Universitat Autònoma de Barcelona

TECHNICAL NOTE 94.5

C1 additional characterization: Test Plan

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Table of Content

1.Scope

As described in COO3, WP 94.5, this Test Plan will cover the testing activities on Compartment 1 additional characterization including the following phases:

- CI start-up

- Test phases at various liquid residence times, to be representative of:

  - Nominal operation: 10 days
  - Natural perturbations: 7 days, 13 days and 5 days

The former issue of this TN the Test Plan included the particular activity regarding the validation of the C1 optimized filtration unit, as this was performed in advance, along 2010.

2.Reference and applicable 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	PID of Compartment 1	MPP-PID-10-1001	B3	05/10/11
AD5	C1 Operation Manual	MPP-OP-12-1001	0	February 12
AD6	C1 Maintenance Manual	MPP-UM-11-1001	0	February 12

7	C1 Acceptance Review Datapackage including HMI and PLC software user manuals	DP94.1	1	October 11
AD8	TN94.41 User Requirements for C1 filtration unit optimization	TN94.41	0	09/12/08

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	01/09/08
RD3	TN94.43 Hardware procurement and upgrading activities	TN94.43	0	16/04/10
RD4	EPAS EWC User Manual	User Manual	1	12.06.07
RD5	General description of the Facilities	MPP-TN-08-0001	3	26/09/11

3.Acronyms and definitions

- PID: Piping and Instrumentation Diagram
- GN2: Gaseous Nitrogen
- COD: Chemical Oxygen Demand
- VFA: Volatile Fatty Acids
- HRT : hydraulic residence time
- RT : residence time

- PLC : programmable logical controller
- UF: ultra filtration

4.Test items

4.1. *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 (AD4) and in the Operation Manual (AD5), 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.

4.2. *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:

- Mechanical hazard (pumps GP_1001_01 and GP_1201_01)
- pressure (gas: up to 6 barg-compressed air and GN2 supplies-, liquid: up to 5 barg)
- temperature (steam sterilization)
- chemical (acid/base for pH control ; base –NaOH- for cleaning)
- biological (biohazard level 2 as a maximum when using faeces for the feeding of C1)

The adequate individual protection measures shall be taken by the operators in order to limit the exposure to these hazards. As detailed in AD5, these measures include :

- wearing of a labcoat
- wearing of safety goggles
- wearing of face shield when pouring corrosive solutions for pH control into the bottles
- wearing of gloves when manipulating materials or equipments

- respect of the user and maintenance instructions

4.3. *Instructions for operation*

See AD5 and AD6

4.4. *Instructions for maintenance*

See AD5 and AD6

5.Test strategy

5.1. *Objective of the tests*

The objective of the C1 characterization test is to collect as many data as possible for the characterization of its performance under different situations (start-up, steady-state,perturbations, etc.) in order to provide the parameters necessary for the understanding of the C1 process behavior, and for the construction of a knowledge model of this compartment., as well as to obtain the required information and expertise needed for the progressive integration of this compartment with the rest of the compartments within the MPP MELiSSA loop.

The final goal is as well to perform, from the test data evaluation, an overall evaluation of the tests with regards to CI hardware, CI control, CI knowledge model, long term operation, ergonomics, degradation efficiency, maintenance, needs of additional characterization, need for future optimization of hardware, software and operating procedures.
One particular objective among the above described ones is as well to validate the long-term performance of the optimized UF membrane that was installed as described in RD4.

5.2. *Applicable requirements*

The following requirements were discussed between ESA and UBP on 29/01/2007 for compartment 1 ; they are not completely finalized but are the best available to date:

Requirement number						Requirement description	Applicability
2						Subsystem requirements	A
2	1					Functional requirements	A
2	1	1				Wastes treatment system = (C1+Fiber Degradation Unit+Wastes Preparation Unit+Wastes Collector Unit)	A
2	1	1	1			The WTS shall handle the solid wastes from the mission	A
2	1	1	2			The WTS shall handle the liquid wastes from the mission	N/A
2	1	1	2	1		The WTS shall handle the toilet flush of the mission	N/A
2	1	1	2	2		The WTS shall handle the urine of the mission	N/A
2	1	1	3			The WTS shall degrade the wastes from the mission	A
						The WTS shall degrade the proteins of the wastes	A
						The WTS shall degrade the lipids of the wastes	A
						The WTS shall degrade the glucids of the wastes	A
						The WTS shall degrade the fibers of the wastes	A
							A
2	1	1	4			The WTS shall produce chemicals that can be used directly by the CIVa and CIVb	A
				1		CO2	A
				2		minerals	A
				3		NH4+	A
							A
						The WTS shall limit the chemicals that cannot be used directly or indirectly by the CIVa and CIVb	A
						CH4	A
						H2S	A
						H2	A
						gas contaminants	A
							A
2	1	1	5			The WTS shall produce chemicals that can be used indirectly by the CIVa and CIVb	A

				1		VFAs	A
				2		NH4+	A
				3		carbonates and bicarbonates	A
							A
						The chemicals produced by WTS that can be used directly by the CIVa and CIVb shall be considered for the ALISSE multi criteria approach	A
						The WTS shall optimize the degradation of wastes into chemicals that can be used directly by the CIVa and CIVb in accordance with ALISSE multi criteria approach	A
2	1	1	4			The wastes compartment shall fulfill the biosafety requirements	A
2	1	1	5			The wastes compartment shall handle all products that can not be used by other compartments or units (e.g. ashes, CH4, H2S,…)	A
2	1	1	6			The WTS shall deliver sterile output to other compartments	A
2	1	1	7			The wastes compartment shall allow for all necessary steps of phase separation (gas, liquid, solid)	A

Among these requirements, the following ones are to be addressed through the characterization test plan TN94.5 and the test protocols TN94.62 to TN94.65 :

- Degradation of organic matter into CO2, ammonium and volatile fatty acids
- Yield of this degradation
- Production of a sterile filtrate by the filtration unit

FU optimization requirements

The User's Requirements for CI Filtration unit optimization were defined in AD8. They are summarized in the following table:

top level requirements		derived requirements			
nber	description	nber	description	nber	description
1	the filtration unit shall retain 100% of all solid particles				
2	the filtration unit shall retain 100% of all microorganisms, i.e. the filtrate shall be sterile	2,1	the filtration unit shall be steam sterilizable in place		

		2,2	the filtration unit shall resist to chemical disinfection		
		2,3	the filtration unit shall prevent contamination of the filtrate side		
3	the filtration unit shall not selectively retain any of the other product compounds (i.e VFA, ammonium, minerals…) that should be further used in the MELiSSA loop	3,1	with the assumption that there is no volumetric concentration factor of CI bioreactor content, then [VFA]bioreactor=[VFA] filtrate		
		3,2	[ammonium] bioreactor=[ammonium]filtrate		
		3,3	idem for other relevant mineral compounds: PO_4^{3-}, SO_4^{2-}, Cl^-, Na^+, Mg^{2+}, K^+, Ca^{2+}, etc.		
4	the filtration shall be performed in continuous mode				
5	Redundancy of membrane modules shall be implemented				
6	filtrate flow shall be kept regular: the critical ratio TMP/flow should be checked with water and with broth, providing different profiles depending on the velocity. At normal velocities (1-2 m/s) , flux should be in the range 30-60 L/m2/h	6.1	mechanical fouling shall be reduced		
		6.2	chemical fouling shall be reduced		
		6.3	redundancy of membrane modules shall be implemented		
		6.4	Hydrodynamics conditions should be optimal to minimise the fouling rates		
		6.5	Presence of exogenous compounds in the filtrate shall be avoided		
		6,6	cleaning shall be optimized; the ability of the membrane to recover its permeability after use will be evidenced by means		

			of a water permeability test. In particular: - In coherence with Req. 5.5, the membrane should not need chemical agents for its cleaning, or should need them in a limited amount. - Frequency of backwashing and cleaning should be reduced as much as possible.		
7	The filtration unit shall not damage C1 consortium micro-organisms				
8	safety of the operators shall be guaranteed				
9	filtration process shall be fully automated in all operation modes				
10	process parameters nominal set points/ranges are	10,1	filtrate flow: 10 l/d up to 15l/d		
		10,2	viscosity: 10 to 20 cP		
		10,3	pH: 4.5 to 6,5		
		10,4	particles size: up to 2 mm		
		10,5	temp: 55 ºC		
		10,6	Dry matter (bioeactor content): 40 g/l in nominal mode		
		10,7	Feed particle size: vegetables, up to 2 mm; straw, up to 0,2 mm		
11	Energy consumption of the FU for long operation periods shall be minimized.				

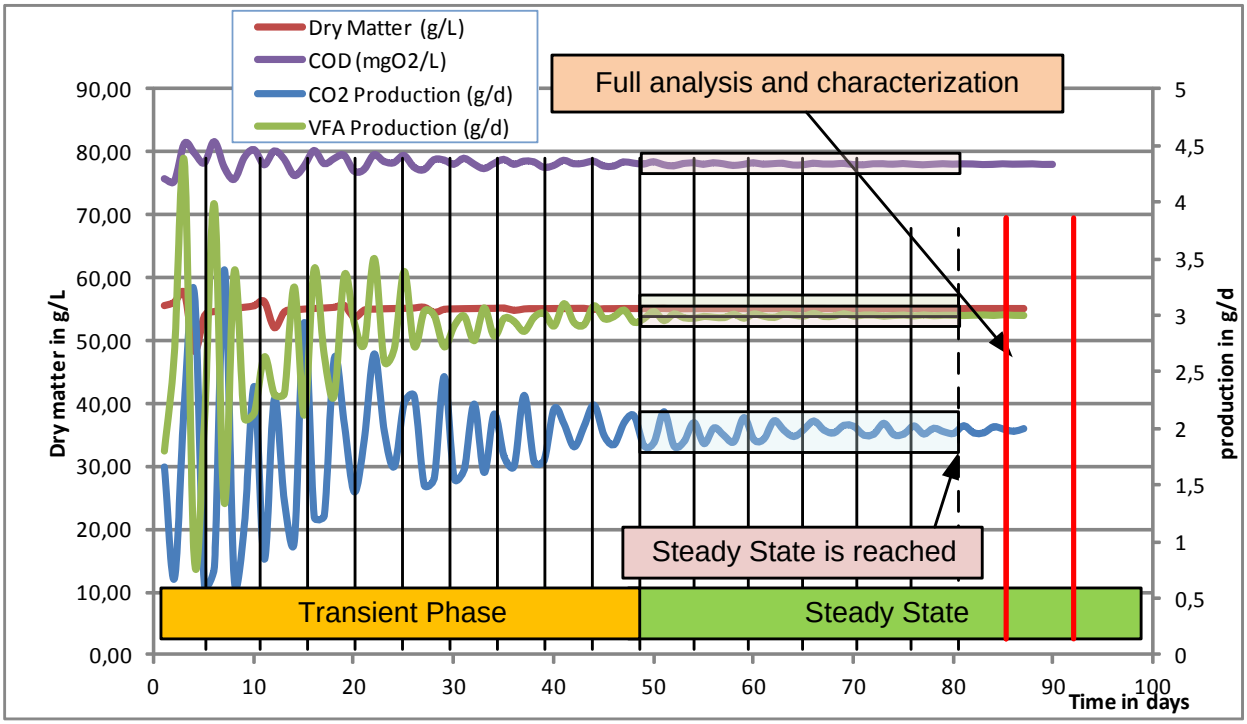
5.3. *Approach followed*

The approach followed during the characterization tests is to operate the C1 reactor in continuous mode and to reach the steady state for several liquid residence times.

In fact, the first objective is to establish a steady state in nominal operation and then to apply realistic perturbations to the system. Among different realistic perturbations considered (residence time / liquid flow rate, substrate composition), obtaining data under various residence times / liquid flow rates was selected as the most important.

Therefore it was decided to operate C1 reactor at 4 different liquid residence times, respectively 10 days (considered as nominal operation), 7 days, 13 days and 5 days, as described in the test protocols TN 94.62 to TN 94.65.
For each condition of liquid residence time, the process is operated during a period of time (i.e. transient phase) long enough to reach a steady state operation.

See the figure below as an example for the logic to be followed for the steady state establishment :



Four indicators have been selected to characterize the overall process performance during this transient phase::

- Dry matter content in C1 reactor
- CO2 production
- VFA production

- Total Chemical Oxygen Demand (COD) in C1 reactor.

For each condition of liquid residence time, steady state will be considered achieved when the four proposed indicators will have remained stable over a duration equal to 3 times the liquid residence time.
Then, when the steady state is proven to be achieved, a proper characterization of this steady state will be performed through an appropriate analysis campaign, as described in TN 94.22 to 94.25. As a general principle, all parameters defined as necessary for a proper characterization of C1 process and C1 compartment operation will be quantified at two times separated by 1 liquid residence time.

For each achieved 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 each chemical element (C balance, N balance, O balance, 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, gas composition in H2, H2S and O2)

Priority 3 (lower 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)

5.4. *Features to be tested*

1. Maintenance of the nominal process conditions in terms of temperature, pH, dry matter content, anaerobiosis (absence of O2 in the gas phase), feeding composition, feeding particle size, sterility of the filtrate output, during the whole test.
2. Continuity of feeding regime according to the established RT for each phase
3. Continuity of filtration regime according to the established RT for each phase
4. Continuity of biogas production, with limited CH4, SH2 and H2 production
5. Continuity and production level of the main products of C1 fermentation process :
   - VFA production rate
   - NH4+ production rate
   - CO2 production rate
6. Evolution of relevant analytical values (elemental analysis, minerals, protein, fibers, etc.) according to the corresponding protocol for each RT period.
7. Long-term performance of the optimised filtration membrane for each RT peeriod.

5.5. *Features not to be tested*

- Realistic perturbations other than residence time/liquid flow rate

- Non-realistic perturbations (ex. VFA, NH3)

5.6. *Success/failure criteria*

1. The characterization tests are considered successful if the specified nominal process conditions have been maintained, as follows :

Parameter	**Measurement means**	**Success/failure criteria**
Temperature 55ºC	Temperature sensor (on-line)	45-58ºC
pH 5,5	pH sensor (on-line)	5,0-6,0

Dry matter > 40 g/L	Dry matter analysis (off-line)	40-70 g/L
Absence of O2 in the gas phase	Gas analysis (on-line)	<0,5%
Feed composition (according to AD5 and AD7)	- Raw materials weight: Food Pilot Plant scale - Volume: measured indirectly by WPU load cells	- Weight: ±5% - Volume: ±5%, including effect of density in the volume calculation
Feed particle size	Laser determination (off-line)	- vegetables, up to 2 mm ±20% - straw, up to 0,5 mm ±100%
Sterility of filtrate output	Sterility check (off-line)(downstream the last dead-end filter)	0 CFU/ 100 mL

2. The characterization tests are considered successful if the feeding regime is maintained according to the specifications for each phase, as follows :

Parameter	**Measurement means**	**Success/failure criteria**
Feeding rate	Influent tank level difference	a) 10 days RT: 210 g dry weight/day +/- 5% b) 7 days RT: 300 g/day +/- 5% c) 13 days RT: 161,5 g/day +/- 5% d) 5 day RT: 420 g/day +/- 5%

3. The characterization tests are considered successful if the filtration regime is maintained according to the specifications for each phase, as follows :

Parameter	**Measurement means**	**Success/failure criteria**
Filtrate production (L/h)	Filtrate tank level difference	a) 10 days RT: (10 L-bleeding volume)/day +/- 10% e) 7 days RT: (14,3 L-bleeding volume)/day +/- 10% f) 13 days RT: (7,7 L-bleeding volume)/day +/- 10% b) 5 day RT: (20 L-bleeding volume)/day +/- 10%

4. The characterization tests are considered successful if the biogas production is maintained during the test, as follows :

Parameter	Measurement means	Success/failure criteria
Biogas production (L/day)	Batch measurement of the evolved gas by the Pressure Volume Temperature (PVT) method in a fixed volume collection vessel	Stable biogas production ± 20% around the average production, for each RT steady state
Limited CH4+ production	CH4+ analysis on-line	< 2%
Limited SH2 production	SH2 analysis (off-line analyser)	< 1000 ppm
Limited H2 production	H2 analysis (off-line analyser)	< 4%

5. The characterization tests are considered successful if the values established for the steady state identification are maintained during the test (see Section 5.7), as follows :

Parameter	Measurement means	Success/failure criteria
Dry matter (g/L/day)	Dry matter analysis (off-line)	Stable dry matter content ± 15% around the average production, for each RT steady state
VFA (g/L/day)	VFA analysis (off-line)	Stable VFA production ± 20% around the average production, for each RT steady state
COD (mg/L/day)	Total COD analysis (off-line)	Stable COD production ± 15% around the average production, for each RT steady state
CO2 (g/day)	Batch measurement of the evolved gas by the Pressure Volume Temperature (PVT) method in a fixed volume collection vessel	Stable biogas production ± 20% around the average production, for each RT steady state

6. The characterization tests are considered successful if the information regarding VFA, CO2 and NH4+ production is available during the whole test, as follows :

Parameter	Measurement means	Success/failure criteria
VFA production	VFA analysis (off-line)	Data available*
Ratio among the different VFA	VFA analysis (off-line)	Data available*
NH4+ production	NH4+ analysis (off-line)	Data available*
CO2 production	CO2 analysis (on-line)	Data available*

* In principle, the availability of data is the only success criterion established

7. The characterization tests are considered successful if the expected data have been collected according to the frequency and conditions specified in the corresponding analysis protocol for each phase, in order to evaluate the following parameters :

Parameter	Measurement means	Success/failure criteria
Elemental balances (C, N, O, H)	Elemental analysis (off-line)	Data available*
Protein degradation	Protein determination (off-line)	Data available*
Fiber degradation	Fiber determination (off-line)	Data available*
Minerals production	Minerals analysis (off-line)	Data available*

* In principle, the availability of data is the only success criterion established

8. The characterization tests are considered successful if the parameters demonstrating the proper performance of the membrane are obtained, as follows:

Parameter	Measurement means	Success/failure criteria
Filtrate side turbidity	VIAMASS probe	Data available*
Retention of solid particles	COD measurement (off-line)	COD retained in the bioreactor must be average > 95% : - COD_{Part} (= COD_{Total} - $COD_{Soluble}$) in the filtrate <5% of COD_{Part} in the bioreactor) - COD_{Part} (= COD_{Total} - $COD_{Soluble}$) in the filtrate <5% of COD_{Total} in the filtrate
Retain 100% of microorganisms	Sterility check (to be	< 100 CFU/ 100mL

	checked upstream the dead-end filter)	
Not selectively retain any of the other product compounds (i.e VFA, ammonium, minerals) that should be further used in the MELiSSA loop.	Specific compounds measurement (off-line analysis)	- concentration of VFA in the bioreactor = concentration of VFA in the filtrate (+/- 10%) - Same for the rest of relevant compounds (NH4+, minerals: PO43-, SO42-, Cl-, Na+, Mg2+, K+, Ca2+)
Feasibility of steam sterilization of the membrane (121-125ºC, at 1-1,3 bar, during 20-30 min)	Temperature measurement (on-line); checking membrane performance as per the rest of parameters described in this table after sterilization	Ability of whistand the sterilisation temperature and maintaining membrane properties (as defined in with the rest of parameters within this table) long-term afterwards
Feasibility of chemical disinfection of the membrane (NaOH 1%)	checking membrane performance as per the rest of parameters described in this table after disinfection	Ability to withstand the disinfection treatment and maintain membrane properties (as defined in with the rest of parameters within this table) long-term afterwards

* In principle, the availability of data is the only success criterion established until the equipment will be considered validated.

9. 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 total mass by the measured/calculated input total mass on the bioreactor should be higher than 90%.

Parameter	**Measurement means**	**Success/failure criteria**
Total mass balance closure	Ratio of measured/calculated output of total mass versus the measured/calculated input of total mass from the analyses and measurements performed as per the	0,9-1,1

	corresponding analytical protocols	

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

Parameter	**Measurement means**	**Success/failure criteria**
C mass balance closure rate	Ratio of measured/calculated output mass of carbon versus the measured/calculated input mass of carbon, from the analyses and measurements performed as per the corresponding analytical protocols	0,8-1,2
N mass balance closure rate	Ratio of measured/calculated output mass of nitrogen versus the measured/calculated input mass of nitrogen, from the analyses and measurements performed as per the corresponding analytical protocols	0,8-1,2

11. The characterization tests are considered successful if the biomass evolution is monitored and the stability of the bacterial consortium is not negatively affected on the long-term by the filtration unit hardware or other:

Parameter	Measurement means	Success/failure criteria
Biomass concentration (g/L)	VIAMASS probe	Data available*
Retentate side turbidity	Turbidity sensor (OPTEK)	Data available*
C1 stable aerobic and anaerobic cell count along the tests	Cell count determination (off-line)	Stable microbial concentration ($10^{6\pm1}$)

* In principle, the availability of data is the only success criterion established until the equipment will be considered validated.

5.7. *Test sequence*

The characterization tests sequence can be summarized as follows :

Phase 1 : maintenance of the inoculum

This phase has been already extensively performed during the first part of 2011, with the following objectives :

- to provide the nominal volume of broth for C1 bioreactor (100L)
- to obtain the adequate level of growth for the RT tests (established in >40g/L dry weight)

Phase 2 : ramp-up of the culture in the C1 bioreactor up to continuous conditions

This phase has been performed starting from summer 2011, and was oriented to the following objectives :

- to establish a continuous feeding regime
- to establish a continuous filtration regime
- to establish a continuous biogas production
- to replace maintenance feed by nominal feed including faeces

- to reach a nominal RT of 10 days, equivalent to 10L/day of feeding and 10 L/day of filtrate production

Phase 3 : 10 days liquid residence time test, and within this phase :

- to reach a steady state, based on the evolution of the bioreactor dry matter, VFA production, CO_2 production, and total COD evolution, including a period of at least three RT of stable performance, meaning 30 days
- to retrieve data from a period of at least one RT at the steady state

Phase 4 : 7 days liquid residence time test, and within this phase :

- to reach a steady state, based on the evolution of the bioreactor dry matter, VFA production, CO_2 production, and total COD evolution, including a period of at least three RT of stable performance, meaning 21 days
- to retrieve data from a period of at least one RT at the steady state

Phase 5 : 13 days liquid residence time test, and within this phase:

- to reach a steady state, based on the evolution of the bioreactor dry matter, VFA production, CO_2 production, and total COD evolution, including a period of at least three RT of stable performance, meaning 39 days
- to retrieve data from a period of at least one RT at the steady state

Phase 6 : 5 days liquid residence time test, and within this phase:

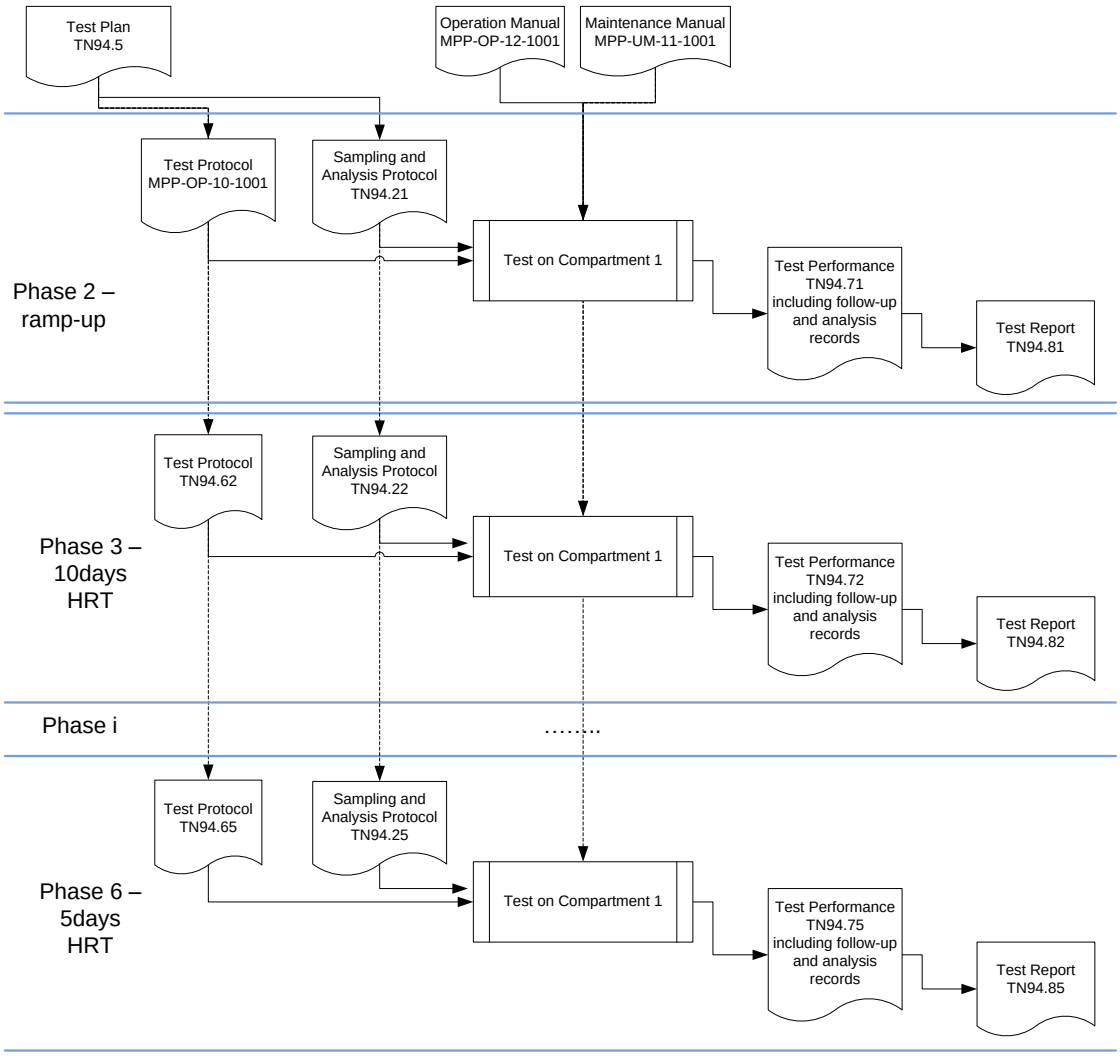
- to reach a steady state, based on the evolution in the bioreactor of the dry matter, VFA production, CO_2 production, and total COD evolution, including a period of at least three RT of stable performance, meaning 15 days
- to retrieve data from a period of at least one RT at the steady state

The protocols for the different phases are the following ones :

Test Phase	Applicable protocol	Applicable sampling/analysis protocol
Phase 1 : maintenance of the inoculum	MPP-OP-10--1001	TN 94.21: CI sampling and analysis protocols- Issue 1- preliminary definition
Phase 2 : ramp-up of the culture in the C1 bioreactor up to continuous conditions	MPP-OP-10--1001	TN 94.21: CI sampling and analysis protocols- Issue 1- preliminary definition
Phase 3 : 10 days liquid residence time test	TN 94.62: “CI test protocol: nominal operation”	TN 94.22: CI sampling and analysis protocols- Issue 2- test with 10 days residence time
Phase 4 : 7 days liquid residence time	TN 94.63: “CI test protocol: natural perturbation (7 days residence time)”	TN 94.23: CI sampling and analysis protocols- Issue 3- test with 7 days residence time
Phase 5 : 13 days liquid residence time test	TN 94.64: “CI test protocol: natural perturbation (13 days residence time)”	TN 94.24 : CI sampling and analysis protocols- Issue 4- test with 13 days residence time
Phase 6 : 5 days liquid residence time test	TN 94.65: “CI test protocol: natural perturbation (5 days residence time)”	TN 94.25 : CI sampling and analysis protocols- Issue 5- test with 5 days residence time

5.8. *Test deliverables*

As-run procedures
Follow-up sheets
Analytical records
Datasheets from server datalogging
Balances calculation sheets
Test results and reports



6.Data collection plan – Sampling plan

6.1. *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, gas mass flow and VFA (see the corresponding protocols for further 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 current method reaches an expanded uncertainty of 44% to 69% with a level of confidence of 95%. For HPLC technique, the standard deviation should be lower than 20%, but the detailed uncertainty budget has not been evaluated.

6.2. *Measurement plan*

The measurement plan as discussed among the partners of call off order 3 includes the three following priority groups (cf. MPP-MOM-08-1007):

Priority 1

Phase	Physical or chemical or biological parameter
Liquid/solid phase	total liquid flow or volumes
	Dry matter
	ashes
	sample volume
	CHON 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+

Gas phase	total mass gas flow or volumes
	CO2
	H2
	CH4
	H2S
	O2
	Sample volume

Priority 2

Phase	Physical or chemical or biological parameter
Liquid/solid phase	EC
	pH
	Temperature
	speed of blenders
Gas phase	Pressure

Priority 3

Phase	Physical or chemical or biological parameter
Liquid/solid Phase	Proteins
	Fibers
	Carbohydrates
	lipids
	alcalinity
	CST
	aerobic count
	anaerobic count
	turbidity
	particles size
	COD soluble
	COD total
	N total
Gas Phase	gas contaminants

6.3. *Sampling techniques*

- 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/solid samples, various ports allow to bleed through manual valves the content of the bioreactor.

- No continuous sampling of liquid/solid phase is planned.

- When making a sampling, the first bled mL are thrown away in order to take a sample that be representative of the sampling point.

- The samplings made on the filtrate circuits and tank, ie downstream the UF membranes, are made in sterile conditions, with a previous steam sterilization, in order to preserve the sterility of the filtrate circuits and to collect a sterile sample.

6.4. *Sample size, frequency, locations*

6.4.1. *Liquid/solid sampling size*

Each liquid sample is taken into a clean container of 100mL volume with a screwable lid (sterile when used for microbiological samples). The exact volume can be lower than 100mL but should be traced every time a sample is taken, preferably recording the mass of the sample (for further details, see the dedicated sampling protocols TN94.22-94.25)

The representativeness should be guaranteed :

- reproductibility of each measurement
- representativity of sampling taken in the reactor (in function of the sampling location)

6.4.2. *Liquid sampling locations*

The following liquid sampling locations can be found in C1 compartment (further details are provided in AD5) :

- Liquid sampling port on Influent Tank :one sampling port is available on the lower part of the influent tank (HV_1000_07).
- Liquid sampling port on Bioreactor Tank: two sampling ports can be used : a lower side port (HV_1007_02) and the bottom port (HV_1007_02).
- Liquid sampling port on Effluent Tank : two sterilizable ports can be used to take filtrate sample from the effluent tank (HV_1204_01 and HV_1204_02),
- and there is one additional sampling port in the filtrate line upstream the filtrate tank (HV_1210_03).

6.4.3. *Analysis frequency on the liquid/solid phase*

The circuit where to sample and the average frequency at which the samples should be taken on the liquid phase will be adjusted in every single sampling and analysis protocols (TN94.22 to TN94.25).

6.4.4. *Analysis frequency on the gas phase*

The circuit where to sample and the average frequency at which the samples should be taken on the gas loop will be adjusted in every single sampling and analysis protocols (TN94.22 to TN94.25).

6.5. *Analysis*

The analytical procedures are described in the corresponding analytical protocols for each test.

7.Resources for the test

7.1. *Personnel: staff qualification and training needs*

The MPP Bioprocess Engineer and 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.

7.2. *Hardware: instruments, specific part, hardware for software operation, calibration certificates*

- C1 Compartment hardware as described in AD5.
- C1 PLC
- Analytical equipment as described in the detailed analysis protocols.

7.3. *Software: verification of software, backup needs*

- All acquisitions have been validated

- PLC is connected to the data acquisition server (Scada / Ifix)
- The software used for C1 PLC control is the Schneider Concept V2.6.
- Microsoft Excel for calculations

No special backup is needed for these tests apart of the nominal server backup.

7.4. *Facilities : environmental needs, test conditions, interfaces needs, utilities needs*

MPP Utilities: steam, compressed air, decalcified and deionised water, cooling water, power, N2 needed as specified in AD4 and AD5. Interfaces: sewage.

For detailed information about MPP Utilities, see RD5.

8.Responsibilities

8.1. *Management team*

Bioprocess engineer and Technical manager: Preparation of test plan, supervision of tests, review test results, reporting.

8.2. *Testing team*

- Maintenance technician: hardware related tests execution and annotation of test procedures and calibration/maintenance records
- Lab technician: analysis and hardware related tests execution and annotation of test procedures, follow-up and calibration/analysis records
- Bioprocess engineer: definition of the protocol, execution and annotation of test procedures, follow-up and calibration/analysis records supervision, test results elaboration and reporting

8.3. *Testing support team*

- N.A.

Test Phase	Author	Checked by	Approved by	Approved by customer
Test Plan TN94.5	E.Peiro	A. Fossen	F. Gòdia	B. Lamaze
Sampling and analysis protocols TN94.21 to TN94.25	A. Fossen	E.Peiro	F. Gòdia	B. Lamaze
Test protocol nominal operation (10 days RT) TN94.62	M. Mansur	E. Peiro and A. Fossen	F. Gòdia	B. Lamaze
Test protocol natural perturbation (7 days RT) TN94.63	M. Mansur	E. Peiro and A. Fossen	F. Gòdia	B. Lamaze
Test protocol natural perturbation (13 days RT) TN94.64	M. Mansur	E. Peiro and A. Fossen	F. Gòdia	B. Lamaze
Test protocol natural perturbation (5 days RT) TN94.65	M. Mansur	E. Peiro and A. Fossen	F. Gòdia	B. Lamaze

9.Schedule

Tests to be finalized by end of September 2012, and reporting by end October 2012. The following planning is foreseen:

	May-December 2011	January-March 2012	April-May 2012	June-August 2012	September 2012
Start-up					
Nominal operation					
7 days RT					
13 days RT					
5 days RT					

10.Risks and contingencies

The overall planning of C1 characterisation tests is based on reaching the steady state within three residence times, what should be confirmed for each test condition.

11.Procedure for review and status reporting

11.1. *Reporting of status for a test*

Annotated as-run procedures and joint decision on final status of the test (failed/passed) in agreement with ESA.

11.2. *Deviations and non conformances*

In case the test sequence cannot be performed as planned or the results are not conforming the expectations, a deviation is opened and appended to the test record.
The deviation is discussed between UAB and ESA to decide on how to address it. In any case, all deviations will be discussed before a decision is taken on the status for the test

In the case that a Non conformity is derived from any of the deviations, the MPP procedure for non conformities management will be followed (MPP-QAP-08-0002)

11.3. *Test readiness review*

Inspection of hardware + review of the dedicated test protocols + AD + RD + as-run procedures of the SAT + MoM

11.4. *Test acceptance review*

Review of the tests results and test reports + MoM

11.5. *Records*

- MOMs
- Data as recorded
- Follow-up records
- Feed preparation records
- Analytical records
- Excel Database for analytical results
- Test results and reports

12.- Comments

TN 94.5

C1 additional characterization: Test Plan

General comments

As a general comment, consistency between TN 94.21, 62 and 5 have to be checked as there are some direct links between paragraphs of these various TNs. To ease this check, we have put an extra remark when identified.

OK. Agreed

Detailed comments

Page/paragraph	Comment
All/ header	Please update identification and number along the TN OK
7/ 2.1	The list of AD should be updated : TN 94.41 is an AD, as well as TN 94.21 List updated: 94.41 as AD; reference to 94.21 removed

7/ 2.1	It is not fully consistent to have only part of the QCPs as AD, either we put everything or nothing. Agree, in fact better all of them removed, as there are specific protocols to refer to this QCPs.
7/ 2.2	With regards to RD6: we understand that this procedure is proposed for the microbiological analysis of filtrates samples; two remarks: such a procedure is indeed and AD , not a RD; either a new procedure has to be written, or the scope/name of this one has to be changed, as we do not check axenicity of a culture but check sterility of a liquid stream. In principle we consider a new procedure for sterility check of filtrate (new QCP drafted); indeed this should be named sterility, not axenicity Removed as AD as there are specific protocols to refer to this QCPs
9/ section 5.1, 1st paragraph	In the top level objective, we should not forget the perspective of the MPP loop integration. OK, rephrased.
9/ section 5.2, 1st table	As discussed over the phone, the requirements proposed there is the best tentative existing today of defining them; please include a sentence explaining this status. Otherwise it could be considered that they are under a definitive version, which is not the case. OK, included.
11/section 5.2, FU reqs	The wording used in this table is not reflecting the final version of the TN 94.41, please doublecheck. OK. In fact, this table corresponds to the initial proposal from ESA, that was then adjusted during the discussion of the TN. So it has been updated as per the final version of the TN.
13/section 5.3, 2nd paragraph	How do you characterize a steady-state: which parameters will support this evaluation? Please precise. As discussed during the TRR, the following parameters should be checked to demonstrate steady conditions: dry matter, VFA, CO2 production rate and COD. TN updated accordingly.

13/3rd paragraph	Please update the formula to include samplings and bleedings. OK, updated.
13/Last paragraph	As mentioned in the comments on TN 94.21, we do not agree with the priorities , the focus on future integration of the MPP loop is completely out of these priorities. We understand this type of priority is not of UBP concern, but it is for the MPP and ESA. Definition of priorities rephrased according to the discussion of the TRR, as in the Analysis protocol TN94.22
14/section 5.6, table under 1.	It is a fair approach to start from EPAS values; we may redefine the values based on the knowledge gained all over the characterisation phase. We have to be careful with the dry matter, we should not forget that the new membrane has an internal channel diameter lower than the one of the previously selected membrane. As agreed during the TRR, the dry matter range has been updated to 40-70 g/L.
15/section 5.6, table under 2.	value for the straw to be discussed. OK, kept as proposed, according to the discussion during the TRR.
15/ table under 1	It is not really correct to call the filtrate check an axenicity check; we should use sterility check. OK, amended.
15/table under 4.	we agree with the proposed values, to be confirmed after further characterization of CI. OK.
16/table under 6.	Everywhere S is mentioned in priority 1, but is not included in the analysis. In fact, S is determined within the minerals analysis; section 6.2 amended.
16/table under 7.	It would be more consistent to name the second parameter "retention of solid particles" assessed thanks to COD total and

	COD soluble measurements, the success/failure criteria being on one hand: COD part in filtrate< x% COD part in the bioreactor, and on the other hand, COD part in the filtralte< x% COD total in the filtrate. OK.
17/table under 7.	Please specify the membrane properties you are considering. OK, precised in the text. Table also updated as per the final version of TN94.41 (see answer to comment nº 11).
17/table under 7.	The last parameter in the table could be discussed and reformulated in 'CI consortium not damaged during operation' or similar. A stable cell count is indeed the only mean to detect any damage, but we will not be able to relate this damage to the membrane, the pump or any other factor. OK, parameter removed from this table and reformulated in new Table 11.
17	Bullet 8. is missing (see page 13/20 of TN 94.62). OK, 8. and 9. of TN94.62 included in the Test Plan.
17/ Section 5.7	Could you please precise how you will decide to move from one phase to the other? After 3 hydraulic residence times automatically you will go to next step, you will base your decision on some success/failure criteria, which ones?. As answered for comment 8, we will base on the four selected variables for the identification of the steady state, the transition period not necessary be 3 RT (this is a fixed time for the steady state period, not for the transition phase).
18/table	We do not fully understand the ref of applicable protocols: where is TN 94.61, and where is TN 94.22?. As discussed in the TRR, TN94.61 cancelled or replaced by existing documents; TN94.22 to be created from TN94.21.
20/Section 6.2	See previous comment : please update the list per priority level taking into account the perspective of future integration; please update according to TN 94.21.

	See previous answer: Definition of priorities rephrased according to the discussion of the TRR, as in the Analysis protocol TN94.22
22/Section 6.3/ 2nd paragraph	The volume bled before taking the sample should maybe be recorded. Do we have a first estimation of the volume we will lost through these bleedings per week, to see if this is significative or not and could potentially impact the calculation of mass balances?. To be updated in the Analysis Test Protocols.
22/Section 6.3/ 3rd paragraph	Samplings are made in sterile conditions, not axenic ones. OK, amended.
22/ Section 6.4.1	Liquid/solid sampling size To be updated in the Analysis Test Protocols.
22/ Section 6.4.2	For the sake of clarity can you include the tags of the sampling ports?. Ok, to be included in the doc.
23/6.4.3	Mentioning liquid only is creating the potential confusion that people consider all sludge samples filtered before analysis. Rephrased as liquid/solid phase.
23/6.4.3	The idea of having sampling and analysis protocols for each test phase is precisely to get the opportunity to check/adjust frequencies, number of samples and any other detial which needs to be updated. So why don't we mention here only that these frequencises are average ones and will be adjusted in every single sampling and analysis protocols?. OK, rephrased; detailed in the analysis test protocols.
23/6.4.3	Two parameter should be mentioned here and/or in each sampling protocol , the time you have available between sampling and actual analysis, the state of your sample (raw, pre-filtered.). Detail to be incorporated in the Analysis Test protocols.
24/ section 6.4.4 Table	Liquid/solid sampling size

	To be updated in the Analysis Test Protocols.
26 / Section 8.3/ EP 25	I guess we speak here about TN 94.61? Creating a protocol retrospectively is maybe not of the utmost importantce; this is maybe more efficient to use the various reports prepared to report precisely what has been done. OK, removed.
26 / Section 8.3 Table	For the sake of clarity can you include the TN numbers?. OK, included.

Second set of comments

Page/paragraph	Comment
5/ Section 1	During our discussion with SHERPA in 2011 it was proposed to change to 5 days RT, and in fact for thye MPP it would be much easier to manage (1 harvest per day of the filtrate tank) than 3 days, so we propose 5 days. Agreed.
6/ Section 2.1	Please update numbering (two times AD6). OK, updated.
6/ Section 2.1	*"AD7 Grinding and mixing of C1 Bioreactor feed with the WPU"* As all the records for feed preparation are in the Test protocol, I would move this AD to the Test Protocol. OK, agreed.
6/ Section 2.1	For full consistency, the OPs 08-1001 (OP for faeces donation) and maybe OP 08-1002 (OP for the handling of faeces samples) should be in AD of the test protocols. OP 08-1002 is already mentioned among the ADs of OP-10-1002, but with another name. Please check OK, checked. AD to be updated in OP-10-1002.
8/ Section 5.1	*"... UF membrane ..."* In other places, you refer to a microfiltration membrane, please

	harmonize OK, harmonized as UF.
10/ section 5.2, first paragraph under the table	*"Test protocol TN94.62."* I would suggest test protocols TN 94.62 to TN 94.65 OK, amended.
16/section 5.6 , bullet 1.	*"Success/failure criteria"* It's not easy to find consistent info within EPAS documents about these limits; we're proposing reasonable limits to be compliant with according to our previous experience, but we have not experience about criticality of some deviations, in fact. Agreed; to be updated in next phases.
16/section 5.6, bullet 1.	*"45-58ºC"* According to TN 71.9.2 from EPAS ; still valid ?. OK
16/section 5.6, bullet 1.	*"5,0-6,0"* Id. OK
16/section 5.6, bullet 1.	*"0 CFU/ 100 mL"* Consistent with the FU optimization values of 100CFU/100mL? The membrane is one thing and the final filtrate is another one : for the last one only 0 could be accepted after the dead-end filter. Agreed.
17/ section 5.6, bullet 4.	Success criterion for biogas production in the QCP or just a calculation of the uncertainty associated to its measurement? Proposed just a deviation around the average production. The current prod. values don't match with the EPAS ones, so it's not easy yet to propose a value. Agreed.
17/ section 5.6, bullet 4.	*"CH_4+ <2%"* Success criteria to be agreed with ESA (in this case is according to TN 71.9.2 from EPAS)

	Agreed.
17/ section 5.6, bullet 4.	*" SH_2 <1000 ppm"* Taken from MPP experience ; in TN 71.9.4 data EPAS reported lower than 300 ppm. Agreed.
17/ section 5.6, bullet 5.	*"Stable dry matter production"* I would say content. Agreed; amended.
18 / section 5.6, bullet 6.	*« The characterization tests are considered successful if the VFA, CO2 and NH4+ production is maintained during the test …"* Please update the wording, it is a bit confusing with 5. OK, amended.
18 / section 5.6, bullet 8.	*"Filtrate side turbidity – VIAMASS probe – data available."* Threshold to be defined with NTE after the validation period of the VIAMASS (the value indicated previously here was defined for off-line turbidity determination, now to be adapted to the on-line VIAMASS measurement.
19 / section 5.6, bullet 8.	*"< 100 CFU/ 100mL"* Just as a remark, As an alternative for the future we could consider as success criteria a given log reduction Agreed.
20 / section 5.6, bullet 11.	*"Cell count evolution constant"* Please rephrase. OK, rephrased: *"Stable microbial concentration"*
21/ section 5.7, Phase 3	*"- to retrieve data along a period of three RT, meaning 30 days"* This wording is confusing , we collect data during the transient phase, including at least three RT of stable performance and then a minimum of 1 RT to measure twice the relevant parameters. Please consider this remark for the other phases Text amended accordingly: *"to retrieve data from a period of at least one RT at the steady state"*
25/section 6.2	*« 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 » To be discussed if we could reduce the list in order to reduce the cost and in case some of them are present only in very small quantities. List to be reconsidered at the end of the first phase.
27/ Section 6.4.1	*"Each liquid sample has a standard volume of 100mL. It is retained into a clean recipient with a screwable lid. The exact volume can be lower than 100mL but should be traced every time a sample is taken."* This should be rephrased to better discriminate among the cases Text amended to discriminate sample and container: *"Each liquid sample is taken into a clean container of 100mL volume with a screwable lid (sterile when used for microbiological samples). The exact volume can be lower than 100mL but should be traced every time a sample is taken, preferably recording the mass of the sample"* This description is taken from TN94.22; referred to this TNs for further details.
28/Section 6.4.3	*"Analysis frequency on the liquid/solid phase"* These frequencies should be adapted for HRT lower than 10 days. The best is to define analyses per residence time instead of per week ; this way we have the same resolution of analyses for all the HRT. So, for 5 days the frequency should be increased.
29/Section 6.4.4	Same remarks for frequency of offline measurements.
32/ 11.5 Records	*"As-run annotated procedures"* Do we have a template of the procedure? do you plan to use an excel database as in BELISSIMA? I checked inside the record for C1 follow-up and it is not covering the necessary information. In this case what we propose is in fact a follow-up record. An excel database is already existing from the previous maintenance period, to be now updated for the testing campaign purpose.