Universitat Autònoma de Barcelona

TECHNICAL NOTE 94.61

Call Off Order 3 – COMPARTMENT I Additional Characterization

Work Package 94.6

Test Protocol – Ramp-up phase

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1. Scope

In the frame of Call Off Order 3, the objective of this document is to summarize the protocol to follow in order to grow the inoculum of compartment 1 up to 100L in the bioreactor and reach the adequate dry weight of 55 g/L to 75 g/L before starting the 10 days hydraulic residence time.

As this growth had to be carried out after the loss of the C1 inoculum, and that an MPP operating procedure was rewritten for that same purpose, it was agreed with ESA to only provide said operating procedure MPP-OP-10-1004 in appendix of the present protocol, for implementation.

The recovery of the lost inoculum is envisaged in two steps : first in the Influent Tank that will be prepared for the semi continuous culture of the recovered inoculum, and once the maximum volume is reached in the Influent Tank, a second semi continuous culture will be started in C1 Bioreactor.

2. Appendix :

MPP-OP-10-1004(0), 13 pages

C1 Inoculum recovery in Influent Tank Semi-continuous Operating Procedure

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Table of content:

1. Scope

This procedure describes the semi-continuous operating procedure of the C1 inoculum to be cultured in the Influent Tank during the recovery and growth start-up phase previous to the transfer to the bioreactor, after the loss of the bioreactor content on 18th Oct 2010 (MPP-NCR-10-1006).

As previously agreed with UBP and ESA (see MPP-MOM-10-1003(0)-AF-20101119), the strategy for the recovery and growth start-up period in order to have the CI inoculum ready for the CI characterisation phase will be as follows:

Step 1 : Restarting 50 L reactor with the available volume of back-up Inoculum (maintained at 4°C)

1. Transfer the inoculum with initial feed (50:50) in the influent tank (working vol. 50L)

2. Feed with only beet (which is easily degradable), until reaching a nominal biomass concentration (close to the one expected for starting continuous operation of the reactor).

3. Progressively, feed with the mix of the 3 higher plants. The frequency / quantity should be adapted to the response of the reactor (probably following the CO2 gas production is sufficient to know the status of the reactor). The objective is to fit a weekly feeding quantity as it is foreseen in the test plan.

4. When the objective of the nominal operation of the reactor with the feeding protocol expected in the test plan is achieved, start the 100L reactor.

Step 2 : Restarting the 100 L reactor

1. Transfer the culture broth from the influent tank into the 100 L bioreactor

2. Adapt the ramp in the frequency / quantity of feed (plant composition identical to the one used in the influent tank) as function of the response of the reactor knowing that the objective is to reach the Nominal 10 days RT as depicted in the test plan.

The procedure includes a general description of the process, the follow up record sheets (MPP-REC-10-1008) filling out instruccions and the analyses to be performed.

2. Reference documents

RD1	CI Software Description and Procedures, September 2010	SHERPA ENGINEERING, CI Software Description and Procedures.

RD2 NTE-MCI-HB-012, Issue 1.0, May 2009. NTE, CI HMI SW User Manual for the MELISSA CS CI.

3. General description of C1 inoculum maintenance process

C1 backup inoculum is maintained in the influent tank of pilot scale C1 located in C1 room. It is a 60L volume tank with 50L working volume. Some hardware modifications were done on the influent tank to adapt it to C1 culture. In process diagram MPP-PID-10-1002-A1, hardware modifications are shown in different colour. This diagram is shown below in paragraph "*15. Process Diagram*."

At present, influent tank is equipped with mechanical agitation and a recirculation loop that it is used to mix the content and to introduce the feeding by the "getting cake process" ([RD1], paragraph 3.7, Procedure 7).

Temperature is controlled by means of the Temperature Control loop and dedicated hardware of the bioreactor, but connected to the influent tank jacket. For this purpose, the following changes were performed:

- The thermal control piping connected to the bioreactor jacket was mounted on the influent jacket

The pH is also controlled by the pH Control loop of the bioreactor, by means of one of its pH probes connected to a pH transmitter/controller (both Mettler Toledo). pH is maintained at 5.3 (±0.2) by the addition of of acid (HCl, 3M) and base (NaOH, 3M) by the two peristaltic pumps installed in the bioreactor skid. For this purpose, the following changes were performed:

- The bioreactor pH probe was mounted on the influent tank directly, and the acid/base addition piping was mounted on the top of the influent tank.
- The influent blender was linked to the pH addition control, to be sure that the blender is ON when is needed to add acid/base.

The tank headspace pressure is maintained at 120 mBar (to avoid the shut down of recirculation pump, which stops above 135 mbar, approximately) by the addition of a nitrogen flow of 100mL/min by a mass flow controller calibrated with Air with a working range of 20 – 1000 $_n$mL/min (Bronkhorst Hi-Tech).

Gas analysis: the piping for the analysis gas loop was mounted in the free ports of the influent tank, to have the values of CO2 and Methane. This point does not affect to the PLC program.

In the Human-Machine Interface (HMI) located in the control room of the MPP, the temperature, pH and pressure control, as well as the agitation and recirculation pump, can be actuated; other parameter values can be visually checked [RD2].

Further details about the software control configuration are given in the Section 14 of this document.

C1inoculum is maintained in semi-continuous mode. The tank is fed once per week with a solid mixture of maintenance feed composed only by beet in the first stage (MPP-OP-10-1005) and then progressively incorporating the complete maintenance feed (MPP-OP-09-1001) Culture nominal volume is 50 L. Solids are returned to the reactor by a centrifugation step and separation of supernatant. Residence time is 20 days and the corresponding load of substrate is 0,58 gDW/(day.L).

4. Personal protective equipment

4.1. Lab coat

4.2. Gloves

4.3. Goggles

4.4. Full face shield

4.5. Half-face organic vapour mask

5. Reagents

5.1. Bleach

6. Equipment

6.1. C1 Influent Tank of 55L working volume

6.2. Steel funnel (the funnel connected to valve H3V_1001_01 can be used)

6.3. Disposal bottle of 10L volume

6.4. Graduated plastic bucket of 20L volume

6.5. Plastic rod

6.6. Infrared gas analyser GA94 (Geotechnical Instruments)

7. Operating procedure

7.1. Inoculum back up and feeding 1 day procedure

7.1.1. On Fridays:

- Defrost one C1 beet feed bag of 2,53-2,57Kg (if only beet is fed, see MPP-OP-10-1005) or 4,1Kg (if the whole vegetable mix is used, see MPP-OP-09-1001) storing it at 3 – 5 ° C for next Monday feeding operation.

7.1.2. On Mondays:

1. Dispose a sample of 80 – 100 mL of reactor content. Take another sample of 80 – 100 mL of reactor content. Label the sample as indicated below and name it BEFORE FEEDING (see 8).

2. Take a sample of 9L of reactor content in order to centrifugate the sample and keep the pellet.

3. Take a sample of supernatant of 80 – 100 mL volume and label it as indicated below. Name it FILTRATE (see 8). Dispose the supernatant as indicated in "9. Disposal of C1 reactor content, samples and supernatant."

4. In case that only beet is used, check the particle size of the defrosted feed bag of 2.53-2.57 Kg of red beet, by sieving it through a 2 mm mesh sieve. Use only feed with particle size lower than this size to feed the CI culture.

5. Mix in a graduated vessel the defrosted 2.53-2.57 Kg feed plus the pellet obtained in the centrifugation with distilled water, until a final volume of 9 L (±0.2).

6. Transfer the feed mixed in the WPU in a graduated plastic bucket of 20L of volume

7. Take a sample of feed of 80 – 100 mL volume and label it as indicated below. Name it FEED (see 8).

8. Open one port of the top of the influent tank. Use the full face visor until step 11.

9. Introduce the content of feed into the influent tank by means of the graduated plastic bucket in three times. Fill the plastic bucket with a maximum of 6L approx. of the content of the bottle each time.

10. Close the top lid of the influent tank.

11. After 1 hour, dispose a sample of 80 – 100 mL of reactor content. Take another sample of 80 – 100 mL of reactor content. Label the sample as indicated below and name it AFTER FEEDING.

12. Fill out the follow up record sheet MPP-REC-10-1008.

8. Labelling

8.1. Samples labelling

- Use the DYMO or a pen to label the samples as indicated below:

MPP	C1	RC BF	*(REACTOR CONTENT BEFORE FEEDING)*	DATE	*(YY/MM/DD)*
		RC AF	*(REACTOR CONTENT AFTER FEEDING)*		
		FEED # ...			
		FILTRATE			

- Examples:

MPP C1 RC BF 090126
MPP C1 RC AF 090126
MPP C1 FEED #12 090126
MPP C1 FILTRATE 090126

8.2. Inoculum back ups labelling

MELISSA C1 INOCULUM BACK UP + DATE *(YY/MM/DD)*

9. ***Disposal of C1 reactor content, samples and supernatant***

To dispose big volumes (>100 mL) use the organic vapour mask.

9.1. Add to the disposal volume a quantity equal to 10% of the disposal volume.

- For example: To dispose 9L of waste use 1L of bleach.

9.2. Close the disposal vessel, shake it gently until complete mixing and throw the content to the liquid waste collector of the MPP.

10. Follow up (MPP-REC-10-1008) filling out instructions

10.1. Fill out the record sheet MPP-REC-10-1008 every day and when it is indicated in this procedure. Fill it out also after taking any action (extra sampling, hardware modification or fixing) or when some parameter is out of the range (although any action is done).

11. Liquid Samples handling

11.1. Analyse pH of all samples at the moment of taking them and note down the result in the follow up record sheet MPP-REC-09-1003.

11.2. Store the samples at 3 – 5 ºC to be further analysed as it is indicated in paragraph "*13. Liquid samples analysis*".

12. Gas analysis

12.1. Analyse influent tank headspace gas with the infrared gas analyser GA94 (Geotechnical Instruments) once per week, just before feeding.

12.2. Connect the gas analyser inlet to the valve HV_1052_02. (If the gas sampling tubing presents condensates, clean it before the measurement).

12.3. Connect to the gas analyser outlet the H_2 probe and connect the probe outlet to the valve HV_1052_03.

12.4. Open the valves HV_1052_02 and HV_1052_03 and start the gas analyser.

12.5. With the analyser pump in OFF mode wait 5 – 10 minutes (unitl a stable measure) and note down the results in the follow up record sheet (MPP-REC-10-1008)

12.6. Repeat the measurement with the H_2S probe and note down the H_2S result in the same follow up sheet.

12.7. Close the valves HV_1052_02 and HV_1052_03 and disconnect the gas analyser.

12.8. Clean the H_2S and H_2 probes: Connect one probe and start the analyser with the pump in ON mode in a clean atmosphere during 5 – 10 minutes. Repeat the process with the other probe.

13. Liquid samples analysis

13.1. Dr Lange analysis

- Every week analyse Feed, Reactor Content and Filtrate samples (as it is shown in Table 1) by ammonium (MPP-QCP-07-00011), total nitrogen (MPP-QCP-07-0020), COD total (MPP-QCP-07-0005) and COD soluble (MPP-QCP-07-0004) using Dr. Lange kits.

- Record the results in MPP-REC-09-1003.

Table 1. Analyses performed on C1 samples

Sample Type	Analysis parameter			
	Total fraction	Soluble fraction		
	COD	COD	NH_4^+	N-total
Feed	X	X	X	X
Reactor Content	X	X	X	X
Filtrate	X			

13.2. Dry weight analysis

- Every week analyse the dry weight (MPP-QCP-07-0007), ashes (MPP-QCP-07-0007) and electroconductivity (MPP-QCP-07-0008) of Feed, Reactor Content and Filtrate samples.

- Record the results in the sheet MPP-REC-09-1005 and MPP-REC-09-1003.

13.3. VFA analysis

- Every week, when analysing the parameters by Dr. Lange kits, store 1mL of each sample soluble fraction at -78/-80°C in GC vials previously labelled for VFA analysis to be performed later on.

13.4. Cell count

- Every two weeks, perform the aerobic and anaerobic count of Reactor content, and perform the same count of each batch of Feed (MPP-QCP-09-1001).

13.5. Elemental composition and Minerals analysis

- Every 1 month take and freeze-dry 100 – 150 mL of Reactor content (MPP-QCP-07-0010).
- Following the annual calendar previously agreed, send the Feed (MPP-OP-09-1001) and Reactor content freeze-dried samples to the "Servei d'Anàlisi Química" (SAQ) of the UAB, located in the Biosciences Faculty.
- Fill out one application for all the samples for minerals analysis. Fill out one application for each sample for elemental composition analysis.

14. CI Software modification for C1 inoculum recovery in the Influent

The following list covers the modifications performed by SHERPA on CI running software version (V01_01_CI) in order to provide the adequate conditions for the process control already described in the Section 3 of this document. The temporary version is called "Temp_CI".

1- In order to have the control of the temperature linked to the bioreactor heater, the PLC address TT_1008_01 is now connected to the TT_1002_01 PLC input (PLC@:300014). The sensor failure tag (TT_1008_01_ERR) is also connected to the TT_1002_01_ERR output.

2- TT_1002_01 is connected to the TT_1008_01 PLC input (PLC@:300015). Alarm previously linked to the bioreactor level is now implemented on the Influent level. In case of Very Low Level in the influent tank, the pH, the blender and the temperature control loops are triggered to OFF mode (the very low level threshold of influent tank is 10 litres).

3- As only one pH probe is used for the control inside the Influent (AT_1011_02), the automatic probe switches in case of failure are removed. If AT_1011_02 fails, the pH control Loop is triggered to OFF mode.

4- If the pH is in auto mode when the Influent Blender stops, the tag "CL1011_Blender_Stopped_A" prevents any injection. It ensures that Base and Acid injections are not done if the broth is not mixed.

5- The valve SV_1003_01 is opened when the pressure reaches -50 mbar (very low threshold) and 120 mbar (Very high threshold).

6- The pH Very Low threshold alarm (which triggers to OFF mode the pH control Loop) is changed from 5 to 4.

15. Process Diagram

Process diagram file:
MPP-PID-10-1002-A1