M E L i S S A

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

Memorandum of Understanding TOS-MCT/2002/3161/In/CL

TECHNICAL NOTE: 1.7

RESULTS OF BATCH EXPERIMENTS ON THE OPTIMISATION OF BOTH UNITS

(PROJECT: A TOTAL CONVERTING AN BIOSAFE LIQUEFACTION COMPARTMENT FOR MELISSA)

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reference/*réference*
issue/*édition* 1
revision/*révision* 0
date of issue/*date d'édition* 30 March 2005
status/*état* Draft
Document type/*type dedocument* Technical Note
Distribution/*distribution*

CONFIDENTIAL DOCUMENT

A P P R O V A L

Title *titre*	Results of batch experiments on the optimisation of both units	issue *issue*	1	revision *revision*	0

author *auteur*	Jeroen Bursens	date *date*	15/10/ 04

approved by *approuvé by*	date *date*

C H A N G E L O G

reason for change / *raison du changement*	issue / *issue*	revision / *revision*	date / *date*

C H A N G E R E C O R D

Issue: 1 Revision: 0

reason for change / *raison du changement*	page(s) / *page(s)*	paragraph(s) / *paragraph(s)*

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1 INTRODUCTION AND OBJECTIVES

The main objectives of the third technical note of the second phase of this project were on the optimization of both methanogenic reactor configurations. To verify the influence of a longer solid retention time of the high-load reactor on the biogas production, additional batch experiments were performed with mixed liquor of the high-load methanogenic reactor. This mixed liquor was supplied with different amounts (0; 1, 1.3; 1.6 and 2 g VS) of pre-digested material (solid digester residue from the high-load methanogenic reactor). Secondly, a fifth closed loop experiment was performed with the methanogenic biofilm reactor treating the material from the Fibrobacter and subsequently the subcritical liquefaction unit. Additionally, toxicity experiments were performed on several effluents of the liquefaction unit to verify whether compounds toxic for methanogenesis were produced during this treatment. The tasks described for this TN are given below:

INPUTS

- Operational high-load and low-load methanogenesis unit
- Required analysis equipment for COD-analysis, DM-analysis, ammonia analysis

Tasks included

- Batch experiments with raw substrate to optimise both reactor configurations: biogas yield, carrier material, solid retention time, …
- Substrate exchange with Partner 2 and Partner 4 (solid digester residue)
- Batch experiments with supernatant from Partner 2 and hydrolysate from Partner 4

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.

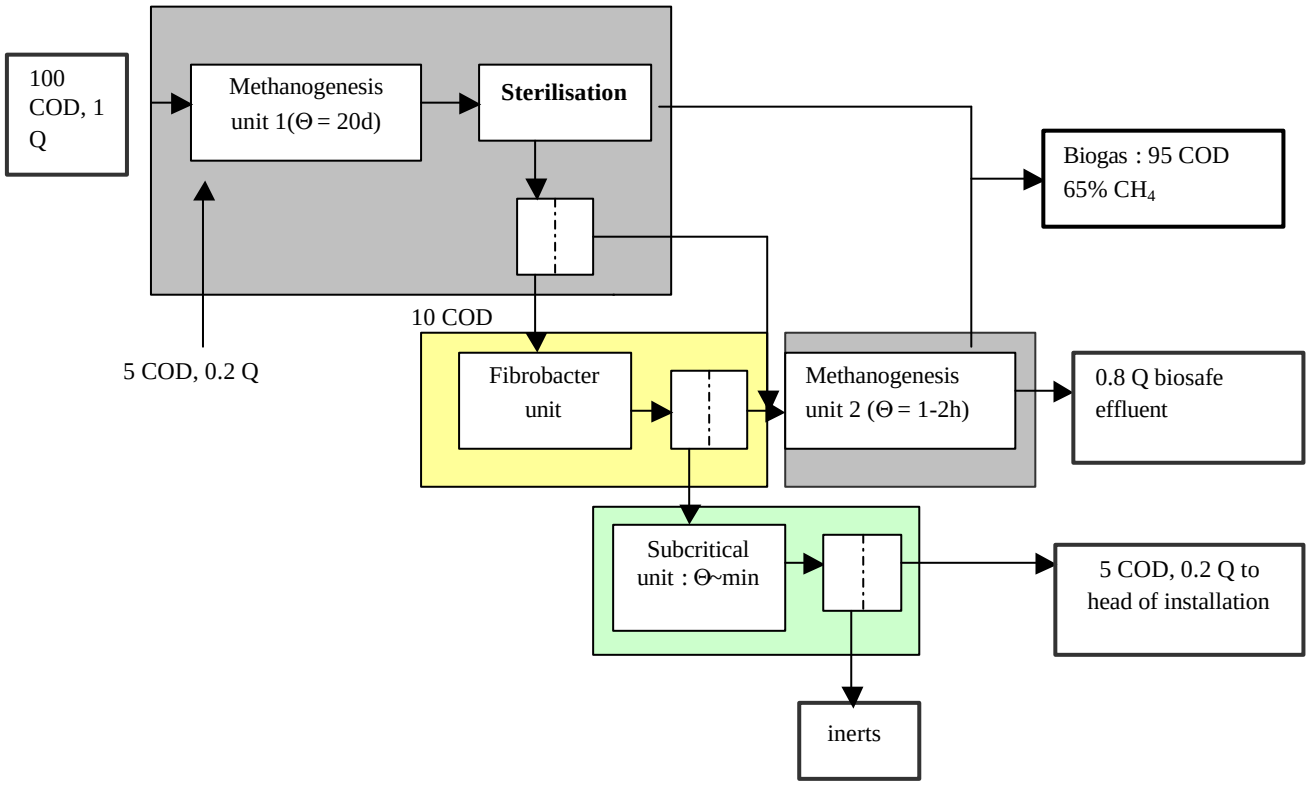

Figure 1: Conceptual scheme of a total converting and biosafe liquefaction compartment for MELiSSA

2 MATERIALS AND METHODS

Substrate composition and preparation of residue (5th 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

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 was set at 7.5 L.

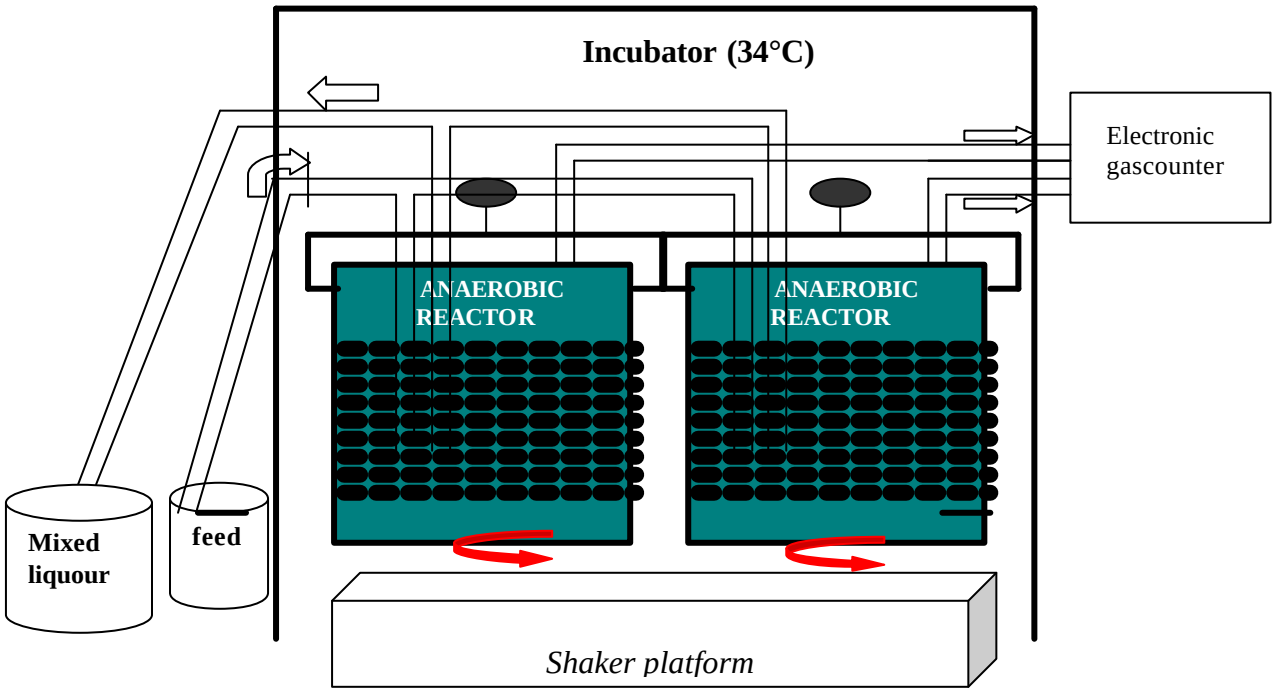

Figure 2: Scheme of the 2 mesophilic digesters for the fermentation and subsequent distribution of the digester residues (approximately 400 + 100 gram DM)

Experimental set-up of the batch 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 fibres from previous fermentations. The liquid phase consists mainly of soluble biopolymers. The experiments were run over a period of 26 days.

The effluent of the main methanogenic reactors was collected and centrifuged (5 min., 7000 rpm) to separate the solid fraction. This solid fraction (7.6 % VS) was added in different amounts (0, 1, 1.3, 1.6 and 2 g of VS) to the batch fermentation tests to evaluate the extra biogas production.

Bottles 1 and 2 were the control samples which were supplied with the raw substrate only (approx. 0.5 g DM). Bottles 3 and 4 were supplied with the raw substrate (approx. 0.51 g DM) and 1 g of VS solid digester residue (corresponding with 13.1 g wet solid digester residue). Bottles 5 and 6 were supplied with the raw substrate (approx. 0.51 g DM) and 1.3 g of VS solid digester residue (corresponding with 17 g wet solid digester residue). Bottles 7 and 8 were supplied with the raw substrate (approx. 0.51 g DM) and 1.6 g of VS solid digester residue (corresponding with 21 g wet solid digester residue). Bottles 9 and 10 were supplied with the raw substrate (approx. 0.51 g DM) and 2 g of VS solid digester residue (corresponding with 26.2 g wet solid digester residue). The volume of biogas was monitored for each bottle. The pH was measured at time zero, after 2 days of incubation and at the end of the test period (after 26 days of incubation). After 26 days of incubation, the biogas was analysed for methane and carbon dioxide. Samples of the mixed liquor were analysed for the dry matter and volatile solids content.

All fermentation trials were performed in duplicate to check for the reproducibility. The reactors were shaken manually three to four times per day.

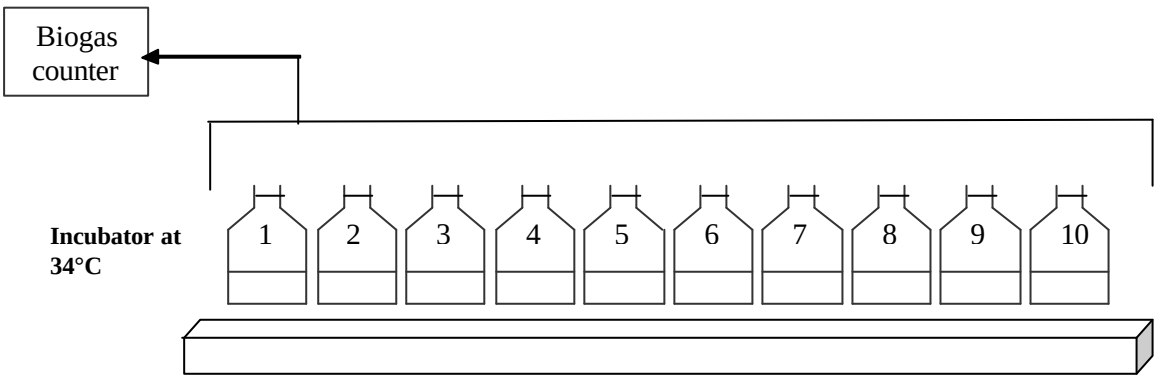

Figure 3: Experimental set-up for batch fermentation tests with raw substrate and raw substrate supplied with 0, 1, 1.3, 1.6 and 2 g VS of solid digester residue

Experimental set-up of the low-load methanogenesis unit

For this experiment, the same reactor set-up as described in the previous Technical Note (TN 1.6) was used. The fixed-bed biofilm reactor had a volume of 1.5 L. The reactor was filled

with 1 dm³ of polyethylene wheels (852 rings in total) (Kaldnes), with a total surface area of 800 m²/m³ and a protected surface area of 500 m²/m³. To initiate the biofilm formation, 1 L of tap water and 500 mL of sludge from the CSTR were added. Subsequently the liquid was continuously recycled at an up flow velocity of 2 m/h and at daily basis between 0.67 and 1.3 g COD/L.day was dosed during a period of 10 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.

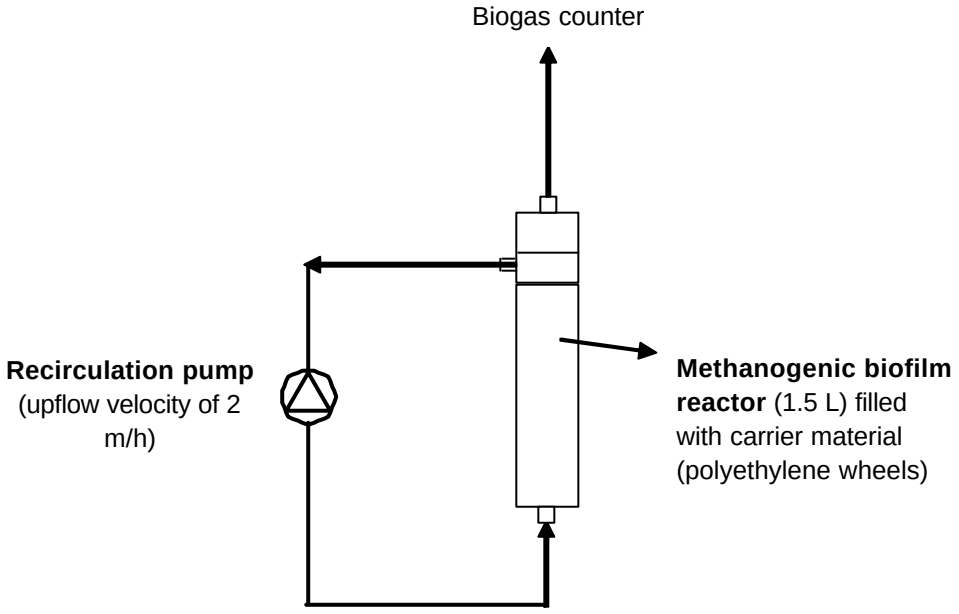

Figure 4. Schematic presentation of the reactor set-up of the low-load methanogenesis unit.

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. This was done in two subsequently performed experiments. In a first experiment, 0.875 L of the returned effluent was diluted to 1.5 L before it was fed to the biofilm reactor. In a second experiment, the recirculation point of the fixed-bed biofilm reactor was lowered to decrease the volume of the reactor to 1 L. 0.8 L of the returned effluent

was diluted to 1 L. 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 regularly basis, during a total period of 7 to 8 days.

Experimental set-up of the toxicity assays

The inoculum used for the toxicity assays was a highly active anaerobic granular sludge, originating from a full-scale UASB reactor of a paper-processing factory. In each reactor of 1 L, 200 ml of seed sludge (about 137 g dry weight sludge/L) was supplied with substrate (different liquefaction effluents with a volume between 570 – 762.5 mL). To each reactor, tap water was added to a total reactor volume of 1 L. In a first control test (C1), only tap water (800 mL) was added to the seed sludge (without substrate) to quantify the background biogas production of the inoculum. To demonstrate the methanogenic activity of the seed sludge, a second control test (C2) with the same amount of seed sludge and the addition of 2.5 g glucose and 3.2 g sodium acetate (together 5 g COD) in 800 ml tap-water (total reactor volume of 1 L), was performed. The other test reactors were T1 to T5 (substrate see Table 7). At time zero, after 2, 3.2, 7 (C1, T2, T3, T4 and T5) and 13 days (C2 and T1), a sample of the supernatant was taken and analysed for CODt (total COD), CODs (soluble COD) and VFA (volatile fatty acids). The biogas production was monitored and the pH of the mixed liquor was measured regularly.

When the biogas production had reached a plateau, 1 g glucose (easy biodegradable compound) was added to the different test reactors and to the control reactors to investigate whether toxic compounds were inhibiting the biogas production. The CODs and pH was measured at time 0' and at the end of the fermentation test.

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 the 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)	9.6	m%
Ash content (AS)	2	m%
Volatile solids (VS)	7.6	m%

The tests were started with 400 mL mixed liquor of the main mesophilic digester. One day before the actual test, 0.51 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.

In TN 1.6 it was found that there was no difference in extra biogas production when 2 or 3 g VS of solid digester residue was added to mixed liquor of the main mesophilic digester. In this work package, the amount of solid digester residue was varied between 1 and 2 g VS. In the experiment, the biogas production of the control reactors (C1) which were supplied with 0.51 g DM of raw substrate, was compared to the biogas production of the test reactors T1, T2, T3 and T4 which were supplied with 0.51 g DM of raw substrate and 1, 1.3, 1.6 and 2 g VS of solid digester residue respectively. This corresponds with 1.3, 1.7, 2 and 2.6 g DM. The volumetric loading rate (Bv) of the control reactors (C1) on time zero was 1.3 g DM/L.d; the pH of the mixed liquor was 7.40 ± 0.04. The Bv of the test reactors T1, T2, T3 and T4 were 4.5, 5.5, 6.3 and 7.8 g DM/L.d respectively; the corresponding pH of the mixed liquor was 7.43 ± 0.02, 7.42 ± 0.04, 7.41 ± 0.02, 7.48 ± 0.08. 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 mesophilic 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)	10.5	g/L
Ash content (AS)	2.5	g/L
Volatile solids (VS)	8	g/L
Kjeldahl Nitrogen	2440	mg/L
TAN*	797	mg/L
TON**	0	mg/L

* **Total Ammoniacal Nitrogen**

** **Total Oxidized Nitrogen (nitrite and nitrate)**

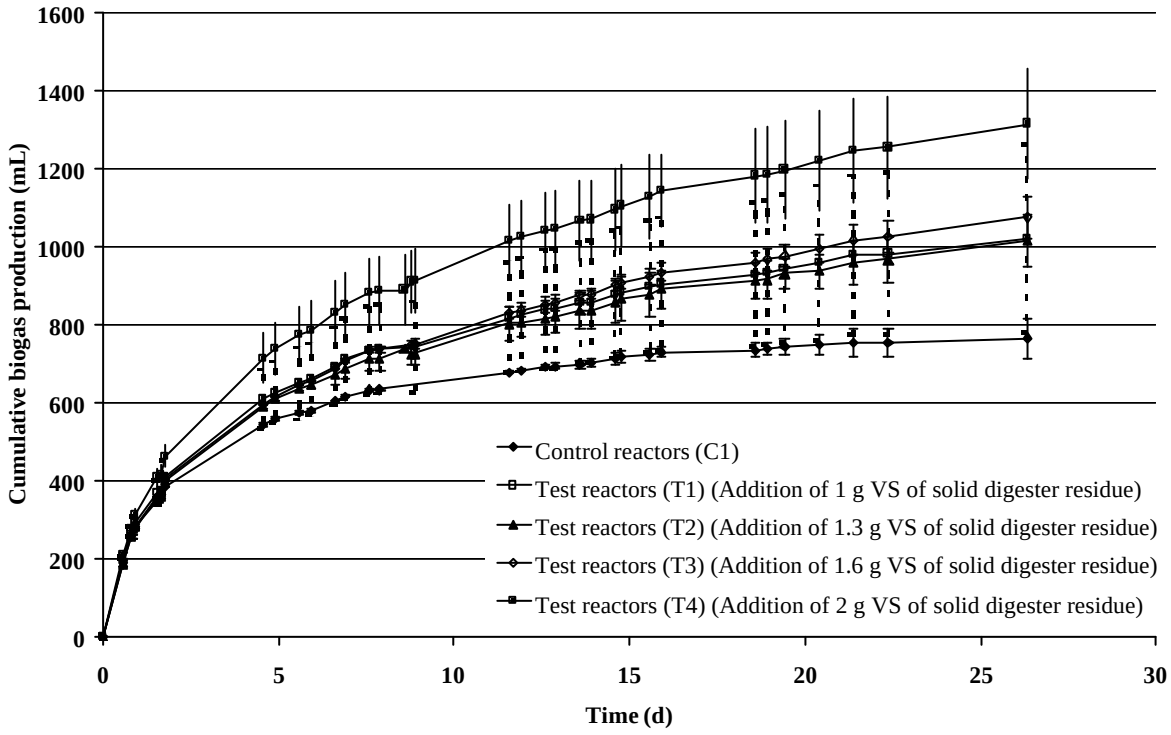

Figure 5: Biogas production (mL) in function of time (days) of the control reactors (C1) versus the test reactors (T1, T2, T3 and T4) (addition of 1, 1.3, 1.6 and 2 g VS solid digester residue). The standard deviation of T1 is marked in dotted lines.

From Figure 5, it can be seen that the addition of an extra 1, 1.3, 1.6 or 2 g VS of solid digester residue to the mixed liquor resulted in a higher biogas yield compared to the control reactors (C1), which were not supplied with solid digester residue. The higher volumetric loading rates of the test reactors at time zero (4.5, 5.5, 6.3 and 7.8 g DM/L.d) were not inhibitory because the biogas production started immediately without a lag phase.

Considering the fact that 1 g VS of solid digester residue equals 1.3 g DM (see Table 1) and hence a maximum production of 0.65 L of biogas, one can calculate the percentage of the added solid digester residue converted into biogas. The results of these calculations, after 15 days and after 26 days of fermentation, are shown in Table 3.

Table 3. Percentage and g of the added solid digester residue converted into biogas after 15 and 26 days of incubation

	After 15 days		After 26 days	
	%	**g**	**%**	**g**
Test reactors T1 (1 g VS of SDR*)	25	0.25	39	0.39
Test reactors T2 (1.3 g VS of SDR)	17	0.22	29	0.38
Test reactors T3 (1.6 g VS of SDR)	18	0.29	29.5	0.47
Test reactors T4 (2 g VS of SDR)	30	0.6	42	0.84

* SDR = solid digester residue

From Table 3, it can be concluded that 30 - 40 % of the added solid digester residue can be further converted into biogas after 26 days of extra incubation. This means that the solid retention time in the main mesophilic digester should be at least 40 days (15 days + 26 days).

A longer residence time would not be beneficial since the figure shows that at about 25 days a plateau-phase is reached. A significant decrease in microbial activity at this point is not expected. As a matter of fact we would only expect such a decreased activity would be expected only when the plateau-phase is maintained for an extended period.

After 26 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

4. Samples of the mixed liquor were analysed for dry matter and volatile solids content. The results of these analyses are depicted in Table 5.

Table 4: Biogas composition after 26 days of fermentation

	Biogas composition	
	CH_4 (%)	**CO_2 (%)**
Control reactors (C1)	73	27
Test reactors T1 (addition of 1 g VS of solid digester residue)	78 ± 1	22 ± 1
Test reactors T2 (addition of 1.3 g VS of solid digester residue)	74 ± 1	26 ± 1
Test reactors T3 (addition of 1.6 g VS of solid digester residue)	74 ± 1	26 ± 1
Test reactors T4 (addition of 2 g VS of solid digester residue)	73 ± 1	27 ± 1

Table 5: Mixed liquor composition after 26 days of fermentation

	Dry matter	Volatile solids	Unit
Control reactors (C1)	13 ± 2	9 ± 2	g/L
Test reactors T1 (addition of 1 g VS of solid digester residue)	15 ± 1	11 ± 1	g/L
Test reactors T2 (addition of 1.3 g VS of solid digester residue)	14 ± 1	10 ± 1	g/L
Test reactors T3 (addition of 1.6 g VS of solid digester residue)	14 ± 1	10 ± 1	g/L
Test reactors T4 (addition of 2 g VS of solid digester residue)	17 ± 2	12 ± 2	g/L

From Table 4 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 ± 2 %. At the end of the fermentation experiment (after 26 days), the pH of the control reactors (C1) were 7.38 ± 0.05. The pH of the test reactors T1, T2, T3 and T4 were 7.26 ± 0.06, 7.23 ± 0.09, 7.18 ± 0.04 and 7.14 ± 0.01. From Table 5 it can be concluded that the dry matter and volatile solids content were more or less the same in every reactor.

Low-load methanogenic reactor (5th 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 has been treated in the Fibrobacter unit followed by the Subcritical unit. This is the 5th closed loop experiment.

The effluent of the subcritical liquefaction test, received from Partner 4, was analysed for pH, COD_t, COD_s, Kjeldahl – N, TAN, TON and VFA. The characteristics are presented in Table 6.

Table 6. Characteristics of the sample received from Partner 4 and treated by anaerobic digestion (solid digester residue) followed by the Fibrobacter unit (Partner 2) and liquefaction unit (Partner 4)

Parameter	Unit	Sample
pH	-	6.6 ± 0.1
CODt	mg/L	2692 ± 439
CODs	mg/L	1099 ± 42
Kj-N	mg/L	98
TAN	mg/L	23
TON	mg/L	0
VFA	mg/L	98
acetic acid	mg/L	46
propionic acid	mg/L	9
isobutyric acid	mg/L	13
butyric acid	mg/L	0
isovaleric acid	mg/L	10
valeric acid	mg/L	8
isocapronic acid	mg/L	5
capronic acid	mg/L	7

As can be seen from Table 6, the sample had a total COD value of about 2.7 g/L. The soluble COD was more or less 1.1 g/L. Consequently, 41 % of the COD was present in solution. These COD values are low in comparison with the subcritical sample received and described

in Technical Note 1.6 (Table 7, subcritical, 5.4 g CODt/L and 2.7 g CODs/L), although the same liquefaction treatment was applied. The volatile fatty acids concentration was 98 mg/L.

Before the experiment was started, the recirculation point of the fixed-bed reactor set-up was lowered so that the reactor volume was 1 L. 0.8 L of sample (received from Partner 4) was diluted to 1 L with tap water. Hence, the effluent sample used in the biofilm experiment was 80 % of the normal effluent. The volumetric loading rate at time 0 was 2.4 g COD/L.d. Prior to the experiment, the pH of the effluent was increased from 6.5 to 8 with NaOH. For a period of 7 days, the liquid was recycled over the fixed-bed biofilm reactor with an upstream velocity of 2 m/h. Parameters such as pH, COD (both total and soluble), VFA, Kjeldahl nitrogen, TAN, TON and biogas production were measured regularly. The results are shown in Table 7.

Table 7. Results of the fixed-bed biofilm reactor experiment with effluent of the sub-critical liquefaction unit.

Time (d)	0	1	2	6	7
pH	8.15	6.74 ↑ 7.88	7.98	-	7.19
CODt (mg/L)	1878	-	-	-	1066
CODs (mg/L)	732	799	-	-	366
VFA (mg/L)	49*	83**	-	-	140***
Kjeldahl – N (mg N/L)	103	-	-	-	107
TAN (mg N/L)	20	-	-	-	52
TON (mg N/L)	5	-	-	-	13
Cumulative biogas production (mL)	0	43	45	70	95

* acetic acid

** 73 mg/L acetic acid and 10 mg/L propionic acid

*** 21 mg/L propionic acid, 25 mg/L isobutyric acid, 20 mg/L butyric acid, 21 mg/L isovaleric acid, 21 mg/L valeric acid, 16 mg/L isocapronic acid, 16 mg/L capronic acid

Data of both non-diluted and diluted effluent are not compatible with respect to VFA concentration. One would expect (based on the 98 mg/L VFA of the non-diluted effluent) a concentration of about 78 mg/L. Only 49 mg/L was measured and only acetic acid. Moreover

the concentration of acetic acid based on the non-diluted effluent would have to be about 36 mg/L). It doesn't seem plausible that other VFA were below detection limit. The only explanation is that there was an error in the VFA measurement.

From Table 7, it is observed that after 1 day of recirculation, the CODs and VFA concentration increased slightly. This can be explained by solubilisation of COD in suspension into soluble COD. After one day, a biogas volume of 43mL was measured. Hence, there was no inhibition in biogas production. Probably there was, next to solubilisation of organic matter, also anaerobic digestion of organic matter into biogas. After 7 days of recirculation, the COD soluble decreased to 366 mg/L. This means a 50 % decrease compared to the CODs value at time 0. The theoretical biogas production, considering the fact that 1 g COD removal results in 0.5 L biogas with a biogas composition of 70 % CH_4 and 30 % CO_2, was 183 mL. The measured biogas volume was 95 mL. This value is 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 because the reactor was regularly opened to take sample and to measure the pH. 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. The composition of the biogas was determined after 1 day and at the end of the test. The results are presented in Table 9.

Table 8. Biogas composition after 1 day of recirculation and at the end of the test

	t = 1 d	t = 7 d
CH_4 (%)	62 ± 1.4	55 ± 0.7
CO_2 (%)	38 ± 1.4	46 ± 0.7

From Table 8, it can be seen that the biogas composition at the end of the test was 55 % methane and 46 % CO_2. Overall, it can be concluded that 50 % of the COD present in the effluent of the liquefaction unit was converted into biogas. This value is comparable to the

results described in TN 1.4 (41 % COD removal after 4 days) and TN 1.6 (41 % removal after 5 days).

Concerning the nitrogen balance, it can be concluded that the Kjeldahl nitrogen remained constant during the experiment. The TAN concentration increased slightly and the TON concentration did not vary significant. Overall, it can be concluded that the nitrogen compounds did not change significantly during the anaerobic digestion in the fixed-bed biofilm reactor.

Toxicity assay on the returned samples of Partner 4

The samples, received from Partner 4 (WP 1.6), were analysed for CODt, CODs, VFA and pH. The characteristics of these samples (T1 to T5) treated by anaerobic digestion (solid digester residue) followed by the Fibrobacter unit (Partner 2) and liquefaction unit (Partner 4) (T1, T2 and T3) or anaerobic digestion (solid digester residue) followed by the liquefaction unit (Partner 4) (T4 and T5) are shown in Table 9 (see also Technical Note 1.6 for detailed VFA analysis).

Table 9. Characteristics of the samples treated by anaerobic digestion (solid digester residue) followed by the Fibrobacter unit (Partner 2) and liquefaction unit (Partner 4) or the liquefaction unit (Partner 4) only

		Treatment by Partner 2 and 4			Treatment by Partner 4	
Parameter	**Unit**	**Near critical**	**Near-critical with oxidant (H_2O_2)**	**Subcritical with oxidant (H_2O_2)**	**Near critical with oxidant (H2O2)**	**Subcritical with oxidant (H2O2)**
		T1	**T2**	**T3**	**T4**	**T5**
CODt	mg/L	7310	546	974	421	520
CODs	mg/L	4999	622	721	398	484
pH		5.79	6.02	5.68	6,81	6,53
VFA	mg/L	124	197	180	24	34
Volume	L	0.763	0.738	0.763	0.575	0.570

From Table 9, it can be seen that 68 % of the total COD was present as CODs in effluent T1. For effluent T2, T3, T4 and T5, the CODs was 100 %, 74 %, 95 % and 93 % of the total COD respectively. As discussed in Technical Note 1.6, it was not possible to treat these effluents in the fixed bed biofilm reactor considering the low amount of CODt and CODs in the samples and considering the amount of effluent available (below 0.8 L) and the reactor volume of the fixed bed biofilm reactor (1.5 L). Therefore, these effluents were used as a substrate in a toxicity assay.

As described in the Materials & Methods, the effluents (T1 to T5) were supplied to a highly active anaerobic granular sludge. Based on the COD analysis of the effluent samples as such (Table 9) and the amount of effluent added to a test reactor (Table 9), one can calculate the theoretical amount of COD (mg) added to the reactor. The theoretical COD (mg) is compared with the measured COD (mg) at time 0 (supernatant reactor) in Table 10.

Table 10. Comparison of the theoretical and measured CODt and CODs of the different reactors.

	Theoretical		Measured at time 0	
Reactor	**CODt (mg)**	**CODs (mg)**	**CODt (mg)**	**CODs (mg)**
C1	0	0	175	98
C2	5000	5000	5467	5043
T1	5578	3814	5797	3571
T2	403	459	709	511
T3	743	550	872	669
T4	242	229	573	326
T5	296	276	253	155

As can be seen from Table 10, the seed sludge had a background CODs of approximately 100 mg COD/L. The measured CODt values are always slightly higher than the theoretical CODt since these analyses were performed on the supernatant of the reactor. Hence, small floating sludge particles can result in higher CODt values than expected. The theoretical CODs values approach the measured CODs values quite well (taken into account the background CODs of the inoculum and an error of 10 % on a COD analysis).

Control reactor 1 (C1) and test reactors T2, T3, T4 and T5 were followed up for 7 days. Because of the higher COD load, control reactor 2 (C2) and test reactor T1 were continued for 13 days. The cumulative biogas production of the reactors is depicted in Figure 6. The results of the CODt and CODs analysis and the cumulative biogas production are shown in Table 9.

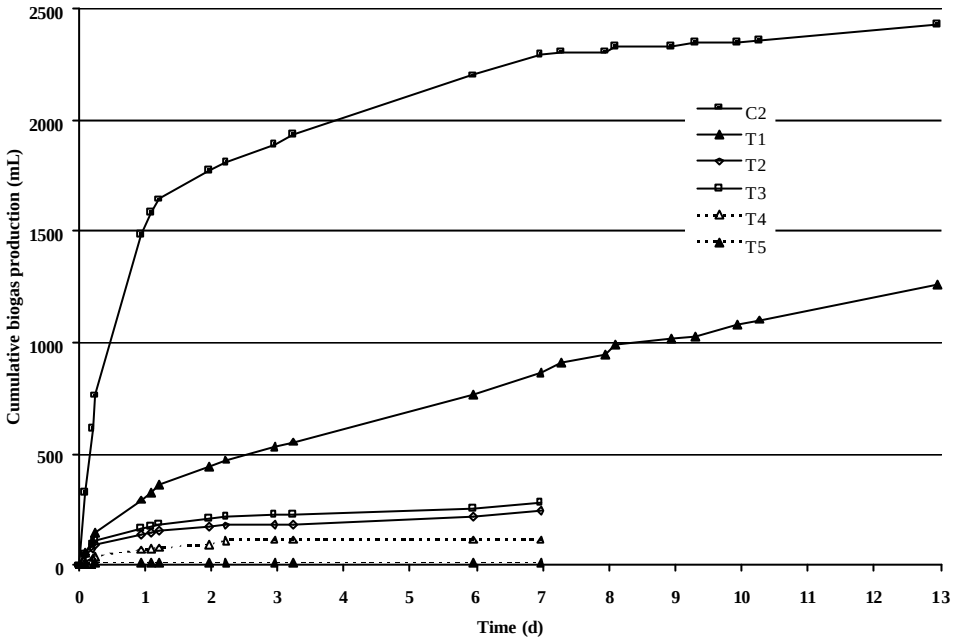

Figure 6. Cumulative biogas production (mL) of the different reactors in function of time (d)

Table 11. CODt, CODs and cumulative biogas production of the different reactors in function of time

	Time (d)	C2	T1	T2	T3	T4	T5
CODt (mg)	**0**	5467	5797	709	872	573	253
	2	1103	2910	296	324	275	256
	3	582	-	198	164	213	183
	7	-	-	180	207	197	139
	13	196	1505				
CODs (mg)	**0**	5043	3571	511	669	326	155
	2	1055	2561	274	290	248	218
	3	421	1823	202	130	198	177
	7	-	-	159	199	173	123
	13	189	1272				
Delta CODs		**4854**	**2299**	**352**	**470**	**153**	**32**
CODs removal (%)		**96**	**64**	**69**	**70**	**47**	**21**
Cumulative biogas (mL)*	**0**	0	0	0	0	0	0
	2	1775	447	177	210	96	13
	3	1933	556	187	232	117	13
	7	2297	869	249	284	117	13
	13	2427	1264				

* Already corrected for the background biogas production of control reactor C1 (no feed)

- Not measured

For the evaluation of the results presented in Table 11, the delta CODs between time 0 and the end of the fermentation (7 or 13 days), was compared with the cumulative biogas production. A mass balance can not be calculated based on the CODt values, since the samples on which the analyses were performed were supernatant samples. Hence, there is always interference of small floating sludge particles. Furthermore, suspended solids present in the feed (difference between CODt and CODs is COD in suspension) will settle during the test period and will result in an overestimation of CODt removal. Therefore, the mass balance was calculated on the delta CODs. In Table 11, the delta CODs between time 0 and 7 (for T2, T3, T4 and T5) or 13 days (for C2 and T1) and the CODs removal percentage were calculated. Considering that the removal of 1 g COD results in 0.5 L biogas with a biogas composition of 70 % CH_4 and 30 % CO_2, these delta CODs values (divided by 2) can be compared with the cumulative biogas production also presented in Table 11. For Control reactor C2, it can be seen that all the CODs removed could be found as biogas. The removal percentage was 96 %. For Test reactor T1 and T5, also a good approach of the theoretical biogas production and the actual biogas production was found. It was found that the degradation of the organic matter in reactor T1

was slow when compared to the control reactor (C2). Hence, the COD was not easily biodegradable. This reactor was fed with effluent of a near critical liquefaction treatment without oxidant. The CODs fraction present after 13 days of fermentation was be considered as being recalcitrant organic matter. This CODs represented 36 % of the CODs present at time 0. Hence the CODs removal percentage was 64 %. The reactor T5 resulted in a removal percentage of 21 %. For Test reactors T3 and T4, the biogas production was a bit higher than the estimated values based on the delta CODs values. This can be explained by the removal of other COD than CODs, namely COD in suspension and the fact that the delta CODs was slightly underestimated due to sludge mineralization (cell decay). Sludge mineralization occurred because the test lasted for one week and the feed was low in COD. The removal percentage of T2, T3 and T4 were 69, 70 and 47 % respectively. Overall, it was concluded that the effluent samples of the liquefaction unit were not toxic to methanogenesis but that a small fraction was recalcitrant (CODs after the fermentation).

In Figure 7, the evolution of the total VFA concentration (mg/L) in the different reactors in function of time is depicted. As can be seen from Figure 7, the total VFA concentration decreased in all reactors in function of time. The high amount of VFA (acetate) in C2 at time 0 was due to the feed composition of this control reactor (3.2 g sodium acetate; hence 2.3 g acetate). The VFA concentration was below 170 mg/L for all the reactors after 3 days of fermentation. After 7 days, no VFA were detected in the reactors C1, T2, T3, T4 and T5 (VFA of C2 and T1 not measured). Hence, all the biodegradable compounds were converted at this point. The VFA concentration of C2 and T1 was measured after 13 days of fermentation and was 0 mg/L for the control reactor and 30 mg/L for test reactor T1.

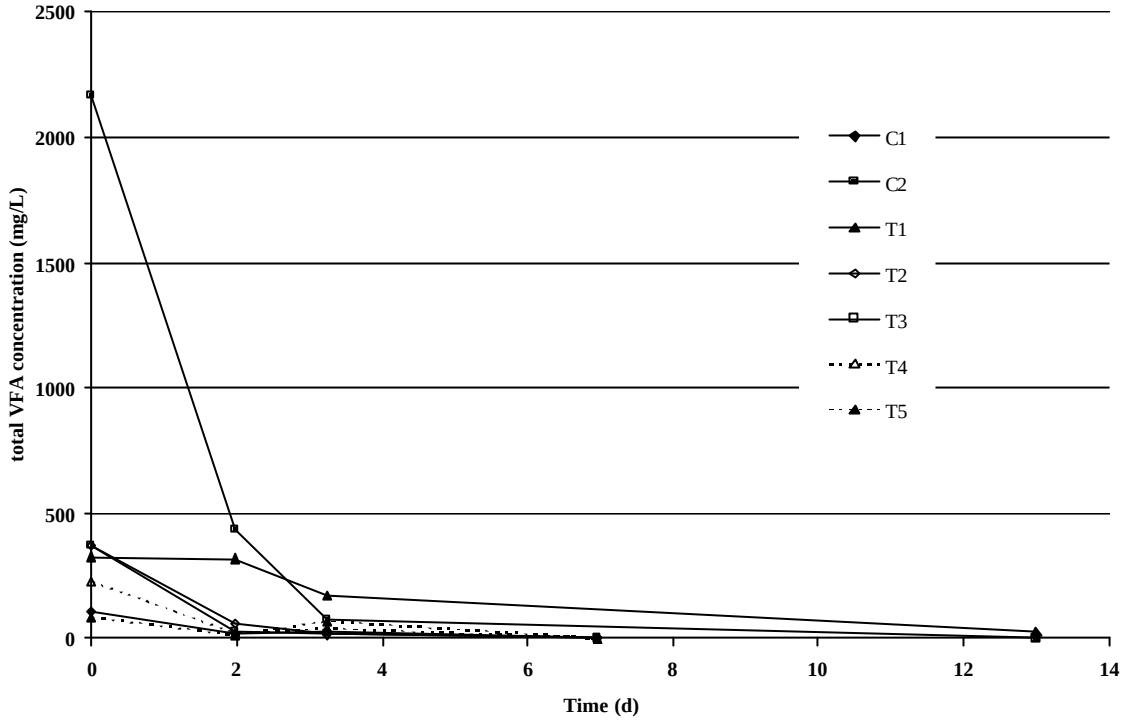

Figure 7. Evolution of the total VFA concentration (mg/L) in the different reactors in function of time (d)

After this fermentation, an easily biodegradable compound (glucose) was added to the different reactors to verify whether the CODs left after the first fermentation test was recalcitrant or whether toxic compounds were formed during the fermentation of organic matter present in the effluent of the liquefaction unit. Therefore, the reactors were supplied with 1 g of glucose (= 1 g COD), the volume of the reactors was adjusted to 1 L and the pH of the reactors was adjusted to 7.3 ± 0.3 with NaOH (5 N). At time zero and at the end of the fermentation, a sample of the supernatant was taken and analysed for CODs. These results are presented in Table 9. Since the volume of the reactor was 1 L, the concentration was easily recalculated into absolute amount (mg).

Table 12. CODs (mg) results of the reactors supplied with 1 g glucose in function of time

Time (d)	C1	C2	T1	T2	T3	T4	T5
0	1254	1317	2234	1259	1185	1113	1160
3	-	266	1209	-	-	-	-
6	169	-	-	189	200	203	224
Delta	1085	1051	1025	1070	985	910	936

- not measured

From Table 12, it can be seen that approximately 1000 mg or 1 g of soluble COD was removed in all the reactors. The higher value at time zero was due to rest COD from the first fermentation. If a biogas composition of 70 % CH_4 and 30 % CO_2 is supposed, a biogas volume of about 500 mL is expected for the removal of 1 g COD. The cumulative biogas production (mL) in function of time is depicted in Figure 8.

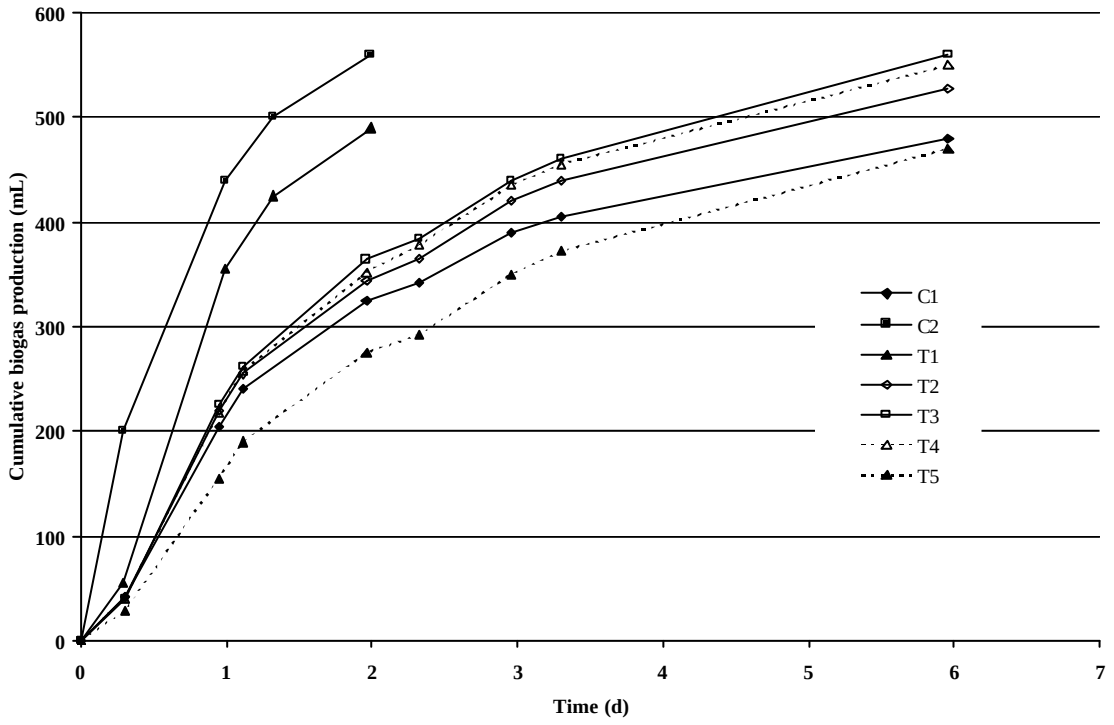

Figure 8. Cumulative biogas production (mL) of the reactors supplied with 1 g glucose (= 1 g COD) in function of time (d)

From Figure 8, it can be observed that the biogas production started immediately in all the reactors without a lag phase. Therefore, it can be concluded that there were no inhibitory compounds to methanogenesis present in the effluent of the liquefaction unit. Theoretically, one can say that 1 g of COD removed results in a biogas production of 0.5 L (70 % CH_4 and 30 % CO_2). The measured biogas volume approached the theoretical biogas volume quite well. Control reactor C2 and test reactor T1 showed a higher removal rate towards the other reactors. This is explained by the higher COD loading rate during the first fermentation. Less starvation and hence less cell lyses occurred in these reactors.

4 CONCLUSIONS AND FUTURE PERSPECTIVES

In this technical note it was shown that the addition of solid digester residue (0, 1, 1.3, 1.6 and 2 g VS) from the effluent of the main mesophilic digester to mixed liquor (400 mL) of this digester gave an extra biogas yield. Overall, it was concluded that 30 - 40 % of the VS added could be converted into biogas. Hence, a solid retention time of 40 days in the main mesophilic digester is recommended.

The fermentation of the returned effluent treated by Partner 1 (anaerobic digestion), Partner 2 (Fibrobacter) and Partner 4 (sub-critical liquefaction) in a fixed-bed methanogenic biofilm reactor resulted in a 50 % CODs removal after 7 days of recirculation. The nitrogen compounds did not change significantly during the experiment.

In a series of toxicity experiments, performed with returned effluent successively treated by Partner 1, Partner 2 and Partner 4 (with and without oxidant) it was shown that there was no inhibition of methanogenesis. The CODs removal percentages varied between 21 and 70 %.

For WP 1.8, there will be a definition of the critical control points and limits. Monitoring procedures for HACCP analysis will be established. The lab-scale methanogenesis units will be constructed for assembly. Furthermore, there will be a substrate exchange with Partner 2 and Partner 4 and batch experiments will be performed with the returned supernatant.