Universitat Autònoma de Barcelona

TECHNICAL NOTE 94.22

Call Off Order 3 – COMPARTMENT I Additional Characterization

Work Package 94.2

Sampling and Analysis Protocols – Issue 2 - Test with 10 days hydraulic residence time

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1. Introduction

The objective of this document is to summarize the sampling and analysis strategy to be implemented on Compartment 1 for the test at 10 days HRT.

This document is defining a first set of samplings and analyses that might evolve depending on the feedback of the C1 bioreactor operation and on the results of said samplings and analyses.

2. Applicable and reference documents

2.1. Applicable documents

Ref.	Title	Reference	Issue	Date
AD1	MPP Proposal for Call Off Order 3 – C1 additional characterization	OFR-ESA-03/07-UAB	1	30/11/07
AD2	MPP Quality Manual	MPP-QA-07-0001	2	
AD3	MPP Rules for Good Laboratory Practices	MPP-QA-07-0003	0	
AD4	Test Plan for C1 additional characterization tests	TN94.5	0	
AD5	PID of Compartment 1	MPP-PID-10-1001	B3	5/10/2011
AD6	MPP Operation Manual for C1	MPP-OP-12-1001	0	9/2/2012
AD7	C1 Acceptance Review Datapackage including HMI and PLC software user manuals	DP94.1	1	October 11
AD8	MPP Maintenance Manual for C1	MPP-UM-11-1001	0	
AD9	Test Protocol for nominal operation with 10 days HRT	TN94.62		
AD10	Analytical Procedure for Ashes determination	MPP-QCP-07-0001	(1)	16/10/2007
AD11	Analytical Procedure for COD soluble determination	MPP-QCP-07-0004	(1)	16/10/2007
AD12	Analytical Procedure for COD total determination	MPP-QCP-07-0005	(1)	16/10/2007
AD13	Analytical Procedure for Dry matter determination	MPP-QCP-07-0007	(1)	16/10/2007

AD14	Analytical Procedure for EC determination	MPP-QCP-07-0008	(1)	16/10/2007
AD15	Analytical Procedure for lyophilization	MPP-QCP-07-0010	(1)	16/08/2007
AD16	Analytical Procedure for NH4-N determination	MPP-QCP-07-0011	(1)	16/10/2007
AD17	Analytical Procedure for NO2-N determination	MPP-QCP-07-0012	(1)	16/10/2007
AD18	Analytical Procedure for NO3-N determination	MPP-QCP-07-0013	(1)	16/10/2007
AD19	Analytical Procedure for O.D. determination	MPP-QCP-07-0014	(1)	16/10/2007
AD20	Analytical Procedure for pH determination	MPP-QCP-07-0015	(1)	16/10/2007
AD21	Analytical Procedure for Protein determination	MPP-QCP-07-0018	(1)	16/10/2007
AD22	Analytical Procedure for Total nitrogen determination	MPP-QCP-07-0020	(1)	16/10/2007
AD23	Quality control procedure for counting bacteria from C1	MPP-QCP-09-1001 0	0	20/10/2009
AD24	Measurement procedure for pH	MPP-QCP-10-1001	(0)	22/02/2010
AD25	Measurement procedure for gas mass flow measurement in C1	MPP-QCP-10-1002	(0)	22/02/2010
AD26	Quality control procedure for Alkalinity determination	MPP-QCP-11-0001	(0)	06/02/2012
AD27	Quality control procedure for the measurement of VFAs by HPLC	MPP-QCP-11-0002	(0)	06/02/2012
AD28	Analytical Procedure for Protein determination from C1	MPP-QCP-12-1001	(0)	01/03/2012
AD29	Procedure for the control of sterility of C1 filtrate line and effluent tank	MPP-QCP-12-1002	(0)	18/6/12
AD30	Quality Control Procedure for total nitrogen determination (total broth)	MPP-QCP-12-0001	(0)	20/6/12

2.2. Reference documents

Ref.	Title	Reference	Issue	Date
RD1	TN 94.11 Compartment I Integration in MPP	TN 94.11	0	13.02.09
RD2	HAZOP on Compartment 1	MPP-TN-08-1001	0	01/09/2008
RD3	Gas Chromatograph User Manual	MPP-UM-09-0009	1	23.10.06
RD4	Portable Gas Analyzer User Manual	MPP-UM-09-0012	0	
RD5	TN 83.7 Expertise of level 0 control loops on the 100 L pilot reactor	TN 83.7	1	23.10.06
RD6	Minutes of meeting MPP/UBP on C1 characterization	MPP-MOM-08-1007	0	16.04.2008
RD7	EPAS EWC User Manual	User Manual	1	12.06.07
RD8	HPLC Ultimate 3000 User Manual	MPP-UM-11-0002	0	25.11.2011

3. Acronyms and definitions

BR: bioreactor
CI : compartment I
CST : capillary suction time
FU: Filtration unit
GL: Gas loop
HMI: human interface
HPLC High Pressure Liquid Chromatography
HRT: hydraulic residence time, equivalent to liquid residence time
ICP-MS : Induced Coupled Plasma Mass Spectrometry
MELiSSA: Micro-Ecological Life Support System Alternative
SFC: Sequential function chart
TAR test acceptance review
TRR test readiness review
UAB: Universitat Autònoma de Barcelona
VFA: volatile fatty acids

4. Responsibilities

For the whole call-off order 3, concerning the characterization tests on C1, the responsibilities of the different organizations are detailed in AD4.

5. Test items

5.1. Description (PID, technical drawings, user manual)

The compartment 1 was delivered in the MPP and installed as described in RD1.
It consists of 3 subunits or modules that are described on the PID AD5 and in the User Manual AD6, namely :

- The bioreactor and influent tank skid
- The gas loop skid
- The filtration unit skid

The system is operated automatically from a programmable logical controller (PLC) as described in AD7.

5.2. Hazards induced by test item and safety measures to be taken

As explained in the hazard and operability study carried out on compartment 1 (cf. RD2), the main hazards induced by the operation of compartment 1 are:

- pressure (gas: up to 3 barg, liquid: up to 5 barg)
- temperature (steam sterilization)
- chemical (acid/base for pH control)
- biological (biohazard level 2 as a maximum when using faeces for the feeding of C1)
- flammable gases (H2, CH4) ;

The adequate individual protection measures shall be taken by the operators in order to limit the exposure to these hazards. As detailed in AD4, these measures include :

- wearing of a labcoat
- wearing of safety goggles
- wearing of gloves when manipulating materials or equipments
- respect of the user and maintenance instructions, in particular the respect of the confined and anaerobic conditions in the bioreactor

5.3. Instructions for operation

See AD6 and AD7, and RD7

5.4. Instructions for maintenance

See AD6 and AD7, AD8 and RD7

6. Test logic

6.1. Objectives of the tests

The objective of the C1 characterization tests is to collect as many data as possible on steady state periods in order to provide the parameters necessary for the understanding of the C1 process behaviour and for the construction of a knowledge model.
For phase 3, the objective is to reach the steady state operating at 10 days HRT and collect all the main parameters characterizing the process at that hydraulic residence time. These parameters will then allow to determine if the success criteria fixed in AD4 have been reached.

6.2. Approach followed

The approach followed for phase 3 of the characterization tests is to operate the C1 reactor in continuous mode in order to reach the targeted suspended matter concentration, and then to reach the steady state at 10 days HRT. For that steady state, a mass balance can be calculated on the bioreactor, as per the following equation

Solid&liquid feed input -> reactor content (liquid+solid) + gas output + filtrate output + reactor samplings/bleedings

This mass balance can be drawn at overall level and for the main chemical elements (by order of precedence C balance, then N balance, then O balance and H balance), and be related to the operational parameters of the C1 unit, which will provide a first set of equations for the knowledge model.

The different parameters to be recorded during the tests have been grouped in three categories by order of priority, as follows :

Priority 1 (high priority): all the data necessary for the characterization of compartment 1 and the long term operation of C1 in the MPP integrated loop including operating parameters measured online (like the pH, the temperature, the pressure, the gas composition in CO2 and CH4), and parameters measured offline (like the sterility checks of the filtrate output, the VFAs, the dry matter, the COD, the pH, the electroconductivity, the bacterial counts)

Priority 2 (medium priority): all the data necessary for computing mass balance on C1 bioreactor as per the hereabove equation (total and soluble nitrogen, soluble COD, ammonium, organic elemental composition, ashes, gas composition in H2, H2S and O2)

Priority 3 (low priority) : the remaining parameters used to refine the models later on (particles size, capillary suction time, proteins in total and soluble fractions, alkalinity, mineral elemental composition, gas contaminants)

The steady state indicators of the bioreactor are measured more frequently than once per HRT in order to detect the establishment of the steady state, these are:

- Dry matter content
- Total Chemical Oxygen Demand (CODtot)
- CO2 production rate
- VFA production rate and, for information, the ratio between the various VFAs compounds

When the steady state is reached, all the parameters of priority 1 and 2 are measured twice, separated by one HRT

6.3. Applicable requirements

See the test protocol AD9.

6.4. Recall of test sequence

The characterization tests sequence can be summarized as follows :
Phase 1 : maintenance of the inoculum, 20 days HRT
Phase 2 : ramp-up of the culture in the C1 bioreactor up to continuous conditions, HRT evolving from 20 days up to10 days, reaching a dry matter content between 40g/L and 70g/L
Phase 3 : 10 days liquid residence time test
Phase 4 : 7 days liquid residence time test
Phase 5 : 13 days liquid residence time test
Phase 6 : 5 days liquid residence time test

6.5. Success/failure criteria for the 10 days HRT

The characterization tests are considered successful if the specified conditions have been maintained and the expected data have been collected as per the sampling plan.

As per AD9, the steady state has been successfully reached if the following parameters are detected stable within the following deviations around their average on a period of 3 HRTs:

- Dry matter content : 15%
- Total Chemical Oxygen Demand (CODtot) : 15%
- CO2 production rate : 20%
- VFA production rate : 20%

The degree of closure of the mass balance is also considered as a success criterion for the sampling and analyses activities. The ratio of measured/calculated output mass by the measured/calculated input masses on the bioreactor should be higher than 90%.

Similar success criteria on C mass balance and N mass balance closures are defined and set to 80%.

For more details see the test protocol AD9.

7. Data collection plan – Sampling plan

7.1. Uncertainty acceptance level

The uncertainty budget has not been exhaustively assessed for all the measurement techniques to be implemented.
A general approach is to accept on all biological samples an uncertainty of 10% due to the natural variety present in the sample.

For three measurement techniques, the uncertainty was assessed, and the budget was calculated :

- pH see AD24 for the procedure details
- gas mass flow , see AD25 for the procedure details
- VFAs by Gas Chromatography , see AD26 for the procedure details

The calculated expanded uncertainties with a level of confidence of 95% are respectively +-0.065 pH unit for pH and 2% for CH4 gas mass flow.
For the VFAs measurement using the gas chromatography, the method reached an expanded uncertainty of 44% to 69% with a level of confidence of 95%, which led to the abandon of this technique for the alternative of VFAs measurements by HPLC that improved the repeatability and reduced uncertainties.

7.2. Measurement plan

The measurement plan as discussed among the partners of call off order 3 includes the following parameters (cf. RD6)

Phase	Physical or chemical or biological parameter
Liquid-solid phase	total liquid flow or volumes
	Dry matter
	ashes
	sample volume and weight
	CHONS total
	Minerals: P, Ca, Mg, Na, K, Si, S, Fe, Al, Ba, Cr, Cu, Mn, Ni, Sr, Zn, Mo, Ti, Be, V, Co, As, Se, Pd, Pb, Cd, Sn, Sb, W, Hg
	VFAs
	NH4+
	COD soluble
	COD total
	N total
	N soluble
	aerobic count
	anaerobic count
	EC
	pH
	Temperature
	Proteins (total and soluble)
	Fibers
	alkalinity
	CST
	turbidity

	Sterility of filtrate solution
	particles size
Gas phase	total mass gas flow or volumes
	CO2 concentration
	CH4 concentration
	H2 concentration
	H2S concentration
	O2 concentration
	Sample volume
	Pressure
	temperature
	gas contaminants

7.3. Sampling techniques

The sampling techniques are used to recover gas and liquid/solid samples.

For gas samples, a dedicated circuit allows to continuously circulate, dry out and analyze the biogas for CO2 and CH4 assaying. A bypass line also allows to force the biogas from C1 bioreactor to a portable analyzer in order to make further assays (CH4,CO2, but also O2, H2 and H2S).

For liquid samples, various ports allow to bleed through manual valves the content of the tanks or circuits.
No continuous sampling of liquid/solid phase is planned.
When making a sampling, the first bled mL are discarded in order to take a sample that be representative of the sampling point. The discarded quantity is weighed. The sampled quantity is weighed as well.

The bleeding is a particular sampling of the liquid-solid phase that intends to regulate the dry matter content in a continuously agitated tank with perfusion. For clarity purposes, the bleeding is distinguished from the other samplings. The bled quantity is also weighed.

All samples are taken in aerobic conditions.

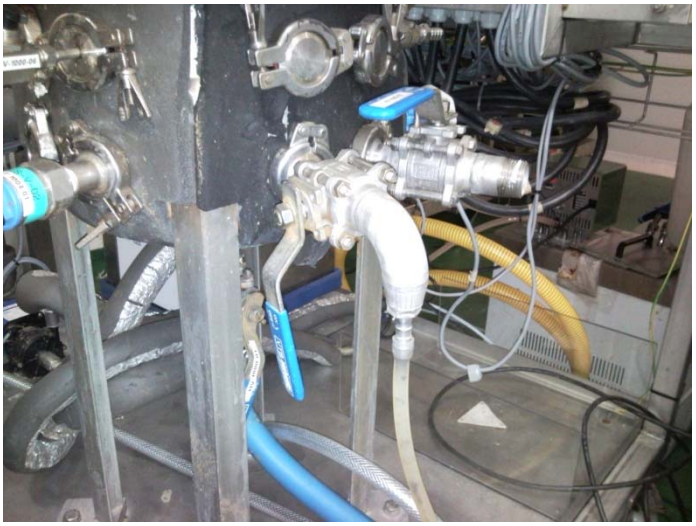
The samplings made on the filtrate circuits and tank, ie downstream the microfiltration membranes are made in sterile conditions, with a previous sterilization, in order to preserve the sterility of the filtrate circuits and to collect a non contaminated sample (see AD29).

7.4. Sampling ports location

For the liquid-solid and gas samplings, various ports can be used.

7.4.1. Liquid sampling port on Influent Tank

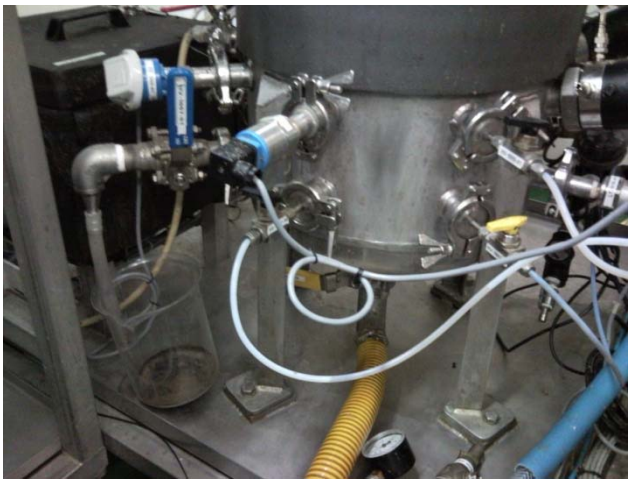
One sampling port is available on the lower part of the influent tank, controlled by HV_1000_07.



Picture 1- influent tank sampling port

7.4.2. Liquid sampling port on Bioreactor Tank

One sampling port is used to make the bleedings and samplings of liquid-solid phase on the bioreactor : the lower side port controlled by HV_1007_02.



Picture 2 – bioreactor sampling port

7.4.3. Liquid sampling port on Effluent Tank

The steam sterilizable port HV_1204_02 can be used to take filtrate sample from the effluent tank VSL2_1204_01 and to proceed to harvest.

Another sterilizable port HV_1204_01 can be used to take smaller quantities of filtrate sample from the effluent tank VSL2_1204_01. It is sterilized applying an ethanol wick on the septum before introducing the syringe into the port.

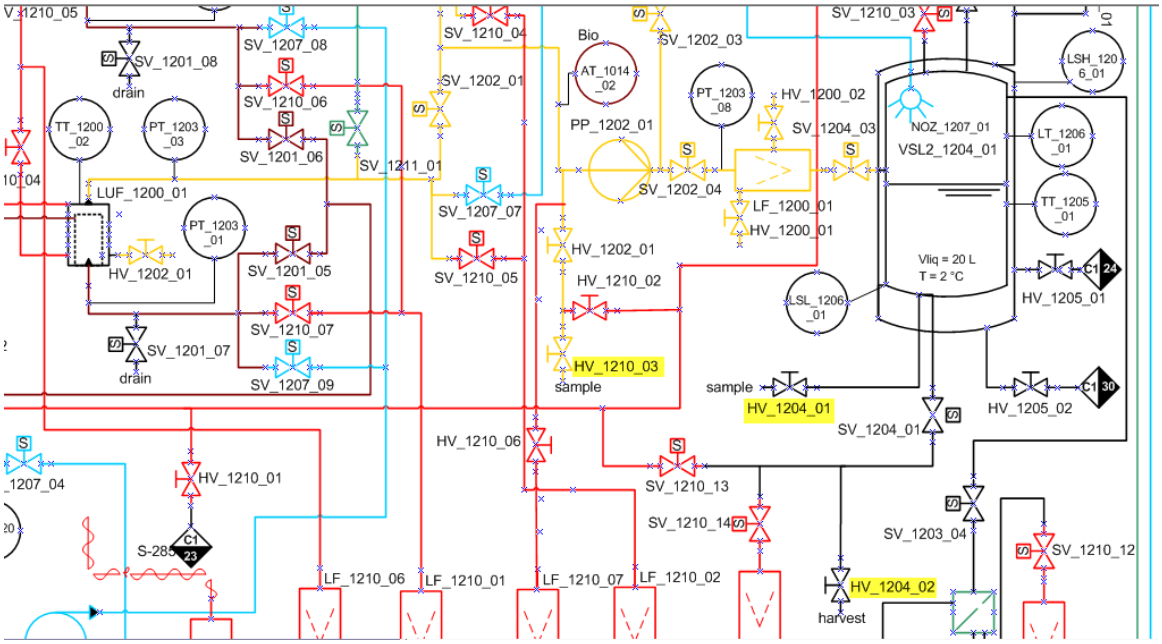
For more details on the steps to follow for sterilization of these ports, see AD29.

Picture 3 – effluent tank harvesting port

7.4.1. Liquid sampling port on filtrate line

The filtrate line downstream the ultrafiltration membranes and upstream the peristaltic pump PP_1202_01 is equipped with a steam sterilizable sampling port HV_1210_03, as shown in the following PID excerpt.
For more details on the steps to follow for sterilization of this port, see AD29.

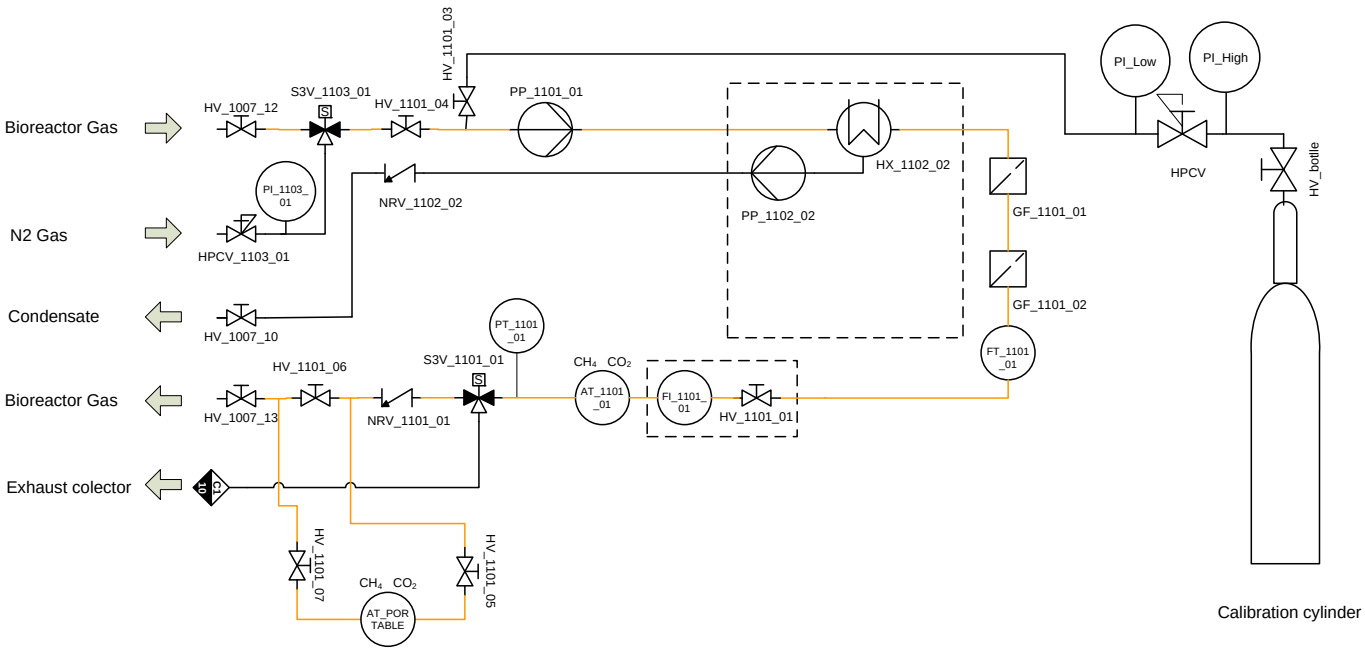
Picture 4 - filtrate line sterilizable sampling port



Picture 5 – sampling ports on effluent tank and filtrate line

7.4.2. Gas sampling ports on the bioreactor

A bypass line allows to force the biogas from C1 bioreactor to a portable analyzer in order to make further assays (CH4,CO2, but also O2, H2 and H2S) and to return it to the bioreactor through HV .



Picture 6 – gas sampling ports for portable gas analyzer

7.5. Sampling recipients

For the bleeding, a clean 2L bottle is used to recover the necessary volume. This bottle can then be used as a dispenser to extract several samples if needed for further analyses.

For the analyses, the samples are collected in a clean plastic recipient, with a maximum volume of 100mL and a screwable lid. The exact volume recovered into this recipient can be lower than 100mL but should be traced every time a sample is taken, preferably recording the mass of the sample.

Then depending on the analyses, specific containers can be used that are adapted to the requirements of the analysis technique :

- for HPLC, 1,5mL single use glass vials are used
- for lyophilized samples, a 50mL single use Falcon tube is used

Some backup samples are taken in case analyses need to be repeated ; they are kept in the following recipients for conservation in frozen state at -20ºC :

- for HPLC samplesfor VFA analysis, one 2mL plastic Eppendorf is used for the deproteinized fraction of the sample
- for soluble fraction : two single use 2mL plastic Eppendorf are used
- for total broth : two single use 2mL plastic Eppendorf are used
- for lyophilized samples : the remaining quantity inside the 50mL single use Falcon tube

7.6. Use of the volumes extracted during the bleeding

The quantity of liquid-solid phase recovered while making the bleeding can if needed be used as a sample to perform further analyses on the bioreactor content.
In that case, the volume of the bled bioreactor content has to be sufficient to provide the volume necessary for samples preparation.

7.7. Conditioning of the samples

Important remark : before taking any aliquot from a sample to perform the mentioned analyses, the full sample should be previously agitated so that it is homogeneous.

The following analyses are made on the raw liquid sample :
pH, EC, COD of total fraction, proteins of total fraction, dry weight, ashes, particle size, anaerobic and aerobic counting, nitrogen of total fraction, particles size.

The following analyses are made on a sample that has been previously centrifuged and filtered on a 0.22 micron filter :
Ammonium, nitrogen of soluble fraction, COD of soluble fraction, proteins of soluble fraction, alkalinity.

For the VFAs analyses, the samples are first treated to degrade their proteins, then centrifuged and filtered on a 0.22 micron filter.

The following analyses are made on a sample that was previously frozen at -80ºC, lyophilized and homogenized by milling:
Minerals analysis, COHNS elemental analysis, fibers composition.

The backup samples follow the same treatment as for the nominal analysis but they are then reserved for further analysis and stored in frozen state at -20ºC.

The following table indicates the minimal volumes of sample needed to perform each analysis.

Item			Preparation for analysis			Backup for later analysis		
	Priority	Type of recipient	Raw sample	Filtered	Lyophilized sample	Volume/content		Type of recipient
Bleeding volume	1	2L glass bottle	variable				1L to 2.5L	Glass bottle
pH/EC	1	100mL plastic container	20mL				N/A (fresh)	
Dry weight/ashes	1		60mL				?	
Total N	2		5mL				2x2mL broth	Eppendorf
Proteins total	3		2mL					
COD total	1		2mL					
Ammonium	2		5mL	3mL			2x2mL soluble fraction	Eppendorf
COD soluble	2							
Soluble nitrogen	2							
Soluble proteins	3		2mL	2mL				
Alkalinity	3		300mL	150mL			N/A	
VFA	1		5mL	4mL			2 mL deproteinized filtered broth	Eppendorf
CHONS	2		200mL		3g		10g-20g	50mL Falcon tube
Minerals	2				3g			
Fibres	3		300mL		5g			
TIC/TOC	2		12mL	10mL				
CST	3		TBD					
Anaerobic count	1		0,5mL				N/A(fresh)	
Aerobic count	1		0,5mL				N/A(fresh)	
Sterility check	1		10mL				N/A(fresh)	
Particle size	3		TBD					

7.8. Labelling of samples

Samples are labelled in such a way that the label allows

- the identification of the originating process part (RC for bioreactor, ET for effluent tank, IT for influent tank, FL for filtration line)
- the identification of the date of the sampling
- the identification of the treatment implemented for the sample (RW raw sample, SF soluble fraction, DP deproteinized, LY lyophilized, DL dilution)

Additionally, the traceability to the record where the preparation steps of the sample are recorded is made on that record.

Example :
RC_dd.mm.yy_RW, ET_dd.mm.yy_DPDL20

The labelling is made by using an appropriate marker or by printing a sticker with the relevant information and protecting the inscriptions with adhesive tape.

7.9. Conservation of the samples :

In case an analysis cannot be performed on a fresh raw liquid sample, the sample has to be stored in the fridge at 4ºC and analyzed within 8h.

In case an analysis cannot be performed on a filtered liquid sample, the sample has to be stored in the fridge at 4ºC and analyzed within 48h ; for VFAs, the samples stored at -20ºC can be analyzed within 2 months.

In case an analysis cannot be performed on a lyophilized sample, the sample has to be stored in dry conditions and analyzed within 6 months.

The samples that are taken as back-ups in order to perform or repeat some analyses are stored at -20ºC and can be analyzed within 2 months.

The gas samples are not stored ; they should be analyzed on the spot.

7.10. Sampling and analyses frequency on the liquid-solid phase

The following six tables summarize the circuit where to sample and the frequency at which the samples should be taken on the liquid-solid phase, when the bioreactor is operated with a HRT of 10days. The frequencies of operation are relative to the applicable HRT.

Remarks :

- the steady state indicators are identified by a yellow highlighting in the following tables
- the operations marked on week-ends can be performed indifferently on Saturdays on Sundays due to operating constraints
- for the transient phase analyses, the analyses that are not required for steady state monitoring might be adjusted to the contingencies of the laboratory
- some frequencies for priority 3 analyses still have to be decided
- analyses or preparations are marked in orange cells when they can be performed on a batch of several samples, collected at distinct dates
- the backup samples are all described as “storage in frozen or lyophilized state” in the preparations and samplings sections
- for the effluent tank, the CHONS analyses will be limited to TIC/TOC technique on the soluble fraction, while soluble nitrogen will allow N determination in the liquid sample
- for the filtrate line and effluent tank, as explained in detail in AD29, in order to validate the sterility of the filtrate line and of the effluent tank, the samplings will be made on a daily basis in order to make sure that sterility can be reached on a 4 weeks period, then the applicable frequency will be the one specified in the tables

During the transient phase :

Transient Phase

	C1 Process Operations	Month 1																		Month 2										.....	frequency per HRT in the planning	frequency specified per HRT
		12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	1	2	3	4	5	6	7	8	9	10	.....		
	Bioreactor																																	
	samplings and preparations																																	
1	Bleeding	●	●	●	●	●	●		●	●	●	●	●		●	●	●	●	●	●	●		●	●	●	●	●		●	●	●	.....	8,6	10
2	Sampling for analysis of total fraction	●	●	●	●		●		●	●		●				●	●	●	●		●		●	●		●	●			●	●	.....	6,1	2
3	Storage of total fraction in frozen state	●					●					●					●				●						●					.....	2,1	2
4	Sampling for analysis of VFAs	●				●			●				●			●				●			●				●			●		.....	2,9	2
5	Preparation of samples for VFA analysis	●				●			●				●			●				●			●				●			●		.....	2,9	2
6	Storage of VFA samples in frozen state	●				●			●				●			●				●			●				●			●		.....	2,9	2
7	Sampling for analysis of soluble fraction		●														●														●	.....	0,7	0,5
8	Filtering of sample		●														●														●	.....	0,7	0,5
9	Storage of filtered sample in frozen state		●														●														●	.....	0,7	0,5
10	Lyophilization	●										●											●									.....	1,1	1
11	storage in lyophilized state	●										●											●									.....	1,1	1
	analyses on total fraction																																	
1	Dry weight	●			●				●			●				●			●				●			●				●		.....	2,9	2
2	COD, total	●							●							●							●							●		.....	1,4	1,33
3	Ashes	●										●											●									.....	1,1	1
4	Nitrogen conc., total sample	●										●											●									.....	1,1	1
5	Protein conc., total sample	●										●											●									.....	1,1	1
6	pH (offline)		●							●							●							●							●	.....	1,4	1,33
7	Electroconductivity		●							●							●							●							●	.....	1,4	1,33
8	Microbial count (aerobic)			●														●														.....	0,7	0,5
9	Microbial count (anaerobic)			●														●														.....	0,7	0,5
10	Particle size			●														●														.....	0,7	TBD
11	VIAMASS																															.....	online	online
	analyses on soluble fraction																																	
12	VFA composition analysis (HPLC)								●											●										●		.....	0,7	2
13	COD, soluble fraction		●																												●	.....	0,4	0,33
14	Nitrogen conc., soluble fraction		●																												●	.....	0,4	0,33
15	Ammonium conc., soluble fraction		●																												●	.....	0,4	0,33
16	Protein conc., soluble fraction		●																												●	.....	0,4	0,33
17	Alkalinity		●																												●	.....	0,4	0,33
	analyses on lyophilized fraction																																	
18	Fibres composition	●														●														●		.....	0,7	0,5
19	Organic elements conc. (COHNS)	●																												●		.....	0,4	0,33
20	Inorganic elements conc.	●																												●		.....	0,4	0,33

Table 1- samplings and analyses on the C1 bioreactor in transient phase

Transient Phase

	C1 Process Operations	Month 1																			Month 2										.....	frequency per HRT in the planning	frequency specified per HRT
		12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	1	2	3	4	5	6	7	8	9	10	.....		
	Influent Tank																																	
	samplings and preparations																																	
2	Sampling for analysis of total fraction	●	●		●		●		●	●		●				●	●		●		●		●	●		●	●			●	●	.....	5,4	2
3	Storage of total fraction in frozen state	●					●					●					●				●						●					.....	2,1	
4	Sampling for analysis of VFAs	●				●			●				●			●				●			●				●			●		.....	2,9	2
5	Preparation of samples for VFA analysis	●				●			●				●			●				●			●				●			●		.....	2,9	2
6	Storage of VFA samples in frozen state	●				●			●				●			●				●			●				●			●		.....	2,9	2
7	Sampling for analysis of soluble fraction		●														●														●	.....	0,7	0,5
8	Filtering of sample		●														●														●	.....	0,7	0,5
9	Storage of filtered sample in frozen state		●														●														●	.....	0,7	0,5
10	Lyophilization	●										●											●									.....	1,1	1
11	storage in lyophilized state	●										●											●									.....	1,1	1
	analyses on total fraction																																	
1	Dry weight	●			●				●			●				●			●				●			●				●		.....	2,9	2
2	COD, total	●							●							●							●							●		.....	1,4	1,33
3	Ashes	●										●											●									.....	1,1	1
4	Nitrogen conc., total sample	●										●											●									.....	1,1	1
5	Protein conc., total sample	●										●											●									.....	1,1	1
6	pH (offline)		●							●							●							●							●	.....	1,4	1
7	Electroconductivity		●							●							●							●							●	.....	1,4	1
8	Microbial count (aerobic)	●														●														●		.....	0,7	0,5
9	Microbial count (anaerobic)	●														●														●		.....	0,7	0,5
10	Particle size	●																												●		.....	0,4	0,33
	analyses on soluble fraction																																	
12	VFA composition analysis (HPLC)								●											●										●		.....	0,7	2
13	COD, soluble fraction		●																												●	.....	0,4	0,33
14	Nitrogen conc., soluble fraction		●																												●	.....	0,4	0,33
15	Ammonium conc., soluble fraction		●																												●	.....	0,4	0,33
16	Protein conc., soluble fraction		●																												●	.....	0,4	0,33
17	Alkalinity		●																												●	.....	0,4	0,33
	analyses on lyophilized fraction																																	
18	Fibres composition	●														●														●		.....	0,7	0,5
19	Organic elements conc. (COHNS)	●																												●		.....	0,4	0,33
20	Inorganic elements conc.	●																												●		.....	0,4	0,33

Table 2 - samplings and analyses on the C1 influent tank in transient phase

Transient Phase

	C1 Process Operations	Month 1																	Month 2										.....	frequency per HRT in the planning	frequency specified per HRT
		12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	1	2	3	4	5	6	7	8	9	10	.....		
	Effluent Tank																																	
	samplings and preparations																																	
2	Sampling for analysis of total fraction	●	●		●		●		●	●		●				●	●		●	●		●	●			●	●			●		.....	5,7	2
3	Storage of total fraction in frozen state	●			●				●			●				●				●			●				●			●		.....	2,9	2
4	Sampling for analysis of VFAs	●			●				●			●				●			●				●			●						.....	2,9	2
5	Preparation of samples for VFA analysis	●			●				●			●				●			●				●			●						.....	2,9	2
6	Storage of VFA samples in frozen state	●			●				●			●				●			●				●			●						.....	2,9	2
7	Sampling for analysis of soluble fraction		●														●													●		.....	0,7	0,5
8	Filtering of sample		●														●													●		.....	0,7	0,5
9	Storage of filtered sample in frozen state		●														●													●		.....	0,7	0,5
10	Lyophilization																															.....	0,0	N/A
11	storage in lyophilized state																															.....	0,0	N/A
	analyses on total fraction																																	
1	Dry weight	●			●				●			●				●			●				●			●				●		.....	2,9	2
2	COD, total	●							●							●							●							●		.....	1,4	1,33
3	Ashes	●										●											●									.....	1,1	1
4	Nitrogen conc., total sample	●										●											●									.....	1,1	1
5	Protein conc., total sample	●										●											●									.....	1,1	1
6	pH (offline)		●							●							●							●							●	.....	1,4	1,33
7	Electroconductivity		●							●							●							●							●	.....	1,4	1,33
24	Sterility control (aerobic)	●			●				●			●				●			●				●			●				●		.....	2,9	3
25	Sterility control (anaerobic)	●			●				●			●				●			●				●			●				●		.....	2,9	3
	analyses on soluble fraction																																	
12	VFA composition analysis (HPLC)								●											●											●	.....	0,7	2
14	Nitrogen conc., soluble fraction		●																												●	.....	0,4	0,33
15	Ammonium conc., soluble fraction		●																												●	.....	0,4	0,33
16	Protein conc., soluble fraction		●																												●	.....	0,4	0,33
17	Alkalinity		●																												●	.....	0,4	0,33
18	Fibres composition																															.....	0,0	?
19	Organic elements conc. (COHNS)	●																												●		.....	0,7	0,5
20	Inorganic elements conc.	●																												●		.....	0,7	0,5
	Filtrate Line																																	
	samplings and preparations																																	
5	Sampling for analysis of total fraction	●				●			●				●			●				●			●					●			●		.....	3,0	2
	analyses on total fraction																																	
3	COD, total	●							●							●							●									.....	1,3	2
24	Sterility control (aerobic)	●			●				●			●				●			●				●			●				●		.....	2,9	3
25	Sterility control (anaerobic)	●			●				●			●				●			●				●			●				●		.....	2,9	3
10	Particle size																															.....	0,0	N/A

Except for the validation period where daily sampling is required

Table 3 - samplings and analyses on the C1 effluent tank in transient phase

After establishment of the steady state phase :

	C1 Process Operations	Steady State – Month n: 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	.....	frequency per HRT in the planning	frequency specified per HRT
	Bioreactor																							
	samplings and preparations																							
1	Bleeding	●	●	●	●	●	●		●	●	●	●	●	●		●	●	●	●	●			8,5	10
2	Sampling for analysis of total fraction	●	●	●	●		●		●	●		●				●	●	●	●				6	2
3	Storage of total fraction in frozen state	●					●					●					●						2	2
4	Sampling for analysis of VFAs	●				●			●				●			●				●			3	2
5	Preparation of samples for VFA analysis	●				●			●				●			●				●			3	2
6	Storage of VFA samples in frozen state	●				●			●				●			●				●			3	2
7	Sampling for analysis of soluble fraction		●														●						1	1
8	Filtering of sample		●														●						1	1
9	Storage of filtered sample in frozen state		●														●						1	1
10	Lyophilization	●										●											1	1
11	storage in lyophilized state	●										●											1	1
	analyses on total fraction																							
1	Dry weight	●			●				●			●				●			●				2,9	2
2	COD, total	●							●							●							1,4	2
3	Ashes	●										●											1,0	1
4	Nitrogen conc., total sample	●										●											1,0	1
5	Protein conc., total sample	●										●											1,0	1
6	pH (offline)		●							●							●						1,4	1
7	Electroconductivity		●							●							●						1,4	1
8	Microbial count (aerobic)			●														●					1,0	1
9	Microbial count (anaerobic)			●														●					1,0	1
10	Particle size			●														●					1,0	?
11	VIAMASS																						online	online
	analyses on soluble fraction																							
12	VFA composition analysis (HPLC)								●											●			1,0	2
13	COD, soluble fraction		●														●						1,0	1
14	Nitrogen conc., soluble fraction		●														●						1,0	1
15	Ammonium conc., soluble fraction		●														●						1,0	1
16	Protein conc., soluble fraction		●														●						1,0	1
17	Alkalinity		●														●						1,0	?
	analyses on lyophilized fraction																							
18	Fibres composition	●														●							1,0	1
19	Organic elements conc. (COHNS)	●														●							1,0	1
20	Inorganic elements conc.	●														●							1,0	1

Table 4 - samplings and analyses on the C1 bioreactor in steady state

	C1 Process Operations	Steady State – Month n: 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	.....	frequency per HRT in the planning	frequency specified per HRT
	Influent Tank																							
	samplings and preparations																							
2	Sampling for analysis of total fraction	●	●				●		●	●		●				●	●		●				4,3	2
3	Storage of total fraction in frozen state	●					●					●					●						1,9	
4	Sampling for analysis of VFAs	●				●			●				●			●				●			2,9	2
5	Preparation of samples for VFA analysis	●				●			●				●			●				●			2,9	2
6	Storage of VFA samples in frozen state	●				●			●				●			●				●			2,9	2
7	Sampling for analysis of soluble fraction		●														●						1,0	1
8	Filtering of sample		●														●						1,0	1
9	Storage of filtered sample in frozen state		●														●						1,0	1
10	Lyophilization	●										●											1,0	1
11	storage in lyophilized state	●										●											1,0	1
	analyses on total fraction																							
1	Dry weight	●			●				●			●				●			●				2,9	2
2	COD, total	●							●							●							1,4	2
3	Ashes	●										●											1,0	1
4	Nitrogen conc., total sample	●										●											1,0	1
5	Protein conc., total sample	●										●											1,0	1
6	pH (offline)		●							●							●						1,4	1
7	Electroconductivity		●							●							●						1,4	1
8	Microbial count (aerobic)	●														●							1,0	1
9	Microbial count (anaerobic)	●														●							1,0	1
10	Particle size															●							0,5	0,33
	analyses on soluble fraction																							
12	VFA composition analysis (HPLC)								●											●			1,0	2
13	COD, soluble fraction		●														●						1,0	1
14	Nitrogen conc., soluble fraction		●														●						1,0	1
15	Ammonium conc., soluble fraction		●														●						1,0	1
16	Protein conc., soluble fraction		●														●						1,0	1
17	Alkalinity		●																				0,5	0,33
	analyses on lyophilized fraction																							
18	Fibres composition	●														●							1,0	1
19	Organic elements conc. (COHNS)	●														●							1,0	1
20	Inorganic elements conc.	●														●							1,0	1

Table 5 - samplings and analyses on the C1 influent tank in steady state

	C1 Process Operations	Steady State – Month n: 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	.....	frequency per HRT in the planning	frequency specified per HRT
	Effluent Tank																							
	samplings and preparations																							
2	Sampling for analysis of total fraction	●	●		●	●	●		●	●		●	●			●	●		●	●			6,2	2
3	Storage of total fraction in frozen state	●				●			●				●			●				●			2,9	2
4	Sampling for analysis of VFAs	●			●				●			●				●			●				2,9	2
5	Preparation of samples for VFA analysis	●			●				●			●				●			●				2,9	2
6	Storage of VFA samples in frozen state	●			●				●			●				●			●				2,9	2
7	Sampling for analysis of soluble fraction		●														●						1,0	1
8	Filtering of sample		●														●						1,0	1
9	Storage of filtered sample in frozen state		●														●						1,0	1
10	Lyophilization																						0,0	N/A
11	storage in lyophilized state																						0,0	N/A
	analyses on total fraction																							
1	Dry weight	●			●				●			●				●			●				2,9	2
2	COD, total	●							●							●							1,4	1,33
3	Ashes	●										●											1,0	1
4	Nitrogen conc., total sample	●										●											1,0	1
5	Protein conc., total sample	●										●											1,0	1
6	pH (offline)		●							●							●						1,4	1,33
7	Electroconductivity		●							●							●						1,4	1,33
24	Sterility control (aerobic)	●			●				●			●				●			●				2,9	3
25	Sterility control (anaerobic)	●			●				●			●				●			●				2,9	3
	analyses on soluble fraction																							
12	VFA composition analysis (HPLC)								●											●			1,0	2
14	Nitrogen conc., soluble fraction		●														●						1,0	1
15	Ammonium conc., soluble fraction		●														●						1,0	1
16	Protein conc., soluble fraction		●														●						1,0	1
17	Alkalinity		●																				0,5	0,33
18	Fibres composition																						0,0	?
19	Organic elements conc. (COHNS)	●														●							1,0	1
20	Inorganic elements conc.	●														●							1,0	1
	Filtrate Line																							
	samplings and preparations																							
5	Sampling for analysis of total fraction	●				●			●				●			●				●			2,9	2
	analyses on total fraction																							
3	COD, total	●							●							●							1,4	2
24	Sterility control (aerobic)	●			●				●			●				●			●				2,9	3
25	Sterility control (anaerobic)	●			●				●			●				●			●				2,9	3
10	Particle size																						0,0	?

Table 6 - samplings and analyses on the C1 effluent tank in steady state

7.11. Sampling and Analysis frequency on the gas phase

The following table summarizes the frequency at which the samples should be made on the gas loop when the bioreactor is operated with a HRT of 10days.

During the transient phase :

		Transient Phase																																
	C1 Process Operations	Month 1																				Month 2										......		
		12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	1	2	3	4	5	6	7	8	9	10	....	frequency per HRT in the planning	frequency specified per HRT
	Bioreactor																																	
	analyses on gas phase																																	
21	CO2 and CH4 production	●				●			●				●			●				●			●				●					.....	2,9	online
22	CO2, CH4, O2, H2, H2S (offline)	●				●			●				●			●				●			●				●					.....	2,9	2
23	gas contaminants																															.....	TBD	TBD

After establishment of the steady state:

		Steady State																					
	C1 Process Operations	Month n																					
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	.....	frequency per HRT in the planning	frequency specified per HRT
	Bioreactor																							
	analyses on gas phase																							
21	CO2 and CH4 production	●				●			●				●			●				●			2,9	online
22	CO2, CH4, O2, H2, H2S (offline)	●				●			●				●			●				●			2,9	
23	gas contaminants																						TBD	TBD

The gas mass flow ejected through the passive gas loop, the bioreactor, and passive gas tank pressures and temperatures, the active gas tank pressure are recorded online.

<u>Some frequencies can be reconsidered after one month of trend analysis.</u>

7.12. Analyses

All the analyses to be carried out in the MPP have a protocol that is detailed in section 2.1 and appendix 1, except for TIC/TOC, CST and particles size that are under development.

There are three analyses that are subcontracted to external laboratories :

- CHONS
- Minerals
- Fibers

The methods used by the subcontracted laboratories for the analyses are given in appendix 2.

Concerning the timing to take into account for the obtention of the results, see the following table.

	analyses on total fraction	Time between sampling and results
1	Dry weight	24h
2	COD, total	Immediate
3	Ashes	36h
4	Nitrogen conc., total sample	Immediate
5	Protein conc., total sample	Immediate
6	pH (offline)	Immediate
7	Electroconductivity	Immediate
8	Microbial count (aerobic)	3 to 7days
9	Microbial count (anaerobic)	3 days
10	Particle size	TBD
11	VIAMASS	immediate
	analyses on soluble fraction	
12	VFA composition analysis (HPLC)	
13	COD, soluble fraction	immediate
14	Nitrogen conc., soluble fraction	immediate
15	Ammonium conc., soluble fraction	immediate
16	Protein conc., soluble fraction	1 day
17	Alkalinity	1 day
	analyses on lyophilized fraction	
18	Fibres composition	1 month to 6 months
19	Organic elements conc. (COHNS)	1 month to 4 months
20	Inorganic elements conc.	1 month to 2 months
	analyses on gas phase	
21	CO2 and CH4 production	Immediate
22	CO2, CH4, H2, H2S (offline)	Immediate
23	gas contaminants	2 months

8. Resources specification for the tests

8.1. Personnel: staff qualification and training needs

The MPP technicians are qualified to operate the C1 compartment.

The MPP Analysis Technicians are qualified to perform the sampling operations and the MPP inhouse analyses (cf. appendix 1)

8.2. Hardware: instruments, specific part, hardware for software operation

C1 Hardware as described in AD6

The portable gas analyzer GA94 as described in RD4.

The gas chromatograph is used for VFA measurement. It is described in RD 3. Alternatively for VFA measurement, a HPLC is used. It is described in RD8.

The preparation of the samples is made with a lyophilizer and other equipments of common use in the Chemical Engineering Department..

8.3. Software : verification of software, backup needs

The software used is the Schneider Concept V2.6. for C1 control.

8.4. Facilities : environmental needs, test conditions, interfaces needs, utilities needs

All hardware involved in MPP utilities for C1 as specified in AD6 and AD5.

8.5. Test conditions

As specified by the test protocol AD9.

9. Measurement and data sampling

9.1. Data logfile

The samplings and analyses are performed routinely and are recorded in written on dedicated record sheet, internal or external to the MPP.
These raw data are then typed into the C1 database for analyses.

9.2. Reporting of status for a test

On a monthly basis, the Bioprocess Engineer or the Technical Manager reviews the raw data, checks the trends and spots the inconsistent values. An example of this database is given in appendix 8.

At the end of the test phase (for example the 10 days HRT test), or at least every 3 months, a report is compiled with all the analyses results related to the same test phase and sent by the Technical Manager to the partners..

9.3. Deviations and non conformances

Records should be maintained for any deviation or non conformity from this Sampling and Analysis Protocol and included in the as-run procedures records or test batch records.

9.4. Record for the performance of samplings and analysis

9.4.1. Records of samplings

The samplings are recorded on the C1 follow-up sheet MPP-REC-10-1001 as per appendix 3.

9.4.2. Records of analyses

Various MPP records are used to trace the results of the analyses on C1 samples :

- For dry weight and ashes : MPP-REC-09-1005 (appendix 4)
- For Electroconductivity, NH4, N total, Nsoluble, COD total, COD soluble : MPP-REC-09-1003 (appendix 5)
- For the CHNS analysis, the analysis report is given in appendix 6.
- For the minerals analysis, the report is given in appendix 7.

10. Appendix 1 – approved MPP protocols for analyses on samples

Parameter analyzed	MPP reference	Principle of the analysis technique
electroconductivity	MPP-QCP-07-0008	potentiometric
pH	MPP-QCP-07-0015 MPP-QCP-10-1001	potentiometric
dry matter	MPP-QCP-07-0007	gravimetric (oven is maintained at 100ºC in UAB)
ashes	MPP-QCP-07-0001	gravimetric (oven is held at 550ºC in UAB)
VFA	MPP-QCP-10-0003	Gas chromatograph
NH4-N	MPP-QCP-07-0011	colorimetric
N total broth	MPP-QCP-12-0001	colorimetric
N soluble	MPP-QCP-07-0020	colorimetric
COD total	MPP-QCP-07-0005	colorimetric
COD soluble	MPP-QCP-07-0004	colorimetric
CO2,CH4	MPP-QCP-10-0002	Online gas analyzer
Protein (broth and soluble fraction)	MPP-QCP-12-1001	Lowry method adapted for Comp. I
H2, O2, H2S	MPP-UM-09-0012	Portable infrared gas analyzer
Anaerobic count	MPP-QCP-09-1001	Petri dish seeding, incubation and counting of colonies
Aerobic count	MPP-QCP-09-1001	Petri dish seeding, incubation and counting of colonies
Filtrate circuits sterility	MPP-QCP-12-1002	Filtering of a volume of liquid, Petri dish seeding, incubation and counting of colonies
VFAs with HPLC	MPP-QCP-11-0002	HPLC
Alkalinity	MPP-QCP-11-0001	Dosimetric method

Missing here, to be defined in an updated edition: particles size ; CST ; TIC/TOC

11. Appendix 2 - External protocols implemented for analyses

11.1. Inorganic elemental composition (minerals) SAQEAt0001_00

SAQEAt0001_00_ele
ments inorgànics_bior

11.2. C,H,N,S organic elemental composition : SAQEAE0001_00_CHNS

SAQAE0001_00_CH
NS_101208.pdf

11.3. Fibers content : not available in English, added in Catalan language

MATÈRIA SECA DE LABORATORI I CENDRES

OBJECTIU

Determinar el contingut en matèria seca i/o cendres d'una mostra un cop preparada per analitzar (és a dir, molturada a 1mm).
Respecte a la matèria seca, s'ha de tenir en compte que s'ha de fer paral·lelament a totes les altres determinacions que es duguin a terme per dues raons: a) tots els anàlisi que es facin s'hauran de referir a aquesta matèria seca; b) la humitat de la mostra pot variar lleugerament amb el temps.

APARELLS I MATERIAL

- Estufa de dessecació a 103ºC±2ºC.
- Gresols de porcellana d'un tamany i forma talls que la mostra ocupi una tercera part.
- Pinces, safata, espàtula.
- Forn de Mufla a 550ºC.
- Dissecador amb silicagel i vàlvula.
- Balança analítica.

PROCEDIMENT

1- Calcinar els gresols a 550º 1h. Deixar que es refredin.
2- Pesar el gresol (P1). Introduir aproximadament 3g de mostra pesats amb aproximació de 0,1mg (P2).
3- Introduir a la estufa de 103º. Deixar-ho un mínim de 12h i un màxim de 24h.
4-Treure-ho de la estufa i deixar-ho refredar dins del dissecador (ATENCIÓ AMB LA VÀLVULA). Pesar (P3).
5- Introduir els gresols (sense safata!) a la mufla. Deixar-ho calcinar a 550ºC fins que les cendres quedin clares (normalment entre 3 i 4 hores).
6- Deixar refredar els gresols dins del dissecador (ATENCIÓ LA VÀLVULA) i pesar (P4).

CÀLCULS

%MS = (P3 - P1) * 100 / P2
%CENDRES = (P4 - P1) * 100 / P2

P1 = Pes del gresol
P2 = Pes de la mostra
P3 = Pes del gresol i la mostra seca
P4 = Pes del gresol i les cendres

OBSERVACIONS

No és estrictament necessari determinar la matèria seca i les cendres al mateix temps, però quan es comença des de zero, és més còmode fer-ho així.
Aquest és un mètode general. Però s'ha de tenir en compte que hi ha algunes mostres que no admeten aquest mètode.
Al realitzar la determinació per calor, les substàncies de punt d'ebullició baix (NH3, alcohols etc.) es determinen com si fos aigua, i per tant cal utilitzar altres mètodes. També les mostres riques en greixos s'han de tractar per altres vies.

DETERMINACIÓ DE LA FIBRA NEUTRE DETERGENT (Fibertec).

FONAMENT

Amb el tractament amb un detergent (SDS) a pH neutre es solubilitza el contingut cel·lular i per tant al residu de la FND ens queda bàsicament la paret vegetal excepte les pectines.

REACTIUS

- Alcohol 1Etil2Hexanol (iso-Octílic, anti-escumant).
- Acetona.

Per 1L d'aigua destil·lada:

- 30 g Sodi Lauril (Dodecil) Sulfat (SDS).
- 18,61 g Àcid Etilen di-Amino tetra-Acètic (EDTA) sal di-sòdica.
- 6,81g Sodi tetra-Borat deca-hidratat.
- 4,65g Fosfat di-sòdic.
- 10 ml Tri-etilenglicol.

Preparació de la sol. ND:

a) Pesar a un vas de precipitats el EDTA i el tetra-Borat. Afegir-hi 1/4 part de l'aigua i dissoldre-ho en calent.

b) Posar a escalfar una altra 1/4 part d'aigua i anar afegint el SDS. Aquest pas s'ha de fer a una campana d'extracció perquè el SDS fa molta pols i és molt irritant.

c) Dissoldre el fosfat.

Barrejar a),b) i c) i afegir-hi el tri-etilenglicol a poc a poc per anar eliminant l'escuma que es va formant. L'endemà comprovar el pH, que ha d'estar entre 6,9 i 7,1.

MATERIAL I APARELLS

- Balança analítica (0,1mg).
- Placa calefactora o Bunsen.
- Aparell complert FIBERTEC HOT EXTRACTOR
- Gresols de vidre amb placa porosa P2 adequats pel fibertec.
- Estufa de dessecació a 103±2ºC.
- Forn de mufla a 550ºC.
- Dissecador amb silicagel, espàtula.

PROCEDIMENT

1- Dins d'un gresol calcinat, pesar 1g (precisió 0,1mg) de la mostra (Pm).
2- Escalfar la sol. ND.
3- Col·locar els gresols amb la mostra al seu lloc a l'aparell, vigilant que no estiguin cavalcats. Fixar-los amb la palanca.
4- Connectar l'aparell i posar les claus en posició CLOSED.
5- Afegir-hi 100 ml del reactiu (fins la primera ratlla) i ajustar el control de calor (HEATER) al màxim.
6- Connectar el refrigerant.
7- Abans de que comenci a bullir, posar-hi dues gotes d'antiescumant. Quan comenci a bullir, ajustar el calor a la posició necessària a fi que bulli suaument..
8- Deixar-ho bullint 1h.
9- Apagar el calor i filtrar: obrir la trompa de buit i posar les vàlvules en posició VACUUM.
10- Rentar 3 vegades amb Aigua Destil·lada calenta i 2 amb acetona.

11- Posar les vàlvules en posició REST, desconnectar l'aparell, treure els gresols amb la pinça corresponent i posar-los a l'estufa de 103ºC tota la nit.
12- Posar els gresols a un dissecador i, quan estiguin freds, pesar-los (P1).
13- Calcinar a 550ºC 3h i quan estiguin freds al dissecador, pesar (P2).

CÀLCULS

%FND = (P1 - P2) * 100 / Pm

OBSERVACIONS

1- Seguir les observacions que s'indiquen per a la fibra bruta.
2- Les mostres RIQUES EN MIDÓ serà molt dificultós, per no dir impossible, filtrar-les (fins i tot amb petites quantitats de midó). Tot i així, i en el cas que s'aconsegueixi filtrar-les, les restes de midó que queden fan que la FND es sobrevalori.
Per tot això es imprescindible afegir-hi 0.25 ml de -amilassa termoestable (SIGMA ref A-3306) per cada 100 ml de sol. ND 20 minuts abans que acabi l'ebullició. En cas que segueixi costant filtrar, es pot repetir la dosis de -amilassa en un dels rentats amb aigua calenta, deixant que l'enzim actui durant un parell de minuts. La resta d'operacions es fan igual.

FIBRA NEUTRO DETERGENTE SEGÚN ANKOM

11.3.1. Reactivos.

Solución Neutro Detergente : 30g SDS, 18.61g EDTA disódico (dihidratado), 6.81g tetraborato sódico 10-hidrato, 4.56g fosfato sódico dibásico anhidro y 10ml trietilenglicol en 1L de agua destilada. Agitar y escalfar para facilitar la disolución. El pH debe quedar entre 6.9-7.1.
Alfa-amilasa termoestable Ankom.
Sodio Sulfito anhidro.
Acetona. Libre de coloración y sin residuo de evaporación.

11.3.2. Precauciones de seguridad.

La acetona es altamente inflamable. Trabajar con campana de extracción y evitar la inhalación o contacto con la piel. Asegurarse de que la acetona se ha evaporado antes de llevar las bolsas a la estufa.

El SDS irrita las membranas mucosas. Se debe usar máscara antipolvo y guantes al manipularlo

11.3.3. Aparatos.

Para la digestión: ANKOM Fiber Analyzer
Sistema de filtración: bolsas de filtración F57 (ANKOM).
Sellador de bolsas.
Desecador.

11.3.4. Procedimiento

Preparación de las muestras en las bolsas.

Pesar la bolsa de filtración (F57), anotar el peso (P1) y tarar la balanza.

Pesar 0.5g (±0.05g) de muestra secada al aire tamizada a 1 mm (P2) directamente en la bolsa. Pesar una bolsa en blanco e incluirla en la digestión para determinar la corrección por el blanco (C1).

Sellar la bolsa a 0.5cm del extremo abierto usando el sellador.

Dispersar la muestra uniformemente dentro de la bolsa. Se debe hacer agitando ligeramente la bolsa eliminando los posibles grumos suavemente.

Se pueden colocar un máximo de 24 bolsas. Se deben usar todas las bandejas independientemente del número de bolsas que se vayan a procesar Colocar tres bolsas por bandeja. Encajar las bandejas en la posición central con una rotación de 120º por nivel. La 9ª bandeja queda vacía y funciona como tope de la 8ª. Situar el peso en el extremo superior de la 9ª bandeja a fin de mantener sumergido el "bag suspender".

ATENCIÓN: MUESTRAS QUE CONTENGAN PRODUCTOS DE SOJA O UNA GRASA > 5%. Extraer la grasa de las muestras colocando las bolsas llenas en un recipiente de 500ml con tapa. Añadir suficiente acetona para cubrir las bolsas y tapar. Agitar el contenedor 10 veces y dejar reposar 10'. Repetir con acetona nueva. Extender las bolsas y dejarlas secar al aire (aprox. 5'). **EXCEPCIÓN: soja tostada.** Colocar las bolsas de soja tostada en un recipiente de 500ml con tapa. Añadir suficiente acetona para cubrirlas y agitar 10 veces y desechar la acetona. Añadir acetona nueva y dejar reposar doce horas. Después de este tiempo, extender las bolsas y dejarlas secar al aire.

Añadir 1900-2000ml de la solución Neutro Detergente al recipiente donde se realiza la digestión. Si se procesan menos de 20 bolsas, añadir 100ml de sol./bolsa con un mínimo de 1500ml. Añadir 20g de Sulfito Sódico y 4ml de alfa-amilasa.

Introducir el "bag suspender" cargado, poner el avisador en 75', poner en marcha el agitador y el calor y poner en marcha el avisador. Después de confirmar que el agitador funciona, cerrar bien la tapa.

Pasados los 75', apagar el calor y la agitación. Abrir la válvula de salida y recoger la solución antes de abrir la tapa. Peligro: dentro del recipiente la solución está bajo presión. La válvula de salida ("exhaust") debe ser abierta para compensar la presión y eliminar la solución ANTES DE ABRIR LA TAPA.

Una vez ha salido toda la solución, cerrar la válvula y abrir la tapa. Añadir aprox. 1900-2000ml de agua destilada caliente (90º-100º)y 4ml de alfa-amilasa a los dos primeros lavados. Poner en marcha la agitación (sin el calor). Cerrar la tapa sin ajustar. Agitar las bolsas 3-5'.Lavar tres veces en total.

Extraer las bolsas del "bag suspender" y apretarlas suavemente para eliminar el exceso de agua. Colocar las bolsas en un vaso de 250ml y añadir suficiente acetona para

cubrirlas. Dejarlas en remojo 2-3', sacarlas y apretarlas suavemente para eliminar el exceso de acetona.

Separar las bolsas y dejarlas secar al aire. Completar el secado en una estufa a 105ºC. Sacar las bolsas de la estufa, introducirlas en un desecador hasta que alcancen la Tª ambiente y pesarlas (P3). Incinerar la bolsa con el residuo en un crisol previamente pesado, enfriar nuevamente en un desecador y pesar.

Cálculos. % Fibra Neutro detergente (libre de cenizas) = $\frac{(P4 - (P1 \times C2)) \times 100}{P2 \times MS}$

Donde:

P1: peso de la bolsa

P2: peso de la muestra

P3: peso del residuo (después de la extracción)

P4: Peso de la Materia Orgánica (pérdida de peso después de la incineración)

C2: Corrección de las cenizas por el blanco (pérdida de peso al incinerar/ peso original blanco)

12. Appendix 3 – example of MPP-REC-10-1001 for the follow-up of the Compartment 1

MELiSSA Pilot Plant				
Document Identification : *C1 in Bioreactor Follow up Record Sheet*	Type	Reference	Chrono	Page : 1 / 1
	MPP-REC	10-1001 (0)	_ _	

Analist	
Date	
Hour	

Checked by:	
Initials	
Date	

HMI - CI BIOREACTOR							
Emergency button		(on/off)		Level	LT_1010_01	(L)	
Agitation	BLE_1012_01	(on/off)		Headspace pressure	PT_1009_01	(mbar)	
On-line pH 2	AT_1011_02			Temperature	TT_1008_01	(ºC)	
C1 ROOM GENERAL							
On-line pH 2	AT_1011_02			ACID bottle:	VSSL_1011_01		
Cooler	HX_1102_01	(on/off)		Observed Level		(mL)	
Condensates pump	PP_1102_01	(on/off)		HCl 3M added volume		(mL)	
Hot bath:	VSSL_1008_01			BASE bottle:	VSSL_1011_02		
Filled with water?		(yes/no)		Observed Level		(mL)	
Temperature		(ºC)		NaOH 3M added volume		(mL)	
GAS LOOP							
N2 supply	HPCV_1052_01	(mbar)		Mass Flow Controller	FRC_1052_01	(%)	
GAS ANALYSIS:							
CH4		(%)		O2		(%)	
CO2		(%)		H2		(ppm)	
				H2S		(ppm)	
LIQUID LOOP							
Feeding: (On Mondays)				**Decantation: (On Fridays)**			
Volume of FILTRATE dumped		(L)		Volume of RC extracted (EFFLUENT)		(L)	
Weight of FEED		(Kg)		**Samples: (total volume in mL)**			
FEED Lot number		#		REACTOR CONTENT		pH off-line	
Volume of FEED + WATER		(L)		FILTRATE		pH off-line	
				FEED		pH off-line	
MELISSA MAINTENANCE FEED							
On Fridays:							
Bag of FEED stored at 3-5ºC		yes/no		Nº of bags left		#	
Weight of Bag		(Kg)					
FEED Lot number		#					
REMARKS							
-							

13. Appendix 4 – example of record for dry weight and ashes : MPP-REC-09-1005

MELiSSA Pilot Plant

Document Identification :	Type	Reference	Chrono	
C1 samples Dry weight and ashes Analysis Record Sheet	MPP-REC	09-1005 (0)	_ _	Page : 1 / 1

Name:	
Date of analysis:	

Project name:					Ec:		
Sample name/date of sampling:							
Crucible nº	Volume (mL)	x1	x2	x3		DW	Ashes
	20						
	20						
Done by: (Inicials)					Average:		

Project name:					Ec:		
Sample name/date of sampling:							
Crucible nº	Volume (mL)	x1	x2	x3		DW	Ashes
Done by: (Inicials)					Average:		

Project name:					Ec:		
Sample name/date of sampling:							
Crucible nº	Volume (mL)	x1	x2	x3		DW	Ashes
Done by: (Inicials)					Average:		

Project name:					Ec:		
Sample name/date of sampling:							
Crucible nº	Volume (mL)	x1	x2	x3		DW	Ashes
Done by: (Inicials)					Average:		

Remarks:	Checked by:	
	Initials	Date

14. Appendix 5 – example of record for Electroconductivity, NH4, N total, COD total, COD soluble : MPP-REC-09-1004 and MPP-REC-09-1003

MELiSSA	MELiSSA Pilot Plant			UAB de Barcelona
Document Identification :	Type	Reference	Chrono	Page : 1 / 1
C1 Filtrate samples Dr. Lange Analysis Record Sheet	MPP-REC	09-1004 (0)	_ _	

Name:		Project name:	
Date of analysis:		Sample name/date of sampling:	
Hour:			

Sample filtrated? Yes/No		No		Sample filtrated? Yes/No			
Parameter:		COD total	(ppm)	Parameter:			
Equipment/ Dr Lange kit code:				Equipment/ Dr Lange kit code:			
Initial time:				Initial time:			
End time				End time			
	Dilution	Raw result	Corrected result		Dilution	Raw result	Corrected result
1				1			
2				2			
3				3			
4				4			
Done by (initials):		Average:		Done by (initials):		Average:	

Sample filtrated? Yes/No				Sample filtrated? Yes/No			
Parameter:				Parameter:			
Equipment/ Dr Lange kit code:				Equipment/ Dr Lange kit code:			
Initial time:				Initial time:			
End time				End time			
	Dilution	Raw result	Corrected result		Dilution	Raw result	Corrected result
1				1			
2				2			
3				3			
4				4			
Done by (initials):		Average:		Done by (initials):		Average:	

Sample filtrated? Yes/No				Sample filtrated? Yes/No			
Parameter:				Parameter:			
Equipment/ Dr Lange kit code:				Equipment/ Dr Lange kit code:			
Initial time:				Initial time:			
End time				End time			
	Dilution	Raw result	Corrected result		Dilution	Raw result	Corrected result
1				1			
2				2			
3				3			
4				4			
Done by (initials):		Average:		Done by (initials):		Average:	

Remarks:	**Checked by:**	
	Initials	Date

MELiSSA Pilot Plant

Document Identification :	Type	Reference	Chrono	Page : 1 / 1
Record for C1 samples analysis	MPP-REC	09-1003 (1)	_ _	

Analyst name:	
Date of analysis:	

Supervised by:	
Date:	

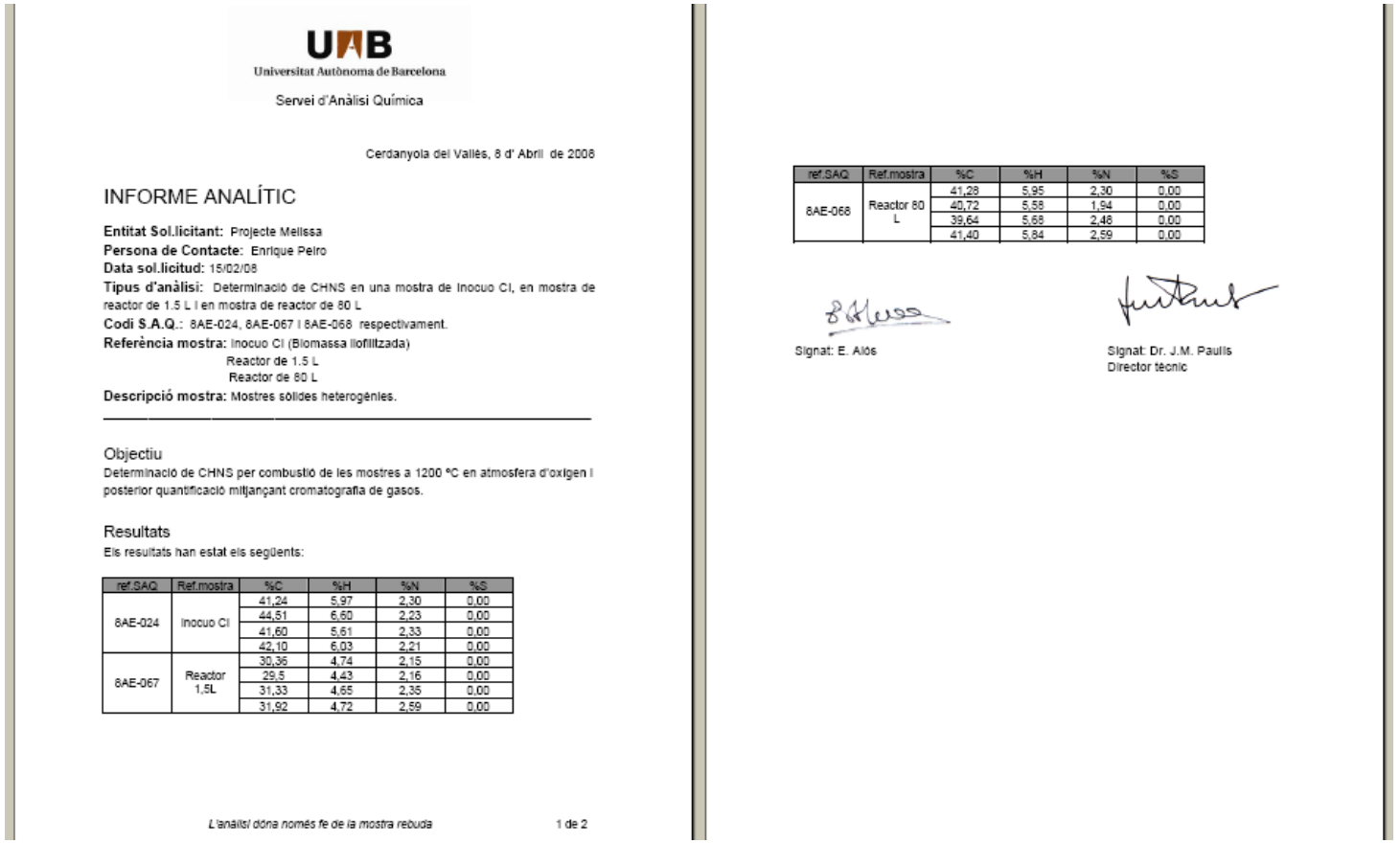
Sample name	Sample date	Tube number	EC (Sample not filtered)	COD total (Sample not filtered)				COD soluble				NH_4^+				N total			
				Kit Code:				Kit Code:				Kit Code:				Kit Code:			
				Kit Batch nº	Dilution	Raw result	Final Result	Kit Batch nº	Dilution	Raw result	Final Result	Kit Batch nº	Dilution	Raw result	Final Result	Kit Batch nº	Dilution	Raw result	Final Result
MPPC1...	dd/mm/aa	#	mS/cm			mg/L	mg/L			mg/L	mg/L			mg/L	mg/L			mg/L	mg/L

Analyses to be done per type of sample:

MPPC1BF	yes	yes	yes	yes	yes
MPPC1AF	yes	yes	yes	yes	yes
MPPC1Filtrate	yes	yes	no	no	no
MPPC1Feed#...	yes	yes	yes	yes	yes

Remarks:

15. Appendix 6 – example of analysis report for the CHNS analysis



Universitat Autònoma de Barcelona

Servei d'Anàlisi Química

Cerdanyola del Vallès, 8 d' Abril de 2008

INFORME ANALÍTIC

Entitat Sol.licitant: Projecte Melissa
Persona de Contacte: Enrique Peiro
Data sol.licitud: 15/02/08
Tipus d'anàlisi: Determinació de CHNS en una mostra de Inoculo CI, en mostra de reactor de 1.5 L i en mostra de reactor de 80 L
Codi S.A.Q.: 8AE-024, 8AE-067 i 8AE-068 respectivament.
Referència mostra: Inoculo CI (Biomassa liofilitzada)
Reactor de 1.5 L
Reactor de 80 L
Descripció mostra: Mostres sòlides heterogènies.

Objectiu

Determinació de CHNS per combustió de les mostres a 1200 ºC en atmosfera d'oxigen i posterior quantificació mitjançant cromatografia de gasos.

Resultats

Els resultats han estat els següents:

ref.SAQ	Ref.mostra	%C	%H	%N	%S
8AE-024	Inoculo CI	41,24	5,97	2,30	0,00
		44,51	6,60	2,23	0,00
		41,60	5,61	2,33	0,00
		42,10	6,03	2,21	0,00
8AE-067	Reactor 1,5L	30,36	4,74	2,15	0,00
		29,5	4,43	2,16	0,00
		31,33	4,65	2,35	0,00
		31,92	4,72	2,59	0,00

L'anàlisi dóna només fe de la mostra rebuda

1 de 2

ref.SAQ	Ref.mostra	%C	%H	%N	%S
8AE-068	Reactor 80 L	41,28	5,95	2,30	0,00
		40,72	5,58	1,94	0,00
		39,64	5,68	2,48	0,00
		41,40	5,84	2,59	0,00

Signat: E. Alós

Signat: Dr. J.M. Paulis
Director tècnic

16. Appendix 7 – example of report for minerals analysis

Muestra	MPP-C1-RC enero	MPP-C1-RC 042010
Codigo SAQ	10EAt153/001	10EAt153/004
	Contingut	Contingut
ug / g Be	< 0.5	< 0.5
% Na (p/p)	6.7	6.2
% Mg (p/p)	0.18	< 0.05
ug / g Al	251	80
% Si (p/p)	< 0.1	< 0.1
% P (p/p)	0.17	0.25
% S (p/p)	0.17	0.25
% K (p/p)	2.2	3.7
% Ca (p/p)	0.43	0.55
ug / g Ti	41	22
ug / g V	0.97	0.58
ug / g Cr	76	32
ug / g Mn	56	41
ug / g Fe	517	177
ug / g Co	0.63	0.55
ug / g Ni	129	35
ug / g Cu	16	15
ug / g Zn	131	146
ug / g As	< 0.5	< 0.5
ug / g Se	< 0.5	< 0.5
ug / g Sr	41	42
ug / g Mo	27	5.9
ug / g Pd	< 0.5	< 0.5
ug / g Cd	0.19	0.22
ug / g Sn	< 0.5	< 0.5
ug / g Sb	< 0.5	< 0.5
ug / g Ba	13	13

ug / g W	1.0	0.58
ug / g Pb	2.0	0.93
ug / g Hg	0.19	0.063

17. Appendix 8 – example of the database used for C1 results of analysis and operational parameters

Time			MPP RECORD SHEET			HMI - CI BIOREACTOR & INFLUENT TANK -						
			Analist	Reference	Chrono	Emergency button	Agitation BLE_1005_01	Level Alarm LSH_1004_0	Recirculation pump GP_1001_01	Level LT_1004_01	Headspace pressure PT_1003_01	Temperature TT_1008_01
Date	hour	day	(name)		Page number	(on/off)	(on/off)	(on/off)	(on/off)	(L)	(mbar)	(ºC)
11/03/2009	16:30	0.0	NM	09-1006 (0)	001	OFF	ON	ON	ON	55.8	-	45.0
11/03/2009	17:30	0.0	NM	09-1006 (0)	002	OFF	ON	ON	ON	56.0	6.6	53.3
11/03/2009	19:00	0.1	NM	09-1006 (0)	003	OFF	ON	ON	ON	56.0	3.9	55.8
12/03/2009	10:20	0.7	NM	09-1006 (0)	004	OFF	ON	OFF	ON	54.5	71.1	57.0
12/03/2009	12:00	0.8	NM	09-1006 (0)	005	OFF	ON	OFF	ON	54.4	50.4	54.7
12/03/2009	20:00	1.1	NM	09-1006 (0)	006	OFF	ON	OFF	ON	54.3	65.5	55.0
13/03/2009	19:30	2.1	NM	09-1006 (0)	007	OFF	ON	OFF	ON	54.3	55.1	55.2
16/03/2009	11:15	4.8	NM	09-1006 (0)	008	OFF	ON	OFF	ON	43.9	86.9	55.2
16/03/2009	16:21	5.0	NM	09-1006 (0)	009	OFF	ON	OFF	ON	43.6	98.4	55.5
16/03/2009	17:00	5.0	NM	09-1006 (0)	010	OFF	ON	OFF	ON	-	28.7	-
17/03/2009	11:00	5.8	NM	09-1006 (0)	011	OFF	ON	OFF	ON	53.3	92.1	50.6
17/03/2009	18:00	6.1	NM	09-1006 (0)	012	OFF	ON	OFF	ON	53.4	79.9	54.7
18/03/2009	13:00	6.9	NM	09-1006 (0)	013	OFF	ON	OFF	OFF	53.9	135.6	54.2

Time			C1 ROOM GENERAL											GAS LOOP						
					HOT BATH			Acid				Base								
			Recirculation pump running	On-line pH	Level	Temperature hot bath	Bottle level	Addition of HCl 3M to the bottle	Addition to the reactor absolute	Addition to the reactor cumulative	Bottle level	Addition of NaOH 3M to the bottle	Addition to the reactor absolute	Addition to the reactor cumulative	N2 Supply HPCV_1003_03	Mass Flow Controller FRC_1003_01	CH4	CO2	O2	H2	H2S
Date	hour	day	(yes/no)		(yes/no)	(ºC)	(mL)	(mL)	(mL)	(mL)	(mL)	(mL)	(mL)	(mL)	(mbar)	(%)	(%)	(%)	(%)	ppm	ppm
11/03/2009	16:30	0.0	YES	5.06	YES	63	1000	-	0	0	1000	-	0	0	-	-	-	-	-	-	-
11/03/2009	17:30	0.0	YES	5.47	NO	63	1000	-	0	0	500	-	500	500	-	-	-	-	-	-	-
11/03/2009	19:00	0.1	YES	5.38	-	-	1000	-	0	0	300	-	200	700	-	-	-	-	-	-	-
12/03/2009	10:20	0.7	YES	5.28	NO	60	1000	-	0	0	100	-	200	900	-	-	-	-	-	-	-
12/03/2009	12:00	0.8	YES	5.29	NO	60	1000	-	0	0	100	900	0	900	-	-	-	-	-	-	-
12/03/2009	20:00	1.1	YES	5.28	NO	60	1000	-	0	0	1000	-	0	900	-	-	0	0.4	0.5	45	3
13/03/2009	19:30	2.1	YES	5.27	NO	60	1000	-	0	0	1000	-	0	900	-	-	0	1.2	2.2	>1000	-
16/03/2009	11:15	4.8	YES	5.26	YES	60	1000	-	0	0	1000	-	0	900	-	-	0	2	4.2	>1000	-
16/03/2009	16:21	5.0	YES		0	-	1000	-	0	0	1000	-	0	900	-	-	0	1.7	1.1	>1000	218
16/03/2009	17:00	5.0	0		0	-	-	-	0	0	-	-	0	900	-	-	0	0.1	0.8	219	-
17/03/2009	11:00	5.8	YES	5.35	YES	60	1000	-	0	0	1000	-	0	900	-	-	-	-	-	-	-
17/03/2009	18:00	6.1	YES	5.37	NO	60	1000	-	0	0	1000	-	0	900	-	-	0	0.2	0.8	79	4
18/03/2009	13:00	6.9	NO	5.33	NO	60	1000	-	0	0	1000	-	0	900	-	-	-	-	-	-	-
19/03/2009	19:00	8.1	YES	5.30	YES	60	1000	-	0	0	800	-	200	1100	-	-	-	-	-	-	-
19/03/2009	19:30	8.1	YES	5.30	YES	60	1000	-	0	0	800	-	0	1100	500	10	-	-	-	-	-
20/03/2009	11:00	8.8	YES	5.28	YES	58.8	1000	-	0	0	800	-	0	1100	500	10	0	0.7	7.8	129	-

			LIQUID LOOP													
			FEED					INFLUENT	REACTOR CONTENT		EFFLUENT		FILTRATE/SUPERNATANT			
Time			Lot	Weight	Total Volume	Sample volume	pH off-line	Total Volume Feed + Solids into C1	Sample volume	pH off-line	Volume	Volume not sedimented or disposed	Volume	Sample volume	pH off-line	
Date	hour	day	#	(Kg)	(mL)	(mL)		(mL)	(mL)		(mL)	(mL)	(mL)	(mL)		
11/03/2009	16:30	0.0	-	0	0	0		0	100	5.03	0		0	0		
11/03/2009	17:30	0.0	-	0	0	0		0	0		0		0	0		
11/03/2009	19:00	0.1	-	0	0	0		0	0		0		0	0		
12/03/2009	10:20	0.7	-	0	0	0		0	0		2000	2000	0	0		
12/03/2009	12:00	0.8	-	0	0	0		0	0		0		0	0		
12/03/2009	20:00	1.1	-	0	0	0		0	0		0		0	0		
13/03/2009	19:30	2.1	-	0	0	0		0	0		0		0	0		
16/03/2009	11:15	4.8	-	0	0	0		0	0		10000		0	0		
16/03/2009	16:21	5.0	-	0	0	0		0	0		0		0	0		
16/03/2009	17:00	5.0	-	0	0	0		0	0		0		0	0		
17/03/2009	11:00	5.8	14	3	7000	80	6.26	10000	130	5.17	0		7000	80	5.19	
17/03/2009	18:00	6.1	-	0	0	0		0	150	5.26	0		0	0		
18/03/2009	13:00	6.9	-	0	0	0		0	0		0		0	0		
19/03/2009	19:00	8.1	-	0	0	0		0	0		0		0	0		
19/03/2009	19:30	8.1	-	0	0	0		0	0		0		0	0		
20/03/2009	11:00	8.8	-	0	0	0		0	0		17500		0	0		