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T E C H N I C A L N O T E

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TECHNICAL NOTE: 1.6

RESULTS ON DETAILED CARBON AND NITROGEN MASS BALANCES OF THE LOW-LOAD METHANOGENIC REACTOR

(PROJECT: A TOTAL CONVERING AND BIOSAFE LIQUEFACTION COMPARTMENT FOR MELiSSA)

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1 INTRODUCTION AND OBJECTIVES

The main objectives of the second technical note of the second phase of this project were on the starting of the optimisation of both methanogenic reactor configurations. The optimisation of the high-load methanogenic reactor was investigated by the implementation of a solid retention mechanism to quantify the influence of solid retention time on extra biogas production. Furthermore, a carbon and nitrogen mass balance was made for the methanogenic biofilm reactor treating the material from the subcritical liquefaction unit. The tasks described for this TN are given below:

INPUTS

- Operational high-load and low-load methanogenesis unit
- Required analysis-equipment for full characterisation of carbon compounds (DTU)
- Analysis equipment for COD-analysis, DM-analysis, ammonia analysis
- Carrier materials for low-load reactor and solid retention system for high-load reactor
- List of stoechiometric parameters from UBP for modelling

Tasks included

- Initial tests with solid retention systems for high-load methanogenesis reactor and with carrier material in low-load methanogenic reactor
- Characterisation of mass balances of the low-load methanogenic reactor
- Substrate exchange with Partner 2 and Partner 4 (solid digester residue)
- Send recommendations to partners with regard to HACCP

In Figure 1, the conceptual scheme of a total converting liquefaction compartment as designed and agreed upon in the latest progress meeting is depicted. The concept combines three

technologies being methanogenesis, *Fibrobacter* liquefaction and thermal sub-critical liquefaction.

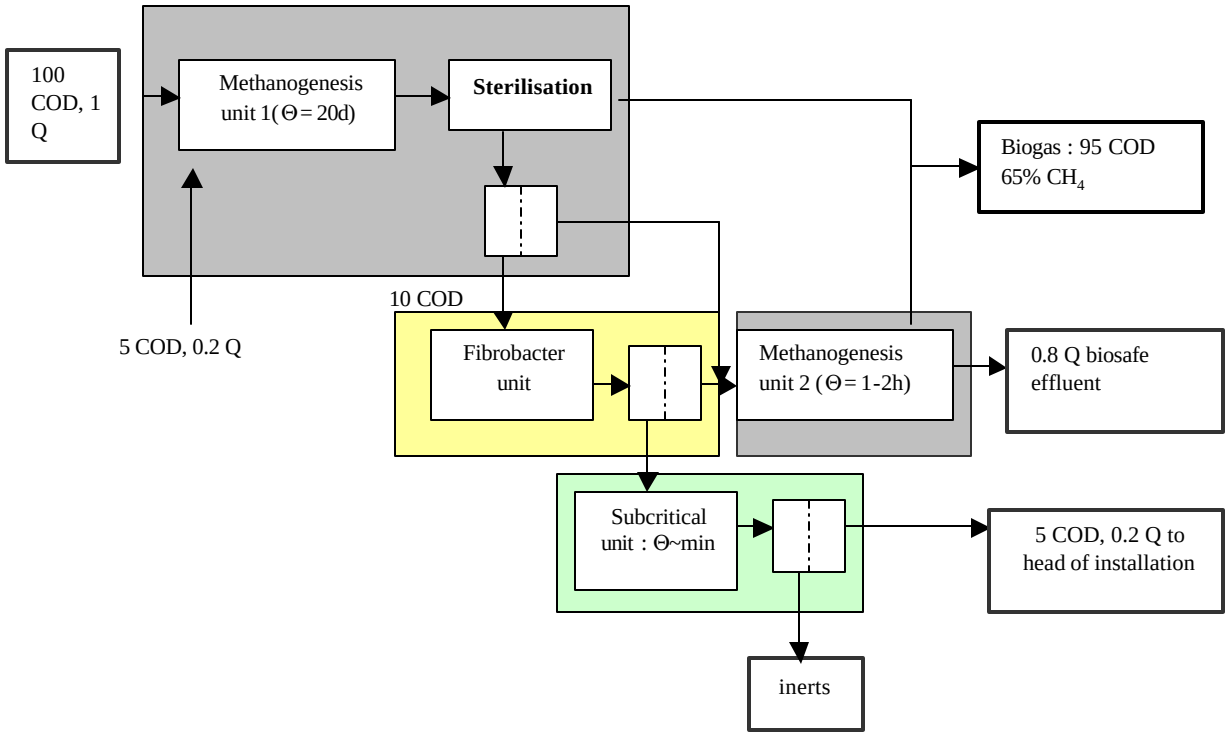

Figure 1: Conceptual scheme of a total converting and biosafe liquefaction compartment for MELiSSA

In this work package, batch experiments with mixed liquor of the high-load methanogenic reactor supplied with different amounts of pre-digested material (solid digester residue from the high-load methanogenic reactor) were performed, to verify the influence of a longer solid retention time of the high-load reactor on the biogas production.

A second objective was to make a mass balance (carbon and nitrogen) of the low-load methanogenic reactor using the exchanged solid digester residue that on the one hand has been treated in the Fibrobacter unit followed by the Subcritical unit and on the other hand in the Subcritical unit only (**4th closed loop experiment**).

2 MATERIALS AND METHODS

Substrate composition and preparation of residue (4th closed loop)

SUBSTRATE COMPOSITION

The composition of the 2% DM substrate was similar to the previous TN's:

10% DM *Spirulina* (95% DM): 2.85 g/L

24% wheat straw (95% DM): 6.65 g/L

22.5% fresh cabbage (9% DM): 6.3 g/L

22.5 % soya (90% DM): 6.3 g/L

21.5 % faeces (10% DM): 6 g/L

After CSTR fermentation, the solids of the digested effluent were separated from the liquid matrix by centrifugation (5 min. at 7000 rpm). One part was collected in a closed vessel, frozen and subsequently distributed to Partner 2 (about 400 g DM solids) and to Partner 4 (about 100 g DM solids). The other part was used to perform the batch fermentation tests with the addition of solids.

Experimental set-up of the high-load methanogenesis unit

MESOPHILIC DIGESTER

A 10 Liter anaerobic PVC-reactor is used for the anaerobic digestion of the defined feed. As indicated in Figure 2, the digester is maintained at a constant temperature of 34°C by placing it in an incubator. The reactor is a CSTR-type (continuously stirred tank reactor) and is shaken on a shaker platform (INNOVA shaker) at a constant 70 rpm.

The reactor is fed batch wise at regular time intervals. For each volume of the feed fed to the reactor, a same volume of stirred mixed liquor is withdrawn simultaneously. The biogas passes

by an electronic milligascounter device (Fachhochschule Bergedorf, Hamburg-Harburg, Germany) with a resolution of 1 ml and an accuracy of 3%. The biogas composition has been monitored during the preparation and fermentation of the batch fibrous residues.

The volumetric loading rate of the mesophilic digester was held at 1.33 g COD/L.day (Chemical Oxygen Demand) over a period of 3 - 4 months in order to obtain the necessary amount of fibrous residue (about 400 g DM (Dry Matter) to distribute to Partner 2 and about 100 g DM to Partner 4). Reactor performance was stable at the given volumetric loading rate.

The dry matter content of the synthetic feed was kept at 2% dry matter. The reactor was fed in quantities of 0.5 L feed/day. In order to maintain a hydraulic retention time of at least 15 days, the liquid reactor volume of both reactors was set at 7.5 L.

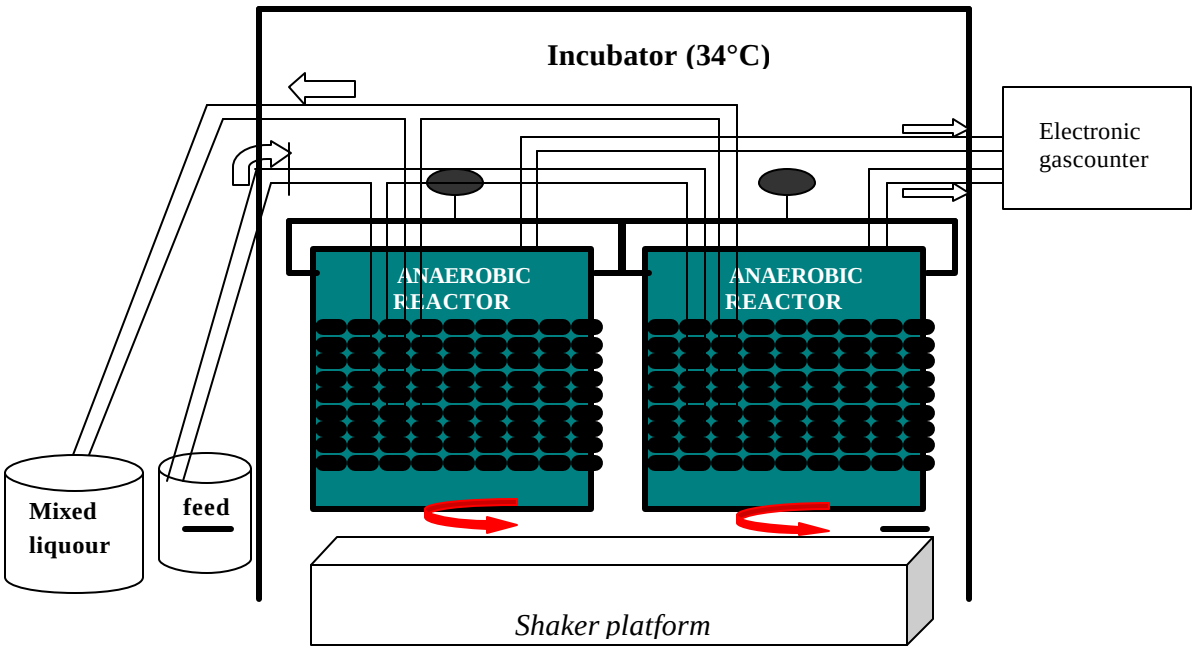

Figure 2: Scheme of the 2 mesophilic digesters for the fermentation and subsequent distribution of the digester residues (approximately 400 + 100 gram DM)

BATCH FERMENTATION TESTS WITH SOLID DIGESTER RESIDUE

Batch fermentation tests were set up with effluent (mixed liquor) from the CSTR reactor. All experiments were performed in 500 mL erlenmeyers containing a fixed amount of mixed liquour from the two main mesophilic reactors. The volume of mixed liquor present in each batch bottle was 400 mL. The mixed liquor contained a solid phase, existing both of flocculated non-granular sludge (methanogenic bacteria) and residual fibers from previous fermentations. The liquid phase consists mainly of soluble biopolymers. The experiments were run over a period of 14 - 20 days.

The effluent of the main methanogenic reactors was collected and centrifuged (5 min., 7000 rpm) to separate the solid fraction. This solid fraction (7.3 % DM) was added in different amounts (0, 1, 2 and 3 g of VS) to the batch fermentation tests to evaluate the extra biogas production.

In a first test series, bottles 1 and 2 were the control samples which were supplied with the raw substrate only (approx. 0.6 g DM). Bottles 3, 4 and 5 were supplied with the raw substrate (approx. 0.6 g DM) and 1 g of VS solid digester residue (corresponding with 13.7 g wet solid digester residue). The volume of biogas and pH was continuously measured for each bottle.

In a second test series, the biogas production of the control samples (bottles 1 and 2), which were only fed with raw substrate (approx. 0.6 g DM), was compared with bottles 3, 4 and 5. These triplicates were supplied with the raw substrate and 2 g VS solid digester residue (corresponding with 27.4 g wet solid digester residue). Subsequently in a third series, the biogas production of the control samples was compared with bottles 6, 7 and 8, which were supplied with raw substrate (approx. 0.6 g DM) and 3 g VS solid digester residue (corresponding with 41.1 g wet solid digester residue). All fermentation trials were performed in duplicate or triplicate to check for the reproducibility. The reactors were shaken manually two times per day.

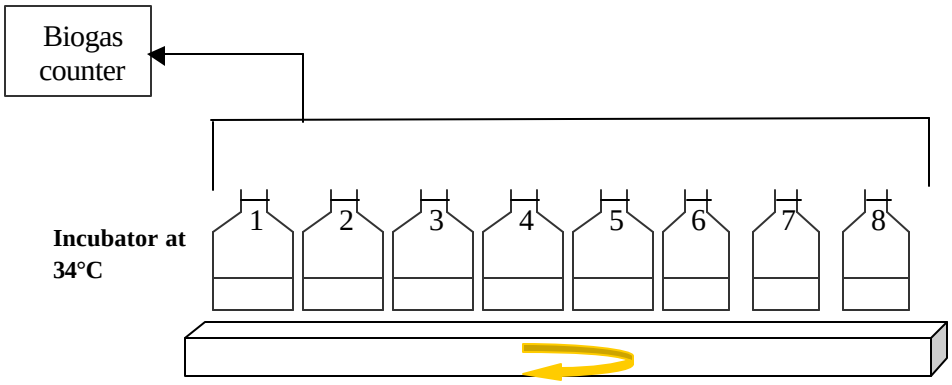

Figure 3: Experimental set-up for batch fermentation tests with raw substrate and raw substrate supplied with 1, 2 or 3 g VS of solid digester residue

Experimental set-up of the low-load methanogenesis unit

For this experiment, the fixed-bed biofilm reactor had a volume of 1.5 L. The reactor was filled with 1 dm^3 of polyethylene wheels (852 rings in total) (Kaldnes), with a total surface area of 800 m^2/m^3 and a protected surface area of 500 m^2/m^3. To initiate the biofilm formation, 1 L of tap water and 500 mL of sludge from the CSTR was added. Subsequently the liquid was continuously recycled at an up flow velocity of 2 m/h and at daily basis 5 g COD/L.day was dosed during a period of 8 weeks. Subsequently, the excess of (free) sludge was removed from the reactor. The methanogenic activity during the experiments is thus mainly propagated by the biofilm. Only minor amounts of the CSTR-sludge couldn't be decanted, because part of the sludge was trapped within the matrix of the polypropylene rings. The initiation of the biofilm and the experiments were performed at mesophilic temperature ranges. A schematic presentation of the reactor set-up is depicted in Figure 4.

After the start-up period of the fixed-bed biofilm reactor, the returned effluent from the Fibrobacter digestion followed by the sub-critical liquefaction was added to the fixed-bed biofilm reactor. The liquids were continuously recirculated with an up flow velocity of 2 m/h. The biogas production and parameters as COD_t, COD_s, VFA and pH were followed on daily basis, during a total period of 8 days.

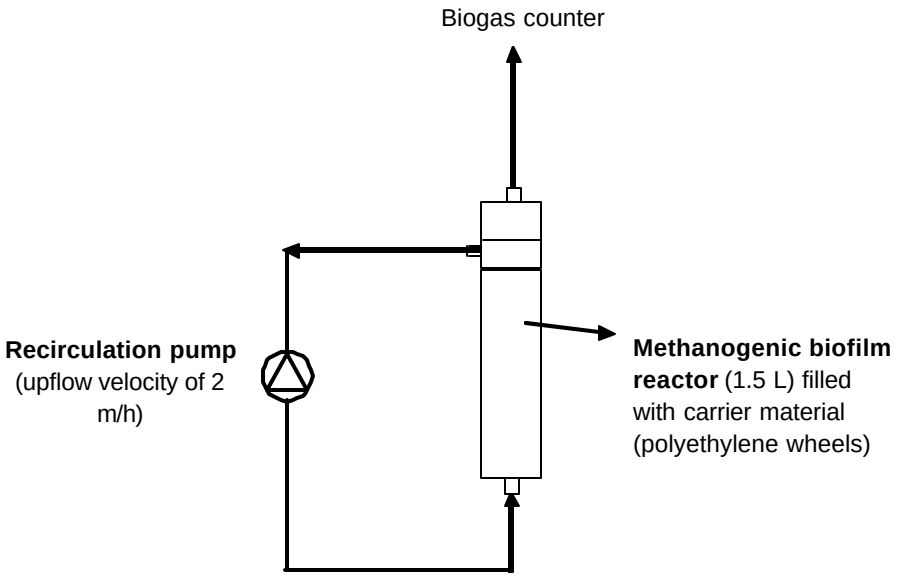

Figure 4. Schematic presentation of the reactor set-up of the low-load methanogenesis unit.

3 RESULTS AND DISCUSSION

BATCH FERMENTATION TESTS WITH SOLID DIGESTER RESIDUE

The solid digester residue used in the batch fermentation tests was originating from the effluent of the main mesophilic digester and was separated from the supernatant by centrifugation. The characteristics of this solid digester residue are shown in Table 1.

Table 1. Characteristics of the solid digester residue used in the batch fermentation tests

	Value	Unit
Dry matter content (DM)	8.9 ± 0.05	m%
Ash content (AS)	1.7 ± 0.01	m%
Volatile solids (VS)	7.3 ± 0.1	m%

The tests were started with 400 mL mixed liquor of the main mesophilic digester. One day before the actual test, 0.6 g DM was added to the reactors in order to verify if the gas tubing and columns were not leaking. All the reactors gave the same gas production after one day.

TEST 1: ADDITION OF 1 G VS OF SOLID DIGESTER RESIDUE

In a first test series, the biogas production of the control reactors (C1) which were supplied with 0.6 g DM of raw substrate, was compared to the biogas production of the test reactors (T1) which were supplied with 0.6 g DM of raw substrate and 1 g VS of solid digester residue (1.2 g DM). The volumetric loading rate (Bv) of the control reactors (C1) on time zero was 1.5 g DM/L.d; the pH of the mixed liquor was 7.3 ± 0.03. The Bv of the test reactors (T1) was 4.5 g DM/L.d; the pH of the mixed liquor was 7.3 ± 0. 03. The volumetric loading rates were calculated on the volume of mixed liquor taken from the main digester (400 mL). In Table 2, the characteristics of the mixed liquor taken from the main digester are shown. In Figure 5, the biogas production in function of time is depicted.

Table 2. Characteristics of the mixed liquor from the main digester used for the batch experiment

Parameter	Value	Unit
Dry matter (DM)	10.7	g/L
Ash content (AS)	4.5	g/L
Volatile solids (VS)	6.2	g/L
Kjeldahl Nitrogen	1306	mg/L
TAN	830	mg/L
TON	0	mg/L

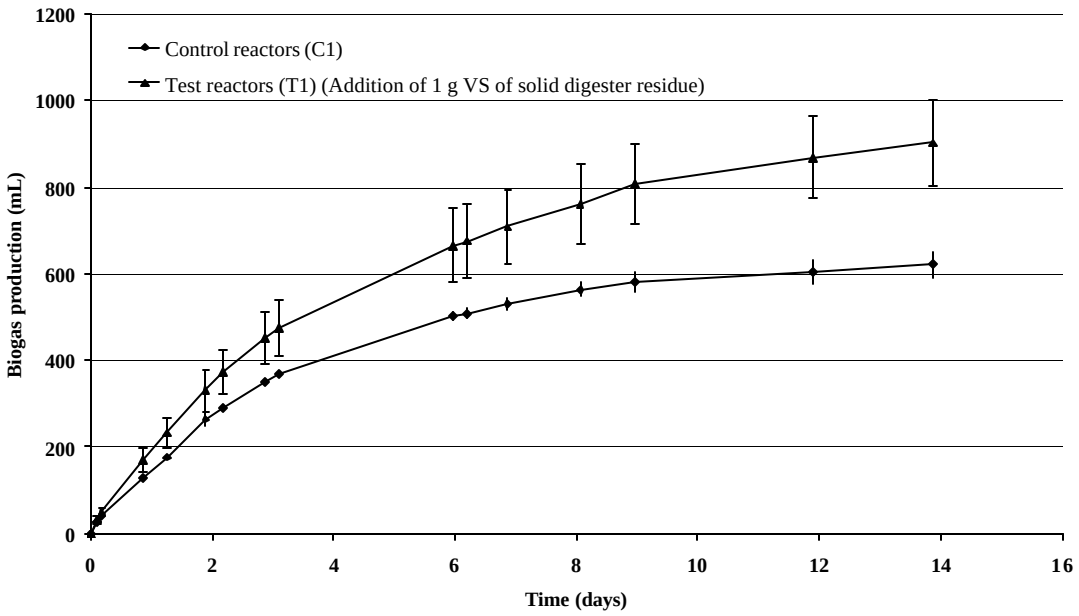

Figure 5: Biogas production (mL) in function of time (days) of the control reactors (C1) versus the test reactors (T1) (addition of 1 g VS solid digester residue)

From Figure 5, it can be seen that the addition of an extra 1 g VS of solid digester residue to the mixed liquor had a higher biogas yield compared to the control reactors, which were not supplied with solid digester residue. The higher volumetric loading rates of the test reactors (4.5 g DM/L) were not inhibitory because the biogas production started immediately without a lag phase. After 6 days, the test reactors had a biogas yield that was on average 32 % higher than the control reactors. Hence, when the retention time of the solid material in the main digester is increased from 15 to 21 days, the biogas yield will increase with 32 %. At the end of the fermentation test (t = 14 days), the test reactors gave on average 45 % more biogas than the control reactors. This means that a retention time of the solid material in the methanogenic CSTR reactor of 29 days will result in a higher biogas yield (45 %). However, a longer retention time will also result in the need for larger reactor volumes.

It can be remarked that the standard deviations of the test reactors were relatively high. This could be explained by the heterogeneity of the solid digester residue. Although the solid digester residue was very well mixed before samples were taken, the mixture is heterogeneous.

After 7 days of fermentation, the biogas composition was determined and the percentage of methane and carbon dioxide were calculated. The results of these analyses are shown in Table 3.

Table 3: Biogas composition after 7 days of fermentation

	Biogas composition	
	CH_4 (%)	**CO_2 (%)**
Control reactors (C1)	76 ± 3	24 ± 3
Test reactors (T1) (addition of 1 g VS of solid digester residue)	71 ± 3	29 ± 3

From Table 3 it can be seen that the percentage of methane in the biogas was comparable for the control reactors and the test reactors and was on average 74 ± 4.2 %. At the end of the fermentation experiment (after 14 days), the pH of the control reactors (C1) were 7.3 ± 0.02. The pH of the test reactors (T1) was 7.2 ± 0.01.

TEST 2: ADDITION OF 2 AND 3 G VS OF SOLID DIGESTER RESIDUE

In a second test series, the biogas production of the control reactors (C2) which were supplied with 0.6 g DM of raw substrate, was compared to the biogas production of the test reactors which were supplied with 0.6 g DM of raw substrate and 2 (T2) or 3 g VS (T3) of solid digester residue (2.44 g and 3.65 g DM respectively). The volumetric loading rate (Bv) of the control reactors (C2) on time zero was 1.5 g DM/L.d. The pH of the mixed liquor was 7.3 ± 0.1. The Bv of the test reactors T2 (2 g VS of solid digester residue) was 7.7 g DM/L.d. The average pH of the mixed liquor was 7.24 ± 0.03. The Bv of the test reactors T3 (3 g VS of solid digester residue) was 9 g DM/L and the average pH of the mixed liquor was 7.3 ± 0.1. The volumetric loading rates were calculated on the volume of mixed liquor taken from the main digester (400 mL). In Table 4, the characteristics of the mixed liquor taken from the main digester are shown. The solid digester residue supplied was the same as in the first experiment. The

characteristics are shown in Table 1. In Figure 6, the biogas production in function of time is depicted.

Table 4. Characteristics of the mixed liquor from the main digester used for the batch experiment

Parameter	Value	Unit
Dry matter (DM)	12.15	g/L
Ash content (AS)	4.05	g/L
Volatile solids (VS)	8.1	g/L
Kjeldahl Nitrogen	1454	mg/L
TAN	936	mg/L
TON	0	mg/L

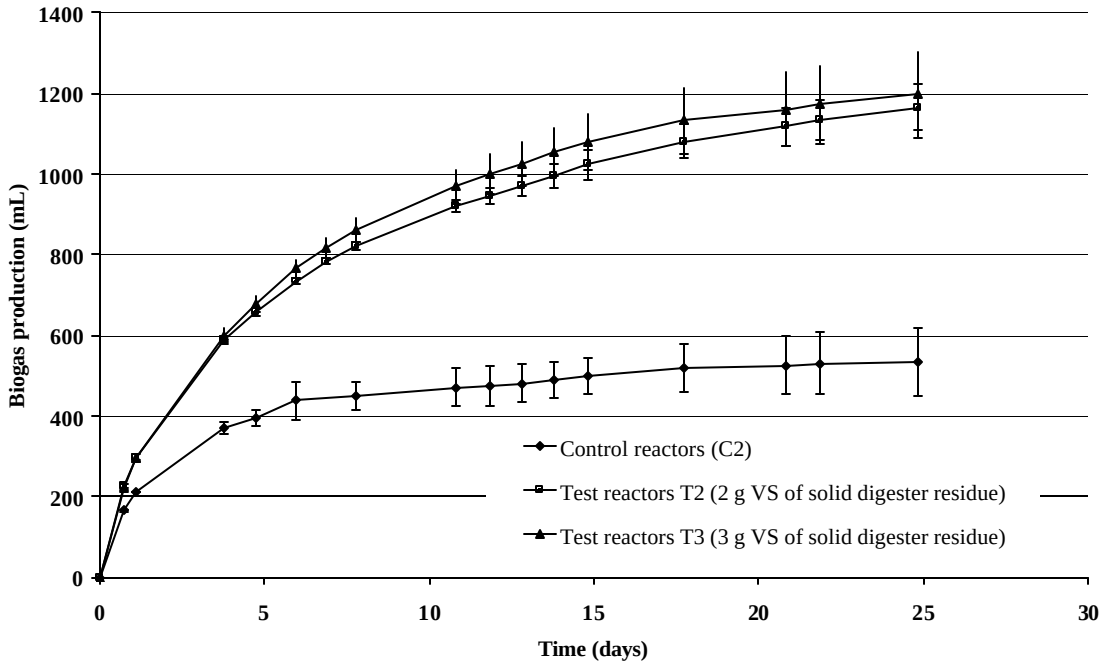

Figure 6: Biogas production (mL) in function of time (days) of the control reactors (C2) versus the test reactors T2 and T3

From Figure 6, it can be seen that the test reactors T2 and T3 showed a higher biogas yield compared to the control reactors (C2). After 6 days, the test reactors T2 and T3 had a biogas yield that was on average 68 % and 75 % higher than the control reactors (C2). After 14 days of fermentation, the test reactors T2 gave 104 % more biogas than the control samples (C2). The addition of 3 g VS solid material (T3) resulted in 116 % more biogas than the control samples (C2). There was no significant difference in biogas production between the test reactors T2 and T3, the addition of 2 and 3 g VS of solid material respectively. The batch fermentation test was stopped after 25 days.

After 25 days of fermentation, the biogas composition was determined and the percentage of methane and carbon dioxide were calculated. The results of these analyses are shown in Table 5. The pH of the mixed liquor was also measured and was 7.5 ± 0.01 for the control reactors (C2), 7.3 ± 0.05 for the test reactors T2 and 7.5 ± 0.1 for the test reactors T3.

Table 5: Biogas composition after 25 days of fermentation

	Biogas composition	
	CH_4 (%)	**CO_2 (%)**
Control reactors (C2)	82 ± 3	18 ± 3
Test reactors T2 (addition of 2 g VS of solid digester residue)	79 ± 2	21 ± 2
Test reactors T3 (addition of 3 g VS of solid digester residue)	78 ± 4	22 ± 4

From Table 5 it can be seen that the methane concentrations at the end of the batch experiment were quite comparable for the control reactors (C2) and the test reactors (T2 and T3) and that the average value was 80 ± 5.

SUMMARY OF THE BATCH FERMENTATION TESTS

In Table 6, a summary of the batch fermentation tests is shown. The extra biogas production after 6 days and 14 days was calculated and these results are also shown in Figure 7.

Table 6. Summary of the biogas production of the test reactors compared to the control reactors

	Control reactors C1	Test reactors T1	Control reactors C2	Test reactors T2	Test reactors T3
g VS of solid digester residue added	-	1 g	-	2 g	3 g
After 6 days					
mL biogas	503 ± 8	665 ± 86	438 ± 45	735 ± 9	766 ± 22
Extra biogas production*		32		68	75
After 14 days					
mL biogas	621 ± 30	902 ± 100	489 ± 44	996 ± 30	1054 ± 61
Extra biogas production*		45		104	116

* In comparison with the control reactors

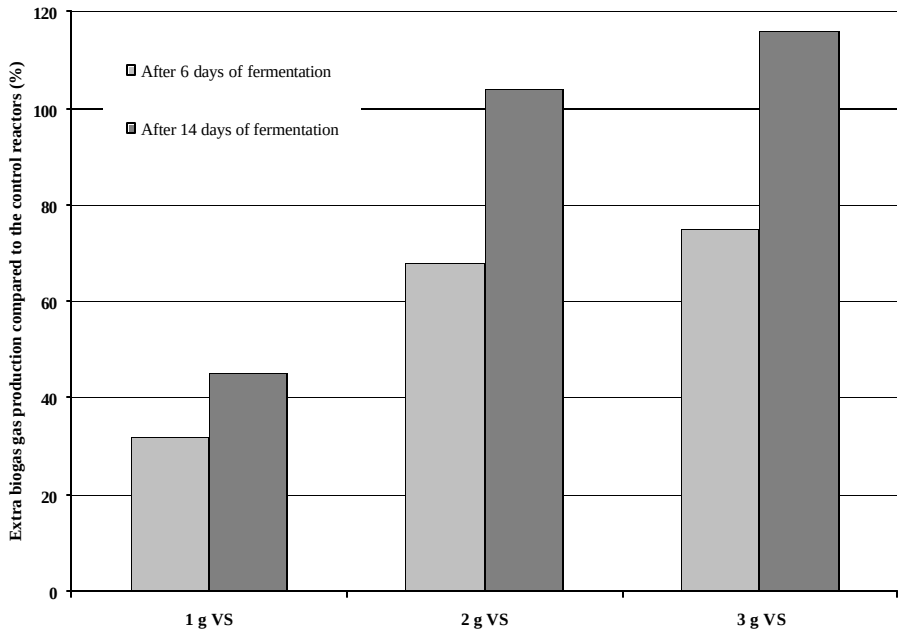

Figure 7. Extra biogas production of the test reactors in function of the amount of solid digester residue added

From Table 6 and Figure 7, it can be seen that the addition of 1 g VS of solid digester residue resulted in 32 % more biogas than the control reactor after 6 days and 45 % after 14 days. The addition of 2 g and 3 g VS of solid material resulted in 68 and 75 % more biogas after 6 days respectively. After 14 days, the extra biogas production was 104 % (addition of 2 g VS) and 116 % (addition of 3 g VS). When the added solid digester residue was increased from 1 g VS to 2 g VS, the extra biogas production after 6 days was more or less doubled (32 % versus 68 %). However, when the amount of solid digester residue added to the test reactors was increased from 1 to 3 g VS, the biogas production did not increase linear with the increase of the amount of solids added (32 % versus 75 %). It is possible that the difference between 2 and 3 g VS of solid digester residue is significant after a longer fermentation period than 25 days. Somewhere between the addition of 1 g and 2 g extra VS, there is a saturation of the conversion rate.

Low-load methanogenic reactor (4th closed loop experiment)

A second objective of this Technical Note was to make a mass balance (carbon and nitrogen) of the low-load methanogenic reactor using the exchanged solid digester residue that on the one hand has been treated in the Fibrobacter unit followed by the Subcritical unit and on the other hand in the Subcritical unit only. This is the 4th closed loop experiment.

The samples, received from Partner 4, were analysed for CODt, CODs, VFA and pH. The characteristics of the samples treated by anaerobic digestion (solid digester residue) followed by the Fibrobacter unit (Partner 2) and liquefaction unit (Partner 4) are shown in Table 7. The characteristics of the samples treated by anaerobic digestion (Partner 1, solid digester residue) followed by the liquefaction unit (Partner 4) are shown in Table 8.

Table 7. Characteristics of the samples treated by anaerobic digestion (solid digester residue) followed by the Fibrobacter unit (Partner 2) and liquefaction unit (Partner 4)

		Treatment (by Partner 4)			
Parameter	**Unit**	**Near critical**	**Subcritical**	**Near-critical with oxidant (H_2O_2)**	**Subcritical with oxidant (H_2O_2)**
CODt	mg/L	7310	**5378**	546	974
CODs	mg/L	4999	**2730**	622	721
pH		5.79	**5.47**	6.02	5.68
VFA	mg/L	124	**207**	197	180
acetic acid	mg/L	55	**133**	187	177
propionic acid	mg/L	11	**35**	5	2
isobutyric acid	mg/L	28	**4**	0	0
butyric acid	mg/L	10	**15**	2	1
isovaleric acid	mg/L	20	**12**	1	0
valeric acid	mg/L	0	**5**	0	0
isocapronic acid	mg/L	0	**0**	0	0
capronic acid	mg/L	0	**2**	1	0

Table 8. Characteristics of the samples treated by anaerobic digestion (solid digester residue) followed by the liquefaction unit (Partner 4)

		Treatment (by Partner 4)	
Parameter	**Unit**	**Near critical with oxidant (H2O2)**	**Subcritical with oxidant (H2O2)**
CODt	(mg/L)	421	520
CODs	(mg/L)	398	484
pH		6,81	6,53
VFA	(mg/L)	24	34
acetic acid	(mg/L)	15	26
propionic acid	(mg/L)	3	4
isobutyric acid	(mg/L)	0	0
butyric acid	(mg/L)		0
isovaleric acid	(mg/L)	6	4
valeric acid	(mg/L)	0	0
isocapronic acid	(mg/L)	0	0
capronic acid	(mg/L)	0	0

As can be seen from Table 7 and 8, the effluent of the near-critical liquefaction treatment showed the highest COD (both total and soluble) values, 7.3 g/L and 5 g/L respectively. Applying sub-critical liquefaction, the CODt was 26 % lower (5.4 g/L) and the CODs was 45 % lower (2.7 g/L). The treatments with oxidant (H_2O_2) resulted in an effluent which was much lower in CODt and CODs (< 1 g COD/L). Because the reactor volume of the biofilm reactor is 1.5 L and the available amount of treated effluent was around 0.8 L, the effluent should be diluted with tap water. This will result in COD values too low for fermentation in the biofilm reactor.

For the experiment with the biofilm reactor, the sample which has been treated by the Fibrobacter unit followed by the Subcritical liquefaction unit (Table 7) was chosen because this loop was already previously tested (see Technical Note 1.4 of MAP 1, p. 16-18). As can be seen from Table 7, the effluent after sub-critical liquefaction contains a relatively low amount of COD, namely 5.4 g CODt/L and 2.7 g CODs/L. During the experiment 0.78 L of

the mixed liquor could be used. Because the reactor volume was 1.5 L, one was obliged to add 0.72 L of tap water in order to make recycling possible. Consequently the effluent sample used in the biofilm experiment was only 52 % of the normal effluent and hence the volumetric loading rate at time 0 was 2.8 g CODt/L.d.

For a period of 8 days, the liquid was recycled over the fixed-bed biofilm reactor with an upstream velocity of 2 m/h. Parameters such as COD (both total and soluble), VFA, pH and biogas production were measured on a daily basis. Kjeldahl nitrogen, TAN (Total Ammoniacal Nitrogen) and TON (Total Oxidised Nitrogen) were measured at time 0 and after 4.8, 5.8, 6.7 and 8 days. The results are shown in Table 9 (general parameters), Table 10 (different compounds of VFA) and Figure 8.

Table 9. General parameters during 8 days recycling of the effluent of the sub-critical liquefaction over the fixed-bed biofilm reactor

Time (d)	0	0.7	1.7	4.8	5.8	6.7	8
CODt (mg/L)	2876	2818	2530	1684	1658	1246	1238
CODs (mg/L)	1488	1932	1684	1334	1175	872	893
VFA (mg/L)	68	320	230	163	138	-	-
pH	6.34	7.18	6.96	6.94	6.99	6.92	-
Cumulative biogas production (mL)	0	0	0	129	150	196	223

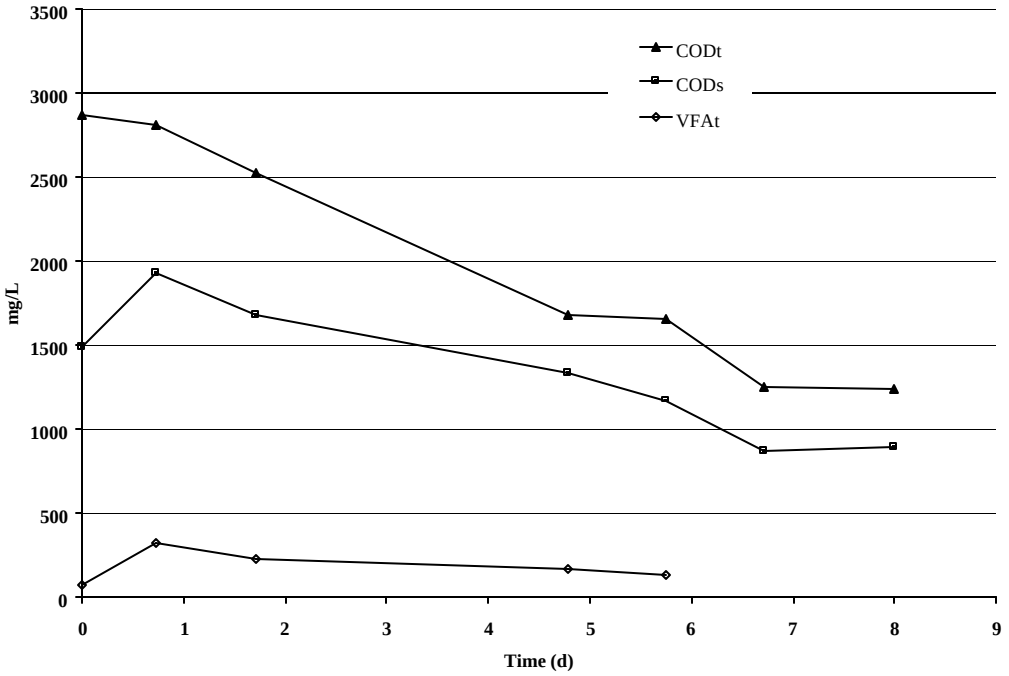

Figure 8. Evolution of the CODt, CODs, and VFA_t during 8 days recycling of the effluent of the sub-critical liquefaction over the fixed-bed biofilm reactor

Table 10. Different compounds of VFA during 8 days recycling of the effluent of the sub-critical liquefaction over the fixed-bed biofilm reactor

Time (d)	0	0.7	1.7	4.8	5.8	8
Acetate (mg/L)	42	287	187	122	105	0
Propionate (mg/L)	13	18	23	24	18	0
Isobutyric acid (mg/L)	1	3	5	5	4	3
Butyric acid (mg/L)	5	3	5	0	0	5
Isovaleric acid (mg/L)	4	6	8	10	9	9
Valeric acid (mg/L)	0	1	1	1	1	5
Isocapric acid (mg/L)	0	0	0	0	0	0
Capric acid (mg/L)	2	2	1	1	0	3
Total (mg/L)	**67**	**320**	**230**	**163**	**137**	**25**

From Table 9 and Figure 8, it can be seen that 30 % of the total COD was solubilised after 1 day of recirculation. At the same time the soluble COD increased from 1.4 to 1.9 g/L and the VFA content increased to 320 mg/L. After 8 days of recirculation, the total COD content decreased with 57 %. The soluble COD removal after 8 days was 40 %. The biogas produced after 8 days of recirculation was 223 mL. This value is however underestimated because of several reasons. Firstly, an overpressure in the reactor is needed before the biogas can be transferred to the gas column. In case of a low volumetric loading rate where a small amount of biogas is produced, this causes a bias. Secondly, the biogas produced is partly entrapped within the polyethylene wheels. Most of the biogas can be released from the rings by manually shaking the reactor but some gas bubbles remain entrapped in the matrix of rings.

If these results are compared to the results of the previous biofilm experiment with effluent treated with sub-critical liquefaction (see Technical Note 1.4, MAP 1), where 50 % removal of the CODt was calculated after 4 days of recirculation, it can be concluded that the removal rate was a bit lower this time (41 % after 4.8 days). This can be explained by the fact that a new biofilm reactor was started for this experiment. It is possible that the amount of biofilm was less than in the biofilm reactor used for the experiment.

At the end of the test, the gas composition was measured and is depicted in Table 11.

Table 11. Biogas composition after 8 days of recirculation

	CH_4 (%)	CO_2 (%)
Biofilm reactor	67 ± 2	33 ± 2

As can be seen from Table 11, the biogas contained 67 % methane after 8 days of recirculation.

During the liquid recycling, the nitrogen compounds Kjeldahl N, TAN (Total Ammoniacal Nitrogen) and TON (Total Oxidised Nitrogen) were measured in function of time. The results are presented in Table 12.

Table 12. Different N compounds during 8 days recycling of the effluent of the sub-critical liquefaction over the fixed-bed biofilm reactor

Time (d)	0	4.8	5.8	6.7	8
Kj-N (mg/L)	112	96	109	104	103
TAN (mg N/L)	17	6	18	18	34
TON (mg N/L)	1	0	0	0	0
Total N (mg N/L)	130	102	127	122	137

From Table 2 it can be seen that there was no significant mineralization of Kjeldahl nitrogen into ammonium. The Kjeldahl nitrogen was more or less constant. The TAN concentrations were always low (< 34 mg N/L). The TON concentrations (nitrite and nitrate) were zero. The nitrogen compounds did not change significant during the anaerobic digestion in the biofilm methanogenic reactor.

Recommendations regarding the HACCP protocol

On the latest progress meeting, the choice of a chemical compound was discussed. The main objective of the chemical HACCP proof is to evaluate the effect of a chemical compound on the ecosystem. To convince people that we have a system that is chemically HACCP proof; we should look for a chemical compound that is inert to biological degradation but possibly susceptible to physico-chemical changes during the physico-chemical treatments investigated. Therefore, the most resistant chemical compounds, namely chlorinated compounds (PCB's) were proposed. Because it was not clear whether these tests could be performed, considering the permission of a laboratory to work with these kinds of chemicals, there was also a need to look for alternative aromatic and/or chlorinated compounds.

Partner 4 proposed to investigate the effect of sub-critical liquefaction on naphthalene because of the permission of their laboratory to work with this compound. The chemical structure is shown in Figure 9. The results of these tests will be presented in Technical Note 6.4.

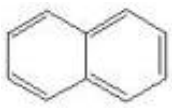

Figure 9. Chemical structure of naphthalene

Naphthalene is a bicyclic aromatic compound that has wide industrial and commercial applications. It is used as the starting material for the synthesis of other compounds, as a moth repellent, soil fumigant and lavatory deodorant. Most exposure occurs through low dose chronic inhalation, dermal contact or ingestion through the food chain. The lungs and eyes appear to be most susceptible to toxicity, although biochemical markers of toxicity can be demonstrated in other tissues, such as the kidney, brain and liver. In addition to lens opacification (cataracts) and histological changes associated with pneumotoxicity, other biomarkers of toxic effects include glutathione depletion, lipid peroxidation, DNA fragmentation and the production of the active oxygen species as superoxide anion and hydroxyl radical. In addition, the urinary excretion of lipid metabolites occurs. A role for the tumor suppressor gene p53 has been demonstrated. Toxic manifestations of naphthalene are associated with its oxidative metabolism to various products including quinones. The ability to protect against the toxic effects of naphthalene by using various antioxidants and free radical scavengers has been demonstrated. Studies have been conducted with vitamin E, vitamin E succinate, melatonin, curcumin, various L-cysteine prodrugs, several aldose reductase inhibitors and spin-trapping agents. The ability to prevent the toxic manifestations of naphthalene is dependent on the pharmacokinetic properties of the agents, which have been studied. The appropriate selection of chemoprotectants can be useful in preventing naphthalene toxicity (Stohs et al., 2002).

As a second model compound, the UGent proposes **Triclosan** as the chemical compound to work with. **Triclosan** (2,4,4'-trichloro-2'-hydroxydiphenyl ether, see Figure 10 for the chemical structure) is a nonionic, broad spectrum, antimicrobial agent that, because of its favorable safety profile, has been incorporated into a variety of many personal care products,

including deodorant soaps, underarm deodorants, shower gels, and health care personnel handwashes. Triclosan exhibits a moderate degree of substantivity to the skin, and, in many products, it imparts a remnant antimicrobial effect. Although direct contact with the material under exaggerated exposure conditions causes dermal irritation in laboratory animals, it has only rarely been associated with skin irritation or sensitization in human being in formulated products. Acute, subacute/subchronic, and chronic toxicity profiles have been established to determine that triclosan is neither an acute oral toxicant nor that it acts as a carcinogen, mutagen, or teratogen. A new application for triclosan is in oral dentifrices for plaque control. Currently under investigation in the United States, it is approved for oral care application in Canada and many European countries (Bhargava and Leonard, 1996).

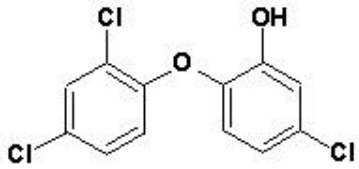

Figure 10. Chemical structure of Triclosan (2,4,4'-trichloro-2'-hydroxydiphenyl ether)

4 CONCLUSIONS AND FUTURE PERSPECTIVES

This work showed that the addition of solid digester residue (1, 2 and 3 g VS) from the effluent of the main mesophilic digester to mixed liquor (400 mL) of this digester gave an extra biogas production. The extra biogas production was depending on the amount of VS added and the fermentation time. After 6 days there was 32, 68 and 75 % extra biogas production for the addition of 1, 2 and 3 g of VS respectively. After 14 days there was 45, 104 and 116 % extra biogas production for the addition of 1, 2 and 3 g of VS respectively.

The fermentation of the returned effluent treated by Partner 1 (anaerobic digestion), Partner 2 (Fibrobacter) and Partner 4 (sub-critical liquefaction) in a methanogenic biofilm reactor resulted in a decrease of CODt and CODs and gave a biogas yield. However, the biogas yield

was lower as described in a previous technical note due to the start up of another reactor and possibly less amount of active biomass and to an underestimation of the captured biogas. There was no significant mineralization of Kjeldahl nitrogen into ammonium nitrogen. Also nitrification did not occur in the biofilm reactor. The COD of the samples treated by Partner 4 with oxidant (H_2O_2) were however too low for fermentation in the biofilm reactor. These samples can be used in the future to do toxicity batch experiments to verify whether the effluent of the liquefaction unit produces compounds toxic for methanogenesis.

For WP 1.7, batch experiments will be performed to optimise both reactor configurations: biogas yield, carrier material, solid retention system… Furthermore, there will be a substrate exchange with Partner 2 and Partner 4. The returned material will be used for batch experiments.

5 REFERENCES

Bhargava HN, Leonard PA (1996) Triclosan: applications and safety. *American Journal of Infection Control* 24 (3): 209-18.

Stohs SJ, Ohia S, Bagchi D (2002) Naphthalene toxicity and antioxidant nutrients. *Toxicology* 180 (1): 97-105