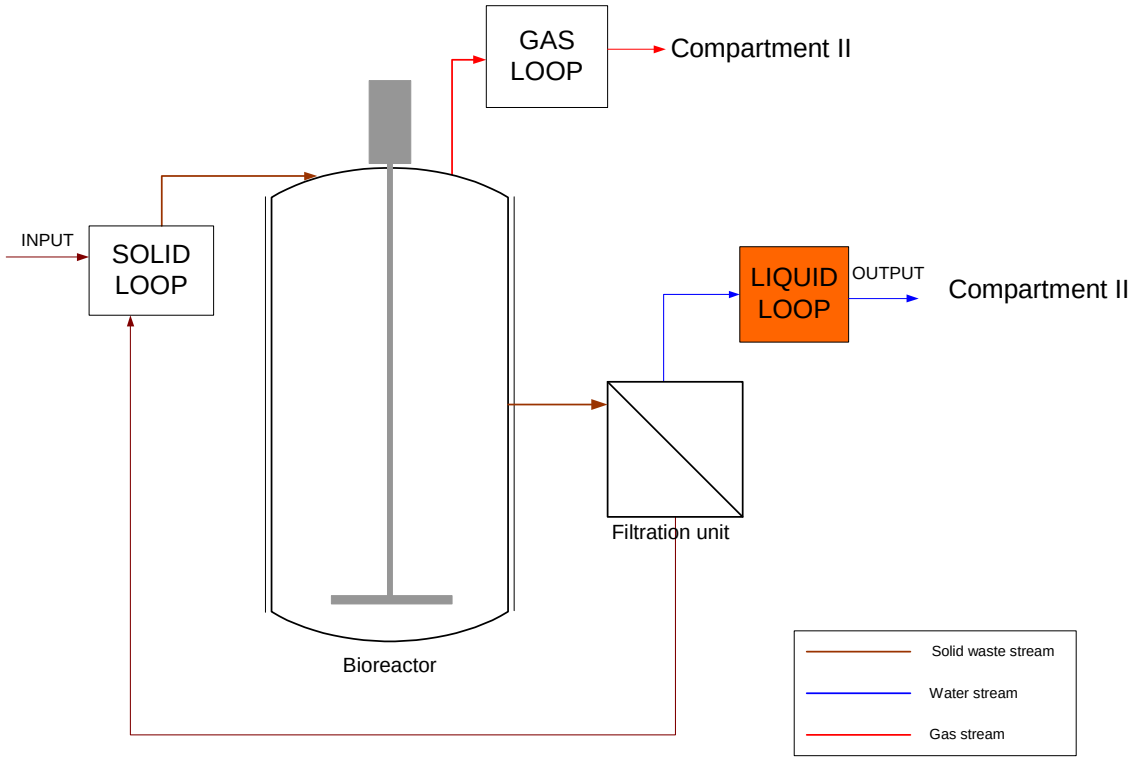


Figure 1: Schematic general design of the waste compartment with indication of the sub-system treated in this technical note

2. Concept of the liquid loop

The liquid loop as presented in this note does not include the filtration unit. The details of the latter, including the instrumentation are presented in technical note 71.2.

General information about the design of the liquid loop is schematised in Figure 3. The different sensors and on-line instrumentation (analysers) are positioned in such a way that the collected data will concern the generated filtrate at the outlet of the filtration unit and after eventual sterilisation of the filtrate before it enters the second compartment. It is not the scope of this note to actually select the on-line instruments. At this level of the study, different measurement techniques and brands are described with their advantages and drawbacks. On the basis of this, an appropriate measurement technique will be selected. The trade-off and selection of the most appropriate instrument will be presented in TN 71.7.

2.1 Requirements of the liquid loop

As mentioned above, the clarified stream originating from the filtration unit has to be sterile with the highest possible nutritional value (high VFA and ammonium concentration) before being conveyed to the photoheterotrophic compartment. To fulfil these requirements, different treatment steps are proposed in this technical note, which may ensure high hygienic conditions. The specific requirements of the liquid loop can be defined as follows:

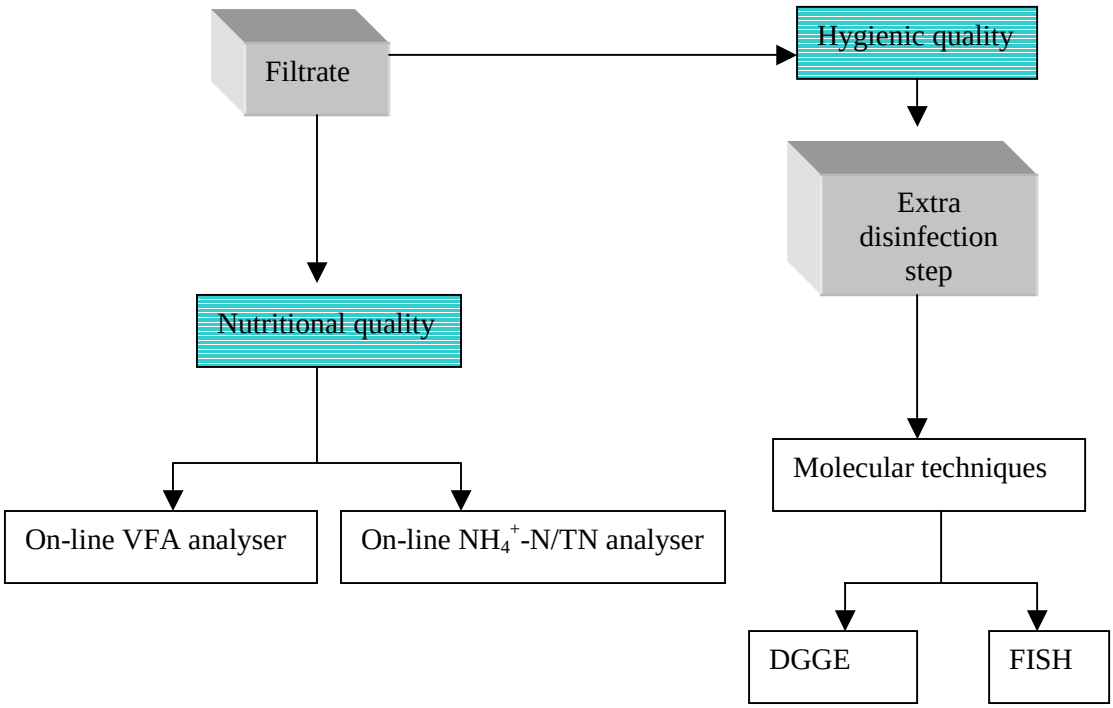


Figure 2: Most important parameters to determine in the filtrate stream before entering the photoheterotrophic compartment

In Figure 3, the placement of on-line instrumentation is proposed. Other parameters could also be followed for example using a total organic carbon (TOC) on-line apparatus. However, the TOC on-line analyser will be considered as optional since the process follow-up is possible using to the on-line VFA analyser.

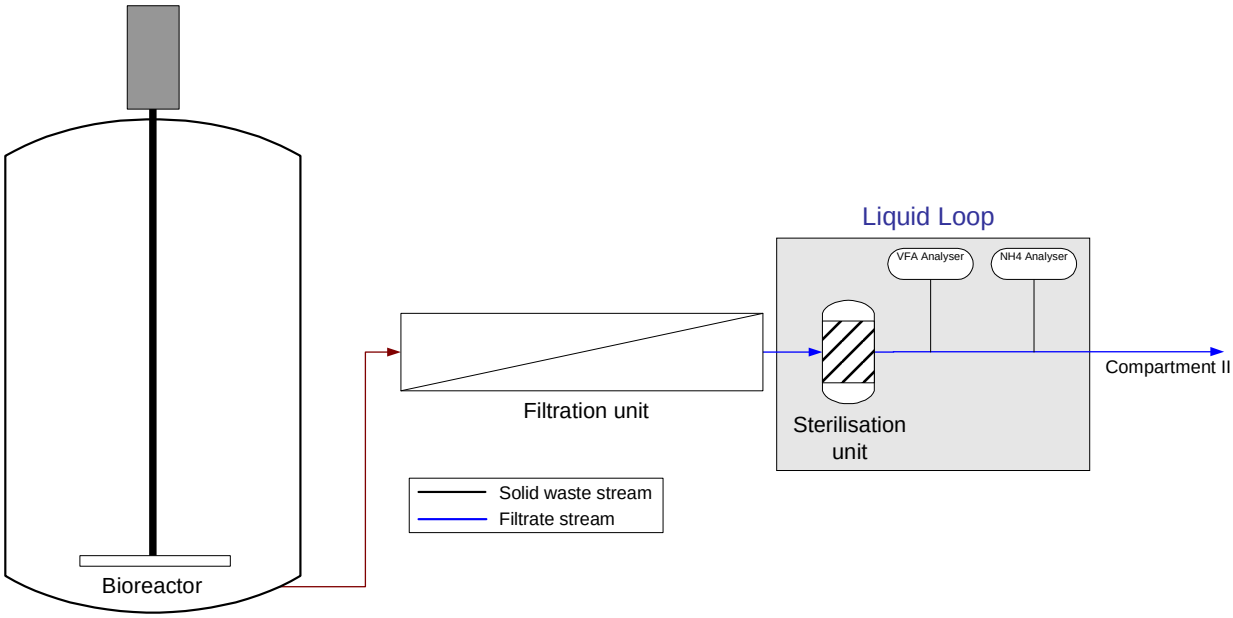


Figure 3: Conceptual scheme of the liquid loop of the waste compartment

3. Review of sensors and instrumentation

For a proper use of the hardware, harmonisation of the selected material is necessary between the different project partners. Instrumentation and sensors, which would be adequate for the first compartment and in parallel would be used for the second compartment have to be selected after close collaboration of both parties (EPAS responsible for the engineering of the waste compartment and UAB responsible for the photoheterotrophic compartment).

3.1 Volatile Fatty Acids (VFA) analyser

3.1.1 Measurement principles

3.1.1.1 Advantages and drawbacks of different instrumentation

Infrared (IR) spectroscopy, or an extraction method followed by gas chromatography (GC) injection can be used for the determination of the volatile fatty acids that are produced in the first compartment. The differences between the two methods are reported in TN 62.1. The advantages and disadvantages of both methods are shown in Table 1.

Ideally, the measurement system should be applicable to measure the liquid and gas phases, because VFA are volatile compounds and it is convenient to have the possibility to measure both in the liquid and in the gas phase. In order to obtain both, it would be necessary to use two IR liquid analysers. GC analysers don't have this limitation, they can quantify the VFA in both the liquid and the gas phase.

Both techniques have the possibility of automatic sample devices, which will enable to samples of compartment I and/or II. These systems allow to automatically switch the measurement from one point to the other. The measurement devices in both techniques are able to supply the measured values to the control system. Infrared spectroscopy can supply the measurement signal both analogically (4-20 mA) and digitally (RS 232, RS 485, Ethernet). Since a GC can measure the different components within a functional group individually, the signal is supplied in digital form only (RS 232, RS 485, Ethernet).

Table 1. Advantages and disadvantages of Infrared spectroscopy and gas chromatography

Technique	Advantages	Disadvantages
IR (FTIR, NIR)	Sampling of product for analysis is simple since flow is through an open cell. Scanning a wavelength range is very quick (response time 10-60s). Are designed for continuous measurement of up to 4 IR functional groups. There are versions for sequential analysis to treat different streams of sample	Very good filtration is required upstream in the sample conditioning. Samples require dilution, because of the weak absorption peaks. Is good at discriminating functional groups but not for quantitative speciation (e.g. only total VFA are determined, not individual species). Calibration samples are still required to quantify the components being measured.
GC	Large number of functional groups able to be identified using latest detectors technology. Are very good at quantitative and qualitative analysis of different species within a functional group. Are designed for continuous measurement of lots of components and supplementary give the concentration of the total VFA. Have a very high dynamic range in that they can measure down to low ppm and up to 100% on the same analyser, depending on the detector. It is possible to analyse solid, liquid and gas samples.	The response factors of the detectors are not the same for different components. Physical separation of components requires specialised columns, sample valves accompanied by accurate timing sequences To minimize GC analyser injection mistakes it's necessary to use an internal standard, which sometimes is difficult to find. Each piece of equipment is complex and needs specialists for any diagnostics

3.1.1.2 Conclusion

Since gas chromatography is most reliable technology for quantitative and qualitative determination of volatile fatty acids, it was concluded to use a GC/FID for the follow-up of VFA in the waste compartment.

3.1.2 Gas Chromatography

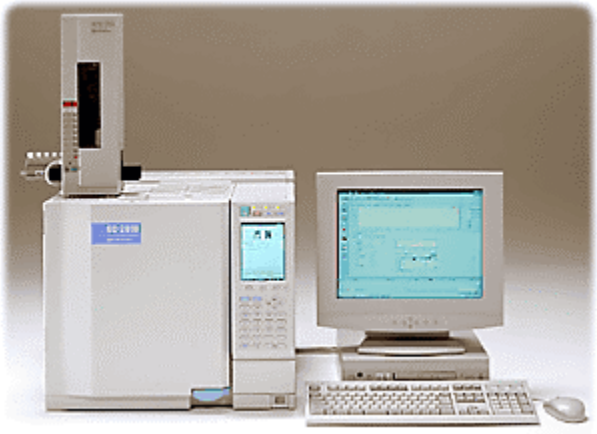


Figure 4. GC-2010 from Shimadzu Benelux

3.1.2.1 Measurement principle

In Figure 5, a schematic overview of the GC measurement principle is depicted. First of all, a tank of compressed gas, a pressure regulator and a valve are needed in order to produce a stable flow and pressure for the mobile phase – carrier gas.

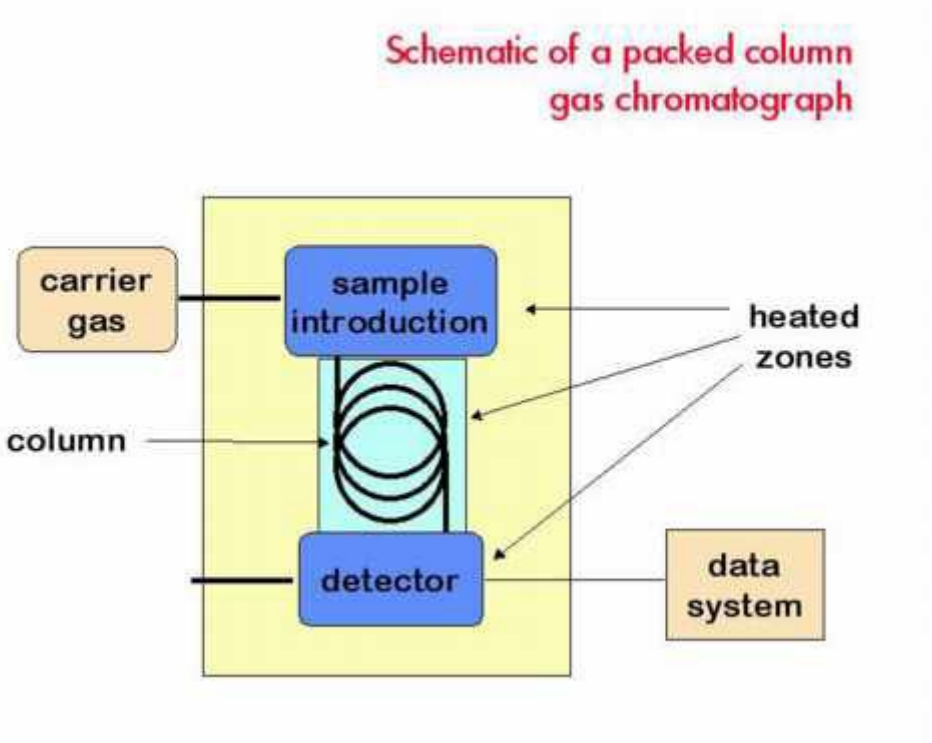


Figure 5: Schematic of a packed column gas chromatograph

The liquid or gas sample is injected by means of a micro syringe through a rubber septum and into a flash vaporiser port at the head of a chromatographic column that contains a liquid stationary phase adsorbed onto the surface of an inert solid. The lifetime of the rubber septum is dependent on the frequency of injections into the column. It should be randomly changed after 300-400 injections (once a month at a frequency of 12 samples injected per day). The sample is transported through the column by a flow of inert carrier gas. The separation is due to the difference in retention time of the compounds when they pass through the column. The effluent of the column goes to the detector, which emits a

signal that is translated into a serial of peaks (chromatogram). The position (retention time) of these peaks is used in qualitative determination and size (area or height) is related with concentration.

The GC ensures that all the operating parameters are set reproducibly, including carrier gas flow, split ratio, all heated zone temperatures, detector combustion and make-up gas flows, and detector output range and current. Analyses are achieved quickly and confidently with electronic flow control of all carrier gas and detector gas functions.

3.1.2.2 Detectors

Different detectors (FID, TCD, ECD, NPD, FPD, MSD) can be used in a gas chromatograph to detect the volatile fatty acids also in very low concentrations, mainly at ppm levels. Detector characteristics and selection are explained widely in TN 62.1. The most popular detector in gas chromatography is the FID. It detects any component which may be oxidised in a hydrogen/air flame, which means any organic compounds. The upper temperature limit is for the best performing GC's is about 450°C. The fused silica jet prevents sample degradation and is designed for insertion of the capillary column (up to 0.53 mm ID) to minimise void volume and prevent peak tailing. The ion collector is easy to clean and the flame is ignited by simple operation of the keyboard. The wide-range FID is provided with a flame monitor.

3.1.3 Sampling methods for on-line analysis

Three possibilities exist for the measurement of VFA in the bioreactor:

3.1.3.1 Filtrate sampling: extraction

The extraction method is used to detect volatile fatty acids in the liquid stream. In this case, one has to ensure that the liquid stream is exempt of particles to allow proper extraction of the components. The extraction itself requires some chemicals (e.g. solvents like hexane or diethylether, NaCl to separate the solvent from the water phase, a strong acid like H_2SO_4) and extra steps (see paragraph 3.1.5.2) before injection in the gas chromatograph. Moreover, the volumes used for the analyses (from some µl to some ml) can not be reintroduced in the liquid stream and thus are lost.

The extraction technique for quantitative and qualitative determination of VFA can in theory be automated. However, the need for chemicals and the high number of manipulations necessary for the extraction is a drawback. Therefore, it is preferred to use another, more adapted, method to determine the VFA in the waste compartment.

3.1.3.2 Headspace sampling

Headspace sampling is used to detect volatile compounds of solid or liquid samples, analysing the vapour phase that is in equilibrium with the sample in a closed recipient. It is especially used to analyse volatile compounds in samples difficult to analyse for conventional GC. This is the case here since we deal with a heterogeneous sample. Furthermore, all VFA of interest are volatile compounds.

This technique has the following advantages:

- it is not necessary to perform a pre-treatment of the sample;
- the column is not damaged by non volatile compounds.

The main disadvantage is the fact that an accurate calibration is required for quantitative analyses of the compounds. This is caused by the vapour-liquid equilibrium that is obtained within the closed recipient. Part of the VFA is still present in the liquid phase after the equilibrium is obtained. The calibration has to account for this. Furthermore, it should be noted that some other volatile components apart from the VFA's will be present in the injected sample, possibly contaminating the column.

In an on-line implementation, the sample is introduced in a glass flask by a robot arm. The flask is closed with a rubber septum and is heated with care in an oven. The applied temperature can range from 40ºC up to 190ºC depending on the thermal stability of the compounds to be measured. The head space is then sampled and introduced into the chromatographic system with a syringe.

3.1.3.3 Direct gas sampling from the gas loop

Since the bioreactor operates at 55 °C, part of the VFA produced will be present in the gas phase. The presence of a gas loop (see TN 71.5) makes is possible to use a flow cell and to sample the gas from there for direct injection into the chromatographic system.

When this sampling would be performed, the measured concentrations would be lower compared to the headspace sampling technique, because of the lower temperature of the liquid. The relation between the gas- and liquid-phase concentrations of the VFA again needs to be established using calibration. This calibration is even more delicate than the calibration in the headspace sampling technique because of the higher concentration differences between gas and liquid phase.

3.1.3.4 Conclusion

For accurate on-line measurement of VFA in the liquid phase of the bioreactor, headspace sampling is preferred. However, occasionally, direct sampling in the gas loop can be performed, since the only measure to be taken to achieve this is to insert a flow cell in the gas loop.

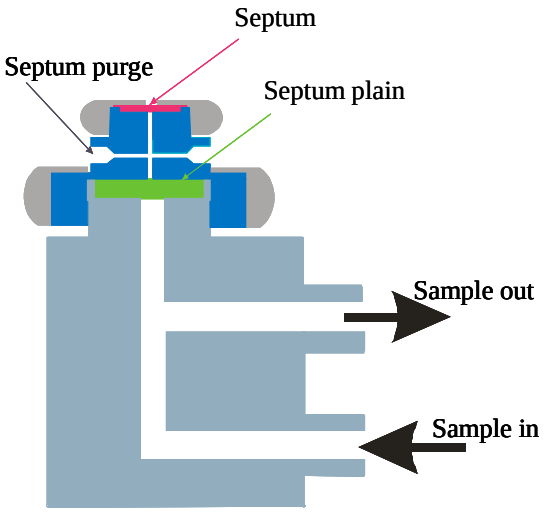


Figure 6: Flow cell for direct sampling of gas

3.1.4 Injection methods for capillary columns using headspace sampling

- split

  This headspace sample is mixed with carrier gas flow. Then it is divided in two different parts, the greatest part is wasted and the smallest one pass through the column. Usual split ratios are from 1:20 to 1:200. It is a technique for major components, because the quantity of sample that is introduced on the column is small, and the use of highly volatile solvents should be avoided whenever possible.

  For quantification, the standard addition of the internal standard method is preferred but the external standard method in which absolute peak areas are compared can be used. Reproducibility will be enhanced by not varying the injected volume.

- split-less

This is a pre-concentration technique for diluted samples such as traces analysis, and for samples containing labile compounds or compounds that are eluted very near to the solvent. The sample is completely introduced on the column.

For quantification, both standard addition and internal standard can be applied. Reproducibility will be enhanced by not varying the injected volume.

All these injections methods for capillary columns could be employed to detect the VFA mix under study. The most adapted method needs to be selected from experimental information gathered in the prototype reactor.

3.1.5 Example of VFA analysis

What follows is an example of a VFA analysis using liquid-liquid extraction. Contrary to the headspace sampling technique, this technique is most frequently used in off-line lab-scale measurements.

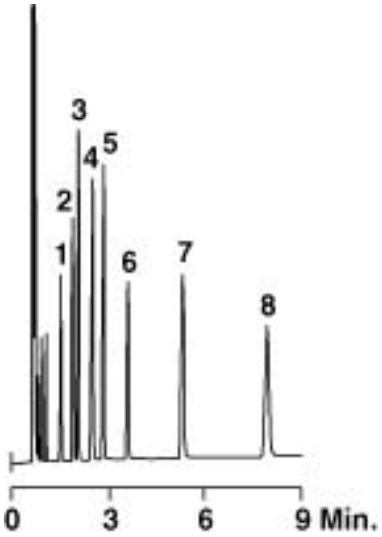
3.1.5.1 Standards for calibration

Product Name: VOLATILE FREE ACID MIX 1X100ML,10M MOLAR, DEIONIZED WATER
Storage Temp: < 0°C
Comments: standard type, saturated fatty acid

Targeted compounds: Low chain volatile fatty acids

1. acetic acid
2. Propionic acid
3. Isobutyric acid
4. Butyric acid
5. Isovaleric acid
6. Valeric acid
7. Isocaproic acid (4-Methyl-n-valeric acid)
8. Caproic acid
9. Hexanoic acid (n-Caproic acid): used as internal standard
10. Heptanoic acid

Short Chain Fatty Acids: Type column from Alltech Associates, Inc.
Column: Econo-Cap™EC-1000, 15mx0.53mmx1.20µm, Part No. 19684
Temp: 150°C
Carrier gas: Helium at 2.85mL/min
Linear velocity: 21.6 cm/sec
Pressure: 1.7 psi
Sample size: 1 µl
Detector: FID at 275°C
Flame gases: hydrogen gas and compressed air (oil free)
Injector temperature: 250°C
Split Ratio: 21:1 used with split-less mode



Peak Identification
1. Acetic acid
2. Propionic acid
3. Isobutyric Acid
4. n-Butyric Acid
5. Isovaleric Acid
6. n-Valeric Acid
7. Caproic Acid
8. Heptanoic Acid

3.1.5.2 Liquid-liquid VFA extraction

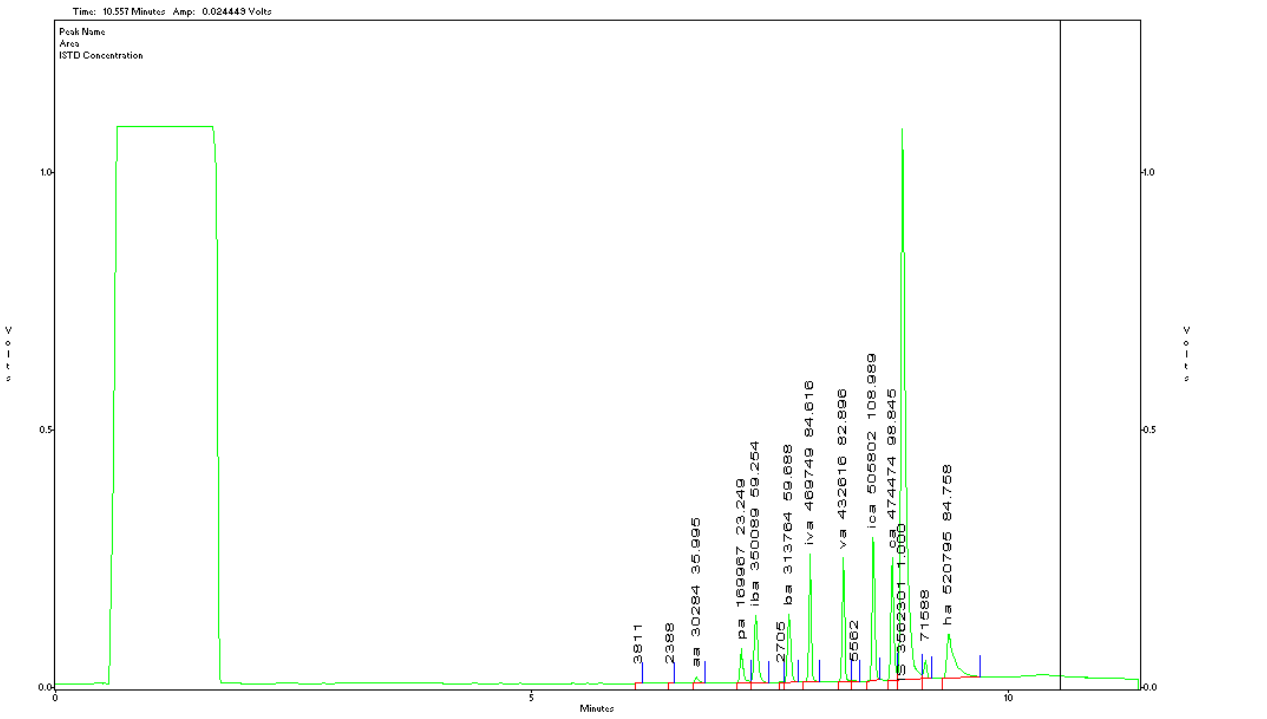
The classical laboratory method for liquid-liquid VFA extraction consists of:

- Acidification of sample with 50% sulphuric acid 96% (V/V)
- Addition of 0.4 g NaCl and 0.4 ml internal standard (Hexanoic acid)
- Extraction with equivalent sample volume of diethyl ether
- Mixing and centrifugation

After extraction of the VFA from the liquid phase into the diethyl ether phase, an aliquot of 0.1 µl is injected into the GC/FID at the above mentioned conditions.

3.1.5.3 Data acquisition

A histogram is displayed after complete analysis of the sample for its content in the targeted volatile fatty acids. The data are expressed in mg/L after relation (area-concentration) taking into account the internal standard and the calibration curve. Figure 7, shows an example of the histogram of one sample. The results are expressed in surface area and data are re-calculated by the software into concentrations expressed in mg/L or in mM.



Method: Vvzmel.met Batch: Mel0805c.seq Data: Mel258 - [Internal Standard Report -- Channel A]

File Edit Method Batch Options Analysis Control Window Help

Method Data Batch Setup Batch Preview Single Batch Recalib Analyze Reports

Pkno	Name	Ret. Time	Conc	Area	Height
1	aa	6.743	35.995 ppm	30284	12347
2	pa	7.212	23.249 ppm	169967	65689
3	iba	7.370	59.254 ppm	350089	131055
4	ba	7.718	59.688 ppm	313764	131038
5	iva	7.935	84.616 ppm	469749	247352
6	va	8.283	82.896 ppm	432616	241783
7	ica	8.598	108.989 ppm	505802	277758
8	ca	8.800	98.845 ppm	474474	237828
9	IS	8.908	1.000 ppm	3562301	1071325
10	ha	9.391	84.758 ppm	520795	84514
Totals			639.289	6829841	2500689

Figure 7: Data acquisition from the GC using the software Class VP4.0 /chrom-Shimadzu

3.1.6 Proposed gas chromatographs

The VFA-analyser will be selected among different candidates, using the requirements listed in Table 2. The candidate analysers are listed in Table 4. The trade-off of the different chromatographs will be presented in technical note TN 71.7.

Table 2. Requirements of the VFA-analyser

COMPONENT REQUIREMENT SHEET	
Device	VFA-analyser
Process Technical Specifications	
Data evaluation software	Yes
Measuring range	µg/L to g/L
Detection limits	Low
Precision	Low
Auto sampling	Yes
multiple sampling points	Yes
measuring frequency	short measuring cycle
auto calibration	Yes
detectors	multiple detectors possible
number of detectors	different detectors simultaneous
Auto cleaning detectors	Yes
Auto cleaning column	Yes
dilution cell and tape filtration	Yes
oven operation temperature	High range
injectors	different injectors simultaneous
Oven rate	High
Solvent Vapour exit	Yes
Programmable temperature vaporiser	Yes
Weight	Minimize
Compactness	Minimize
Safety	
Housing	Dust and Splash proof
Power supply	240 V, 50 Hz
Maintenance	
Self Diagnosis	Yes
Scheduled Maintenance	Yes
Availability Spare Parts	Yes
Certification	
EC Certificate	Yes
Costs	
Cost of the analyser	Minimize
Guarantee	1 Year

Table 3. Proposed gas chromatographs

	Criteria	Biospectra Ingenierìa Analìtica	ABB (Vista2-Model 2000)	GC –2010 Shimadzu	Siemens RGC 202
1	Data evaluation Software	Chemstation Software	Vistanet	GC Solution	Windows, EZ Chrom
2	Maintenance	Every 6 months	8 years	1 year	1 year
4	Measuring Range	detector (selectable) dependent	detector (selectable) dependent	detector (selectable) dependent	detector (selectable) dependent
5	Detection limits	detector and sample dependent	detector and sample dependent	detector and sample dependent	detector and sample dependent
6	Precision	RSD < 2%	RSD < 1%	RSD < 2 - 5%	RSD < 1%
7	Multiple sampling points	Yes	Yes	Yes	Yes
8	Measuring frequency	2-12 injections/hour	5 - 8 injections/hour	application dependent	application dependent
9	Auto calibration	Yes	Yes	Yes	Yes
10	Detectors	FID, TCD, ECD, NPD, FPD, MSD	FID, TCD, FPD	FID, TCD, FPD, ECD, NPD, ECD	FID, TCD, FPD, ECD, PID, ECD
11	Number of detectors	up to 2 simultaneous	up to 2 simultaneous	up to 4 simultaneous	up to 2 simultaneous
12	Dilution cell and tape filtration	Yes	Yes	Yes	Yes
13	Oven operating temperature	4°C - 450°C	30°C - 180°C	min 90°C - 450°C	min 50°C - 450°C
14	Injectors	up to 2 simultaneous	up to 5 simultaneous	up to 4 simultaneous	up to 2 simultaneous
15	Volatiles inlet	Yes	Yes	Yes	Yes
16	Oven rate	up to 120°C/min	up to 120°C/min	up to 100°C/min	up to 25°C/min
17	Solvent vapor exit	Yes	Yes	Yes	Yes
18	Programmable temperature vaporiser	Yes	Yes	Yes	Yes
19	Dimensions (m, wxdxh)	0,8 x 0,6 x 2	0,496 x 0,34 x 1,75	51,5 x 43,7 x 52,0	Unknown
20	Cost (Euro)	40,000 extras dependent	42 - 48,000 without sample treatment system	40,000 extras dependent	40,000 extras dependent
Comments:		Cleaning of non-volatiles components after each injection	Low flexibility because of the T° limits of the oven. Once the application is specified, it is difficult to change	Additional devices: methanizer and oxygen specific detector	Cleaning of non-volatile components after each injection

3.2 On-line Ammonium analysers

Ammonium is one of the major by-products in the fermentation process in Melissa loop, which makes its on-line measurement important. Since the produced ammonium, during the fermentation in the waste compartment, will further be oxidised into nitrate in the aerobic- nitrifying compartment, which in turn will be consumed in the photosynthetic compartment, its measurement is one of the majors key-parameters in the process.

3.2.1 Type ammonium analysers

3.2.1.1 Ammonia Gas sensing ion-selective electrode (ISE)

The measurement of ammonium ions with an ion-sensitive electrode requires adjustment of the sample to pH 11 or higher to convert the ammonium ions to ammonia gas. The hydrophobic membrane allows the ammonia gas to pass through to the inner chamber of the electrode to be converted back to ammonium ions that registers as a pH change in the internal filling solution. This pH change is calibrated against known ammonium standards.

An example of a sensor using this technology is the Applikon 2018 Process Ion Analyzer. This is making use of the standard addition method. To a quantity of sample in the measurement cell an amount of buffer solution (NaOH + EDTA) is added. The Analyser takes an initial reading from the electrode and from that it calculates and dispenses an shot of standard solution into the measurement cell. It takes a second reading and calculates the original concentration using the Nernst equation (Figure 8). Doing so, each analysis is validated and unaffected by the other ions in the sample. Due to the variable addition of standard solution, the instrument is auto-ranging. Furthermore, the analysis results are temperature-compensated.

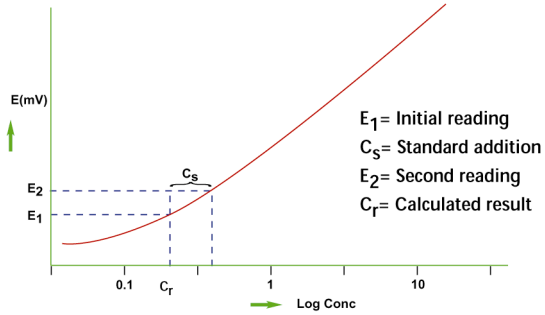


Figure 8: Measurement principle with the standard addition method

Advantages:

- Relatively low volumes are required per analysis;
- Reasonable price compared to the other on-line ammonium analysers (e.g. certain colorimetric methods);
- High accuracy and rapid response.

Disadvantages:

- Recalibration regularly needed;
- Use of chemicals (caustic + EDTA as buffer solution);
- Destructive method, recuperation of sample is not possible;
- Interference of high protein concentrations.

3.2.1.2 Colorimetric techniques

Two methods have been implemented in automated ammonia analysers using colorimetric techniques.

- Sparging method with subsequent photometric pH indication

  The ammonium is converted to the gas phase in the reaction cell by the addition of an alkaline medium, then transferred as ammonia to another vessel containing a solution of pH indicator. The colour change of the indicator is a measure of the ammonium concentration of the original sample.

  Advantages:
  - Relatively low volumes are required per analysis;
  - Reasonable price compared to the other on-line ammonium analysers;
  - High accuracy;
  - Non-destructive. Only the ammonia is sparged, the rest can be recuperated.

  Disadvantages:
  - Recalibration regularly needed;
  - Use of chemicals (caustic).

  An example of a sensor using this technology is the Dr Lange AMTAX-compact.

- Segmented flow colorimetry

  In this method, a colouring compound like indophenol blue compound is produced. The intensity is proportional to the amount of NH_4^+-N present in the sample. This colour intensity is measured directly with a photometer.

  Advantages:
  - High accuracy;

  Disadvantages:
  - Destructive method, recuperation of sample is not possible;
  - Relatively high volumes are required per analysis;
  - High price;
  - Recalibration regularly needed;
  - Sample colour sensitive.

  An example of a sensor using this technology is the SKALAR on-line process analyser.

3.2.2 Proposed on-line ammonium analysers

After discussion with Melissa partners, mainly UAB-University, about the more useful technique for on-line determination of ammonium in the bioreactors, it was decided to focus on the most important requirements for the selection of the analyser. These requirements are listed in Table 4. Requirements listed in red are indispensable and thus can rule out a sensor (e.g. segmented flow colorimetry is ruled out on the basis of its colour-dependency). Some on-line ammonium analysers were thus proposed by both partners as reported in Table 5. The final decision on the type ammonium analyser will be taken after common agreement of Melissa partners on the basis of the trade-off reported in technical note TN 71.7.

Table 4. Requirements of the NH_4^+-analyser

COMPONENT REQUIREMENT SHEET	
Device	NH_4^+-analyser
Process Technical Specifications	
Range	0-1500 mg N/l
Analytical accuracy/reproducibility	1 mg N/l
Interferences	little amount of interferencing substances
Sample colour dependent	independent of sample colour
Automatic sampling	Yes
Sampling/Measurement interval	programmable measurement interval, minimal every 10 min.
Sampling volume	limited sampling volume
Sample dilution	no dilution
Destructive measurement	preferably sample re-use possible
Auto calibration	Yes
Calibration interval	Programmable
Auto cleaning	Autocleaning available
Auto cleaning sampling system	Autocleaning available if applicable
Chemicals consumption	Low
Analogue output	4-20 mA output
Serial output	Preferred
Alarm output	RAL alarm
Display on analyser	data display on instrument
Control panel	control panel for programming on instrument
Data logging	Preferred
Weight	Minimize
Compactness	Compact Set-up
Safety	
Sample-resistant materials	Resistant to medium
Chemicals storage	Safe storage
Temperature ranges	50°C
Ex-proof	No
Housing	Dust and Splash proof
Power supply	240 V, 50 Hz
Maintenance	
Self Diagnosis	Yes
Crew time	Minimize
Scheduled Maintenance	Yes
Availability Spare Parts	Yes
Accessibility	components visible and accessible
Certification	
EC Certificate	Yes
Costs	
Cost of the analyser	Minimize
Guarantee	1 year
Cost Chemicals/Year	Minimize

Table 5. On-line ammonium analysers proposed by EPAS and UAB

Criteria	Aquamonia: AQUA/MCA	Dr Lange AMTAX-compact	APPLIKON 2018
Main principle	ion-selective NH3 electrode, pH adjustment	photometric pH-indication after NH3 stripping	ion-selective NH3 electrode, pH adjustment
Detection limits	0.01 mg/L NH_4^+ without dilution	Depending on Reagentia (minimal 0.2 mg N/l)	0,01 mg/L NH_4^+-N depending on sample volume
filtration		Needed if Suspended Solids present	Needed if Suspended Solids present
Process Technical Specifications			
Range	0 to 200 mg/L	50 to 1200 mg/L	0.01 to 17000 mg/L
Analytical accuracy/reproducibility	+/- 5%	+/- 2.5%	< 1% depending on measurement interval
Interferences	volatile amines, surfactants, proteins > 1 g/l	volatile components (possibly volatile amines)	volatile amines, surfactants, proteins > 1 g/l
Sample colour dependent	No	No	No
Automatic sampling	Yes	Yes	Programmable
Sampling/Measurement interval	at least 8 min	at least 10 min	at least 5 min
Sampling volume		minimum 100 ml/h	5-10 ml per sample
Sample dilution	Yes	No	No
Destructive measurement	Yes	No	Yes
Autocalibration	Yes	Yes	Yes
Calibration interval		programmable	Programmable
Autocleaning		Yes at programmable intervals	Yes at programmable intervals
Autocleaning sampling system		Yes if filtration is present	Yes if filtration is present
Chemicals consumption		1 set / 6 to 12 weeks (dep. of sampling interval)	about 3 litres/month
Analog output		0/4 - 20 mA	4 - 20 mA
Serial output		RS 232	RS 232C / RS 485 (optional)
Alarm output		Optional alarm contacts	alarm contacts
Display on analyser	Yes	Yes	Yes
Control panel		Yes	Yes
Datalogging		continuously	last 100 measurements
Weight		10 kg	30 kg
Compactness		350 x 640 x 220 mm	650 x 400 x 350 mm
Safety			
Sample-resistant materials		Yes	Yes
Chemicals storage		Refrigerated inside analyser	outside analyser
Temperature ranges		0-40 °C (higher might be possible)	0-50 °C
Ex-proof		No	No
Housing		IP65	IP54 (dust and splash proof)
Power supply		100-240 V, 50 - 60Hz	115-240 V, 50-60Hz

Maintenance			
Self Diagnosis	Yes	Yes	Yes
Crewtime		replacement of tubing	replacement of tubing
Scheduled Maintenance		each 6 months	each 6 months
Availability Spare Parts		OK	OK
Accessibility		OK	OK
Certifictation			
EC Certificate		CE - ISO 9001	CE
Costs			
Cost of the analyser		+/- 10.000 EUR	+/- 10.000 EUR
Guarantee			
Cost chemicals / year			
References / Services			
Belgium	No (Barcelona-Spain)	Dr. Lange BELGIË bvba. www.langegroup.be	AppliTek NV. www.applitek.com
	aquatec@agbaring.com	info@langegroup.be	info@applitek.be
	ADASA Sistemas, S.A. Spain, SERES-France, …	Motstraat 54, 2800 Mechelen-Belgium	Venecoweg 19, 9810 Nazareth
	Tel: +34 93 487 40 41 Fax: +34 93 215 43 49	Tel: +32 15 42 35 00 Fax: +32 15 41 61 20	Tel: +32 9 38 34 02 Fax: +32 9 36 72 97

3.3 Note on measurement frequency

For the operation of on-line sensors, samples need to be taken on regular time intervals. From a practical control point-of-view, it is needed to sample faster than T/10, where T is the time constant of the process under study. Too frequent sampling should also be avoided, since there is a risk that the controller will be influenced too much by the high-frequency noise on the measurement signal.

In TN 71.2, it is stated that the reactor of 100 litres, will be fed with a flow rate of about 10 l/d. This means the hydraulic retention time in the reactor is 10 days. However, biological processes can be significantly faster, so it is important to highlight the governing time-constant of the biological process when this process is subject to a load change. This governing process is the hydrolysis of the soluble organic matter. The maximal hydrolysis rate, given as $k_{Ac,Ss0}$ in TN 71.1, has a value of 1.4 d^{-1}. This means the time constant of the process is approximately 17 hours. Following this reasoning, one measurement of VFA and ammonia every 1.5 hours or 90 minutes should suffice for process evaluation and control.

When for example 10-15 ml of sample is taken for one ammonium measurement, about 160-240 ml is needed every day. Assuming a concentration of 500 mg NH_4-N/l in the filtrate, about 120 mg NH_4-N or 2.4 % of the total NH_4-N production is lost via the analysis apparatus, since the nitrogen cannot be recuperated after analysis. For the VFA, this ration is only about 0.5 %, because less sample is needed for this analysis when a GC is used.

4. Safety aspects

The filtrate leaving the waste compartment should be free of micro-organisms in order to avoid contamination of the other compartments. Moreover, unwanted chemicals such as hormones, drugs and salts can be transported to the other compartments. The fate of these compound will be studied in future dedicated projects. The construction of the necessary hardware to remove such compounds will not be delivered in the EWC project.

4.1 Screening of pathogens

Micro-organisms are all supposed to be retained after passing the filtration unit. At this point samples will be taken to be analysed with molecular techniques to verify the absence of micro-organisms. Tests dealing with the screening of the microbial communities in the waste compartment are actually under investigations at VITO, SCK and LABMET using several molecular techniques. Two techniques were proposed in the framework of EWC, namely Fluorescence In-Situ Hybridisation (FISH) and Denaturing Gradient Gel Electrophoresis (DGGE).

4.1.1 Fluorescence In-Situ Hybridisation

Fluorescence in situ hybridisation (FISH) is a method used to label cells or chromosomes according to the sequences of nucleic acids contained within them. The nucleic acid that is labelled is usually the RNA of the ribosomes and the target is usually the complete cell. The process works by taking fluorescently labelled pieces of DNA or RNA called probes that are around 20 nucleotides in length. The probes are incubated in the presence of cells under appropriate conditions to permit specific hybridisation of probe to target nucleic acid. Cells that contain ribosomes with complementary RNA sequences are labeled by the binding of the fluorescent probe in situ. These labelled cells can be visualised by flow cytometry or fluorescence microscopy.

In the study of the contaminants leaving the waste compartment, the use of generally labelled probes will be necessary since the method allows the visualise all living organisms present in solution.

4.1.2 Denaturing Gradient Gel Electrophoresis DGGE

The theory behind DGGE is very simple, the two strands of a DNA molecule separate, or melt, when heat or a chemical denaturant is applied. The temperature at which a DNA duplex melts is influenced by two factors:

1. The hydrogen bonds formed between complimentary base pairs. GC rich regions melt at higher temperatures than regions that are AT rich. The attraction between neighbouring bases of the same strand is called "stacking". Consequently, a DNA molecule may have several melting domains with characteristic melting temperatures (Tm) determined by the nucleotide sequence.

2. DGGE exploits the fact that otherwise identical DNA molecules, which differ by only one nucleotide within a low melting domain will have different melting temperatures. When separated by electrophoresis through a gradient of increasing chemical denaturant (usually formamide and urea), the mobility of the molecule is retarded at the concentration at which the DNA strands with low melt domain dissociate. The branched structure of the single stranded moiety of the molecule becomes entangled in the gel matrix and no further movement occurs. Complete strand separation is prevented by the presence of a high melting domain, which is usually artificially created at one end of the molecule by incorporation of a GC clamp. This is accomplished during PCR amplification using a PCR primer with a 5' tail consisting of a sequence of 40 GC.

Advantages

- High detection rate and sensitivity.
- The methodology is simple and a non-radioactive detection method is used.
- PCR fragments may be isolated from the gel and used in sequencing reactions.

Disadvantages

- Computer analysis and preliminary experiments are essential when setting up DGGE.
- Primers are expensive because of the 40 bases of GC clamp. Additional primers may be required for sequencing.
- Genes which are exceptionally GC rich are not easily analysed by DGGE.
- Method involves the use of formamide.

4.2 Filtrate disinfection

Possibly, when break-through of micro-organisms occurs, an additional barrier needs to be installed after the filtration unit. There is also a possible risk for clogging, breakthrough and deterioration of the filtration module. The clogging problem can be solved by applying backwash at regular intervals, using process water and detergents.

Candidate technologies to be used as an additional disinfection step are:

- thermal treatment: autoclaving, pasteurisation, steam distillation
- oxidative chemical treatment: chlorine, hydrogen peroxide, ozone
- Ultra Violet disinfection
- Dead-End filtration via hollow fibres

A thermal treatment seems desirable, but only high temperatures during long time intervals suffice to successfully remove micro-organisms. If the necessary heat to destroy them would be applied at temperatures exceeding 450°C, a major part of the VFA would be destroyed.

Chemical treatment techniques are reliable, but not desirable since chemicals need to be provided and residues can influence the operation of the following compartment. Furthermore, oxidative treatment will also destroy part of the VFA in the filtrate.

The following techniques remain possible:

4.2.1 UV disinfection

Ultra Violet (UV) radiation can inactivate bacteria and viruses. Ultraviolet light (UV) destroys micro-organisms by changing their genetic information (DNA), but does not produce residual or hazardous by-products, nor does it affect the odour or colour characteristics of the treated water. It is light with very high energy levels and wavelength of 200-400 nanometer (nm). The most effective ultraviolet light for disinfection is UV-C (200-280 nm), specially with a wavelength of 254 nm.

The liquid is exposed to light from a mercury arc vapour lamp which emits UV light in the range 0.2 to 0.29 µm (maximum biological disinfection occurs at 0.254 µm). The disinfection power depends on the penetration of light into the water. Power inputs of 30mW/cm^2 of water film surface are usually applied and can give 4 log or higher reduction in coliforms.

UV systems come in a variety of configurations, some pass the liquid through tubes with the UV source shining in, while some have UV lamps encased in quartz with the liquid flowing over the surface.

It should be noted that breakdown of a certain low portion of the VFA during UV treatment can not be excluded. Therefore, this technique along with its influence on the nutritional value of the filtrate should be further investigated on the prototype reactor actually running at EPAS laboratory.

4.2.2 Membrane filtration

Membrane processes can be classified according to the driving force and pore size. Depending on the pore size, micro-, ultra- and nanofiltration and reversed osmosis can be distinguished. In Table 6, the classification of the membrane processes according to the pore size is shown.

Table 6. Classification of membrane processes according to pore size

Membrane process	Range	Permeable for	Application
Microfiltration	10^2-10^4 nm	Ions, macromolecules, sugars, water	Complete removal of bacteria
Ultrafiltration	1-10^3 nm	Ions, salts, sugars, water	Removal of macromolecules
Nanofiltration	1 nm	Monovalent ions, water	Desalination, COD reduction
Reversed osmosis	< 1 nm	Water	Desalination

In order to successfully remove micro-organisms from the filtrate, an extra dead-end ultrafiltration via hollow fibres (pore size 0.2 µm) is proposed. It should be noticed that he membranes will saturate after a period of time. Therefore, the membrane should be regularly cleaned and replaced. The frequency of renewal of the membrane could be defined after testing the system in the prototype reactor.

To avoid increased pressure drop over the membranes, a gear pump will be used to circulate the filtrate over the membranes and to build up pressure. Speed control will be done via a frequency converter and the flow will be read off the flow meter in the membrane loop. The pressure before and after the membranes will be visualised by pressure sensors.

4.3 Removal of unwanted chemicals in solution

Unwanted chemicals such as hormones, xenobiotics and drugs can be transported to the other compartments. The fate of these compound will be studied in future dedicated projects. The presence of this compounds in the liquid stream, originated from the waste compartment, can not be excluded and therefore, has to be carefully studied to avoid the introduction of these unwanted products in the other compartments.

The list of trace contaminants or endocrine disrupters stemming from human activity found in wastewaters is long (Concil, 1999; Keith, 1998) and the problem has been apparent since the 1970's (Kirchner et al., 1973). The most relevant compounds for research activities are based on four criteria: (1) abundance in waters and wastewaters, (2) high persistence in the environment, (3) high potency as endocrine disrupters and (4) analyses to below ng/L levels are feasible. Compounds that suit those criteria best are natural and synthetic estrogens excreted by men and women in urine and faeces and with increased levels during pregnancy and hormone replacement therapy. Those compounds are excreted in conjugated form and are reactivated during biological treatment (Ternes et al., 1999). While those compounds have an average persistence, synthetic hormones and chemicals have a much higher persistence, but lower potency. Natural hormones can be expected to be present in all municipal wastewaters and hence also in the effluent generated from the waste compartment. Some technologies exist to remove hormones and xenobiotics from water streams, mainly in the case of wastewater treatment technologies.

It is not in the scope of this note to define the best technology for the removal of hormones and xenobiotics from the filtrate of the waste compartment. This study is dedicated to other projects, which are running in parallel to this Melissa-project.

4.3.1 Membrane biosorption

Membrane biosorption is a new integrated process for xenobiotics removal from water environment. Biosorption takes place at the biofilm that covers the membrane surface during MF/UF processes (Koltuniewicz & Bezak, 2002). This technique is especially feasible for removal of diluted components with high retention. The naturally occurring microorganisms may be applied as a sorbent. This option is however, not suitable in the actual study since the liquid stream is supposed to be exempt of microorganisms. The option therefore, is to use another type of sorbent to remove the unwanted chemicals. This may lead to use inorganic ion exchangers and/or activated carbon in its specific forms. The membrane biosorption takes place in two different regions, i.e., at the vicinity of the membrane surface and in the bulk of the system. The biosorption mechanisms are enhanced near the membrane surface because the biosorbent concentration is higher than in within the bulk. The purified liquid passes through the biofilm (biosorbent/sorbent) and eventually leaves the membrane as a permeate. To achieve satisfactory results, the recovery, size of the circulation tank and the surface of membrane are also independent engineering factors that must be carefully selected to achieve the best results of the sorption process.

The sorption packed-column configuration is the most effective mode of application for the purpose. Recovery of the deposited material from saturated biosorbent can be accomplished because they can often be easily released from the biosorbent in a concentrated wash solution which also regenerates the biosorbent for subsequent multiple reuse. This supposes the use of an extra step in the whole process, where the biosorbent, the washing solution and membranes should be included in the concept of the liquid loop. The filtrate coming from the waste compartment, after being disinfected, will pass through an adsorptive membrane

4.3.2 Reverse osmosis

Reverse osmosis, despite the fact that it removes completely xenobiotics and hormones from liquid streams, is not suited in the actual project because it retains salts and some minerals, which are suited to be transferred to the second compartment.

4.3.3 Steam distillation

A possible technique to remove these unwanted chemicals is presented schematically in Figure 9. A steam distillation unit is installed, that transfers a large part of the VFA and the liquid to a liquid phase B. The C phase, containing a concentrated mixture of unwanted compounds can then be treated at a higher temperature (350°C-400°C) to obtain full destruction of contaminants. Afterwards, stream C and B can be joined again to reach the next compartment (stream D). This system requires a modular build-up to enable fumigation of the separate compartments if required.

In the vaporizing compartment, a membrane can be installed that lets the gas stream through. Onto the membrane, condensation will occur, further optimising the distillation and retaining possible bacteria.

This technique may be suitable in the present technology since the destructive effect of high temperatures on volatile fatty acids is avoided by the recuperation of the VFA via the distillation system. Even by applying high temperatures (350-400°C), the major VFA compounds might not be destroyed. The application of this technology should however, be studied carefully, before being selected definitely mainly by considering the fate of the major VFA after being exposed to high temperatures, the behaviour of the accumulated salts inside the non vaporised stream and their amount that will deposit on the walls of the boiler.

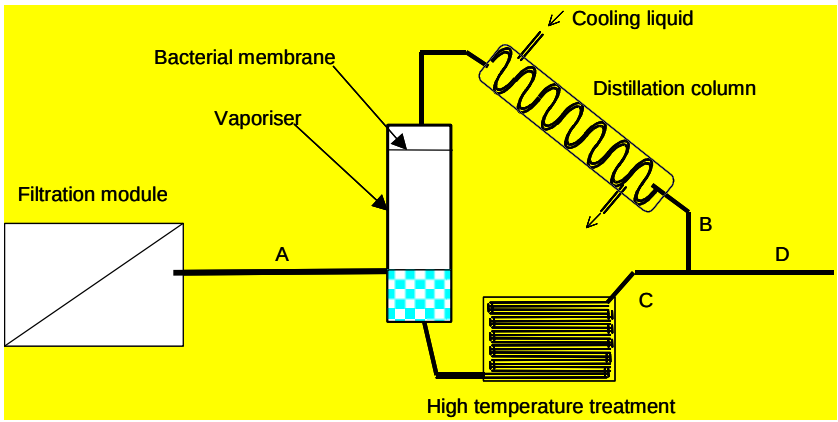


Figure 9: Overview of a possible liquid treatment after the anaerobic treatment

5. Design proposal

For clarity, the filtration unit is treated in technical note 71.2, where also an overview of the components necessary to construct the bioreactor and the filtration unit (including materials, sensors, fittings, valves, pumps, mixing of the reactor,…) is given. Here in Table 7 and Figure 10, this information is repeated, in order to have a general overview of the liquid loop.

Table 7. Filtration unit design and instrumentation

Reference	Description	Type	Sub-type
V-F-001	Valve for control of filtration module feeding	Pneumatic control valve	Bürkert 2000 A 20,0 RVS
V-F-006	Control valve for transmembrane pressure	Pneumatic control valve	Kämmer 20037
V-F-011	2-way valve for regulating effluent recirculation to bioreactor	Solenoid valve	Bürkert 6014
V-F-013	2-way valve for regulating effluent recirculation to bioreactor	Solenoid valve	Bürkert 6013
TS-F-001	Temperature sensor in the filtration loop	Endress+Hauser sensor	TST42, 0 – 100 °C
TT-F-001	Temperature transmitter	Endress+Hauser iTemp	TMT187
LD-F-001	Level Switch	Endress+Hauser Liquiphant T	FTL 260
SS-F-001	Suspended solids sensor	Sensor	Turbidity sensor via light scattering
ST-F-001	Suspended solids transmitter	Transmitter	
PD-F-001	Pressure transducer with ceramic membrane	Endress+Hauser Cerabar T	PMC 131, 0 – 6 bar
PD-F-002	Pressure transducer with ceramic membrane	Endress+Hauser Cerabar T	PMC 131, 0 – 6 bar
PD-F-003	Pressure transducer with ceramic membrane	Endress+Hauser Cerabar T	PMC 131, 0 – 6 bar
FD-F-001	Flow transducer for control of the flowrate through the filtration	Endress+Hauser Promag	50H80, 20 – 2000 l/h
WD-F-001	Scale for control of filtrate production	Sartorius	CP3202S
PMP-F-001	Pump for recirculation over filtration module, nominal flow rate 724 l/h (equivalent to cross-flow velocity of 4 m/s)	Progressive cavity pump	Seepex 2-12BN/A1-C1-C6-F0-A, 0 – 1 m^3/h

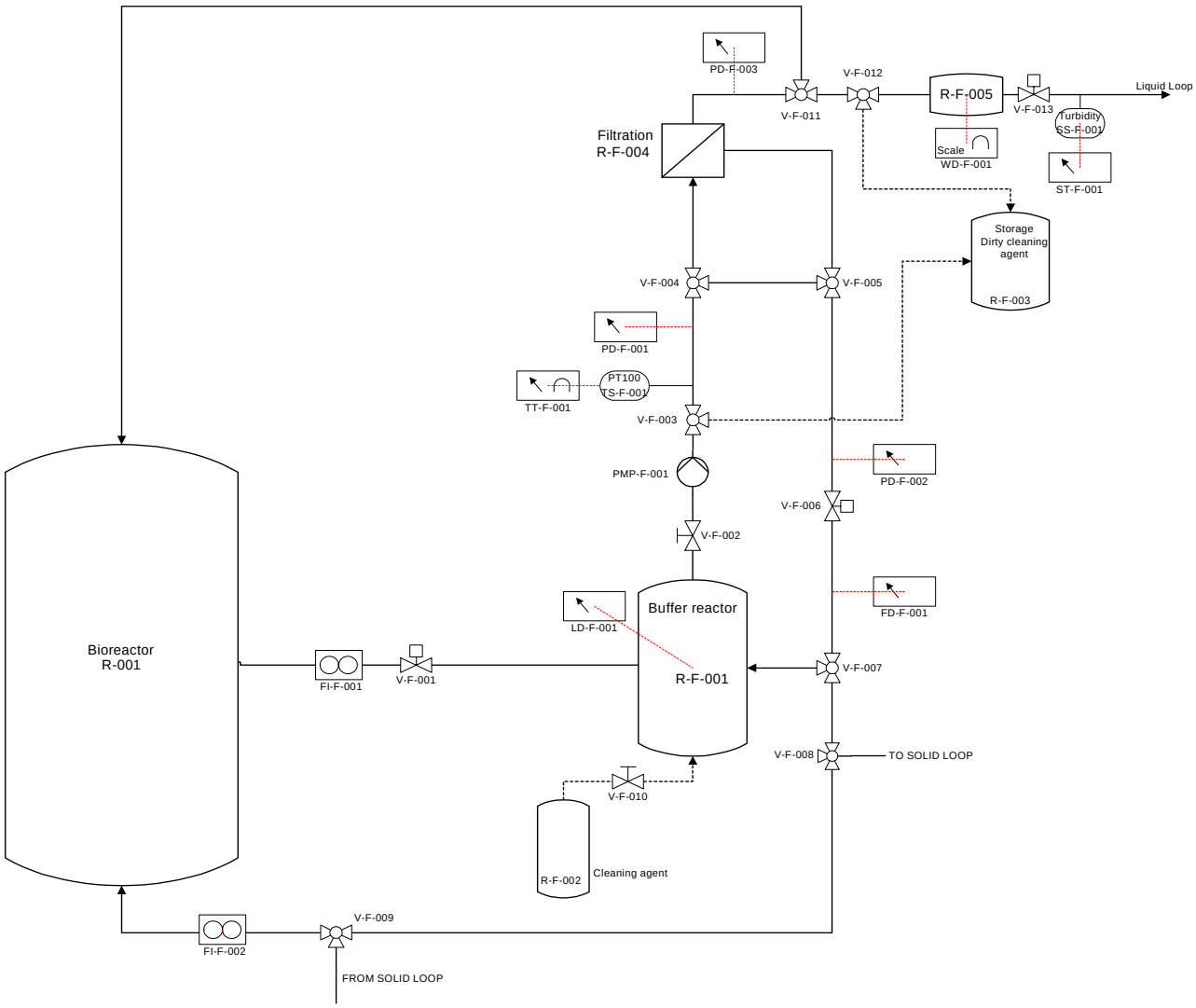


Figure 10. Conceptual design of the whole liquid loop including the reactor

6. References

Keith L. H. (1998) Environmental Endocrine Disruptors. Pure and Applied Chemistry **70**, 2319-2326.

Kirchner M., Holsen H. and Norpoth K. (1973) Fluorescence Spectroscopic Determination of Anti-Ovulatory Steroids in Water and Waste Water on the Thin Layer Chromatography Plate. Zbl. Bakt. Hyg. I Abt. Orig. B 157, 44-52.

Koltuniewicz and Bezak (2002) Engineering of membrane biosorption. Desalination, **144**, 219-226.

National Research Council (1999) Hormonally active agents in the environment. National Academy Press, Washington DC.

Ternes T. A., Kreckel P. and Mueller J. (1999) Behaviour and occurrence of estrogens in municipal sewage treatment plants - II. Aerobic batch experiments with activated sludge. The Science of the Total Environment, **225**, 91-99.