MELiSSA

TECHNICAL NOTE

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MANUAL COUPLING AND DEMONSTRATION OF METHANOGENIS UNITS IN THE TC-UNIT

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

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1 INTRODUCTION AND OBJECTIVES

Scope of this work
Physical coupling of both methanogenesis units with sub-creitical liquefaction unit of Partner 4

Secondly, lab-scale methanogenesis units constructed. In this phase of the project the high-load methanogenic reactor was run at a solid retention time of 40 d, as recommended by the outcome of the results in TN 1.7. The performance of the reactor under these conditions was monitored.

A comparison between treatment sequences with or without *Fibrobacter succinogenes* treatment was made.

The tasks described for this TN are given below:

INPUTS

- Operational and optimized high-load and low-load methanogenesis unit
- Required analysis equipment
- Monitoring equipment for the off-line detection of pathogens

Tasks included

- Follow-up of both methanogenesis units in TC-unit
- Determination of liquefaction and biosafety efficiency of the TC-unit (with and without Fibrobacter treatment)
- Theoretical calculations for methane conversion into SCP and carbon dioxide

2 MATERIALS AND METHODS

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

Analysis of the feed shows a feed concentration of 21g COD.L^{-1}, 24g Volatile solids (VSS).L^{-1} and an ash-content of 4.4g.L^{-1}. Nitrogen concentrations of the feed showed 0.41 g.L^{-1} of ammonia (distillation method); 1.2g.L^{-1} Kjeldahl N (distillation method).

Experimental set-up of the high-load methanogenesis unit

A 10 Liter anaerobic glas reactor is used for the anaerobic digestion of the defined feed. As indicated in Figure 1, 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 For each volume of the feed fed to the reactor, a same volume of stirred mixed liquor is withdrawn simultaneously.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 a stable nominal regime and to follow up solid build-up in the system. 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. The solid retention time (SRT) was maintained at 40 days following the results from T.N. 1.7. In order to achieve a SRT of 40d, 0.625L/L of extracted mixed liquor was centrifuged and returned to the reactor.

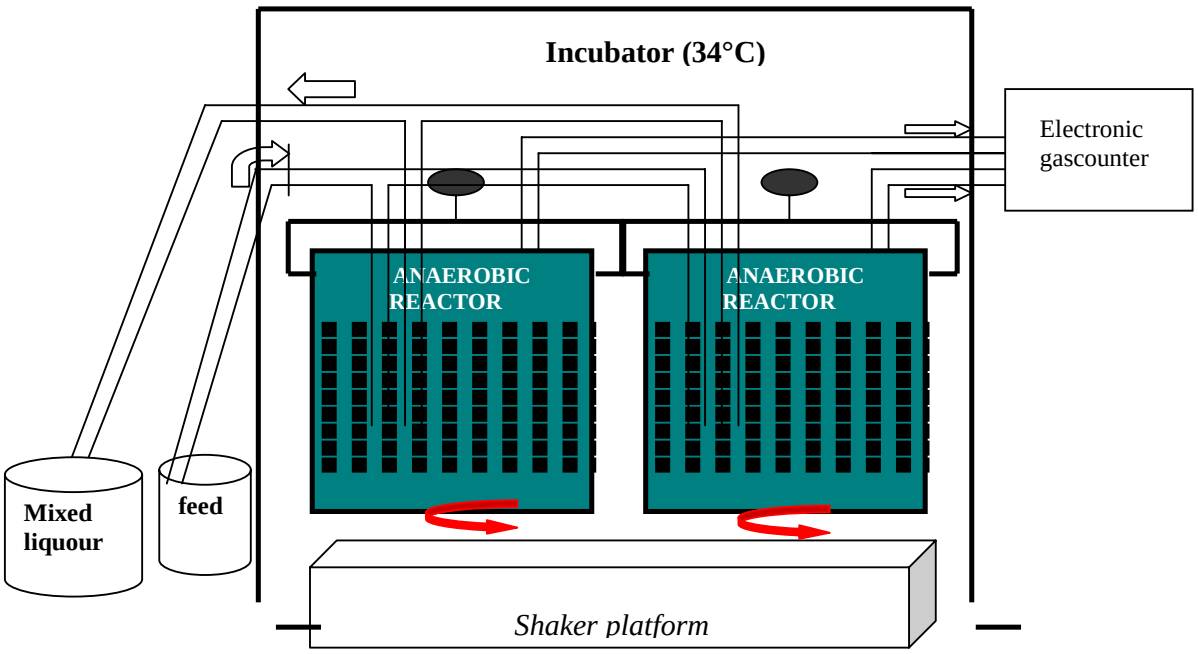

Figure 1: Scheme of the 2 mesophilic digesters for the fermentation and subsequent distribution of the digester residues (approximately 400 + 100 gram DM)

Experimental set-up of the 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 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 were added. 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 2.

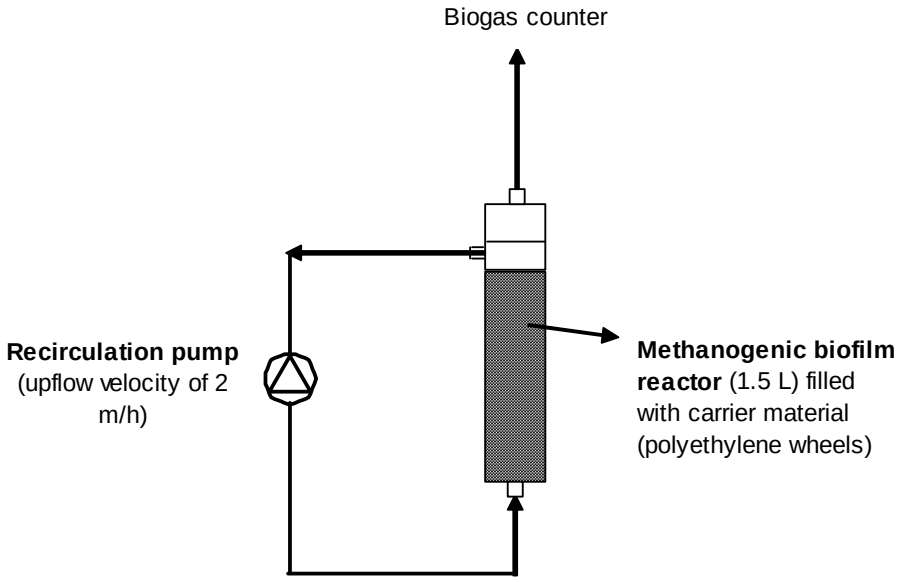

Figure 2. Schematic presentation of the reactor set-up of the low-load methanogenesis unit.

After the start-up period of the fixed-bed biofilm reactor, the returned effluent from the 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 regularly basis, during a total period of 7 to 8 days.

3 RESULTS AND DISCUSSION

3.1 Follow up of high-load methanogenesis unit (CSTR) under optimised conditions

As a result of the experiments in TN 1.7 and to a lesser extent TN 1.6, the hydraulic retention time was uncoupled from the solid retention time, allowing for a longer contact time of the fibrous materials in the standardized feed and should result in a higher removal efficiency of the methanogenic system. Early fears that feed-back inhibition at these higher solid concentrations might occur, were not seen in the exploratory experiments of the above mentioned technical notes, and extended running of the reactor system up to 100 days showed stabile process parameters as shown below.

Influent and effluent analysis of the high-load methanogenesis unit (CSTR)

VFA-analysis (Volatile Fatty Acid), DM-content (dry matter), VS-content (volatile solids), COD (chemical oxygen demand), Kjeldahl-N (KjN), Total Ammonium-N (TAN), and Total Oxidized-N (TON) were measured prior to and after fermentation.

The amount of biogas was monitored continuously with an electronic gas counter and the biogas composition was determined by means of gas chromatography (GC-TCD).

CHARACTERISATION OF THE INFLUENT

Table 1: Feed characterisation

DM-content	COD	TAN	Kj-N	VS	ash-content
2.8%	21 g/L	0.41 g/L	1.2 g/L	24 g/l	4.4 g/l

CHARACTERISATION OF THE MIXED LIQUOR IN THE REACTOR

The dry matter (DM) content profile of the mixed liquor in the digestor is shown in Figure . Although in the start-up phase there was a built-up of DM, the value reached a plateau after 20 days and remained stable during the further operation of the system. Figure 3 shows the graph of the DM evolution.

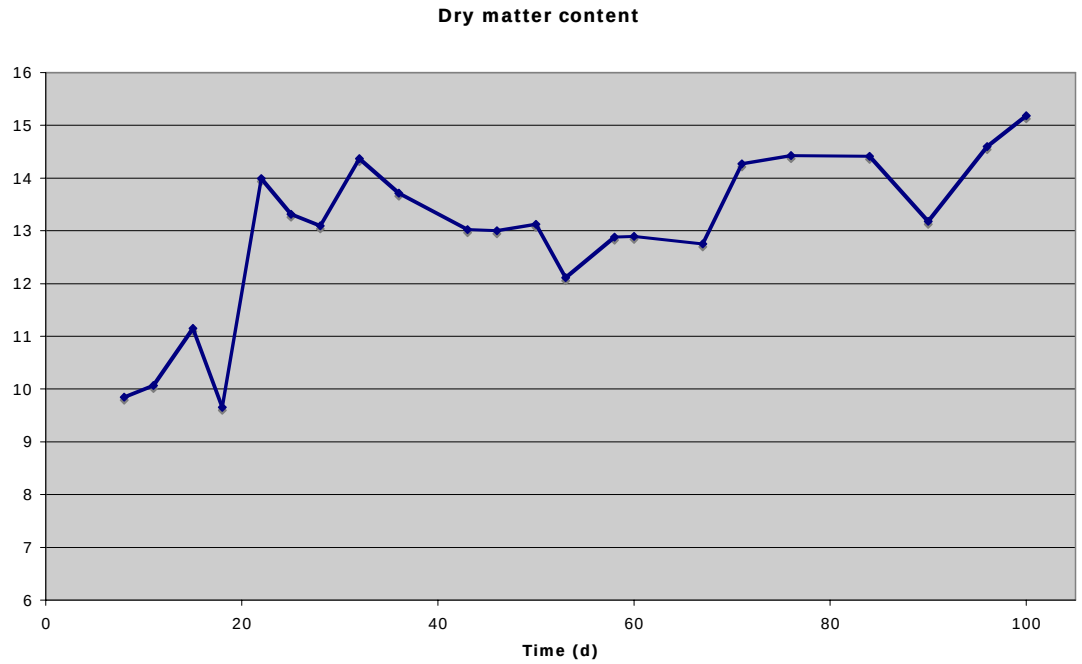

Figure 3 Dry matter profile of the mixed liquor in the digestor

Due to the higher solid retention time, DM content increased compared to values obtained for TN 1.7. and TN 1.8. However, equilibrium was reached after a start-up period of 20 days.

BIOGAS PRODUCTION

During the fermentation of the raw substrate, the biogas production was constantly monitored with an electronic gas counter (Figure 4).

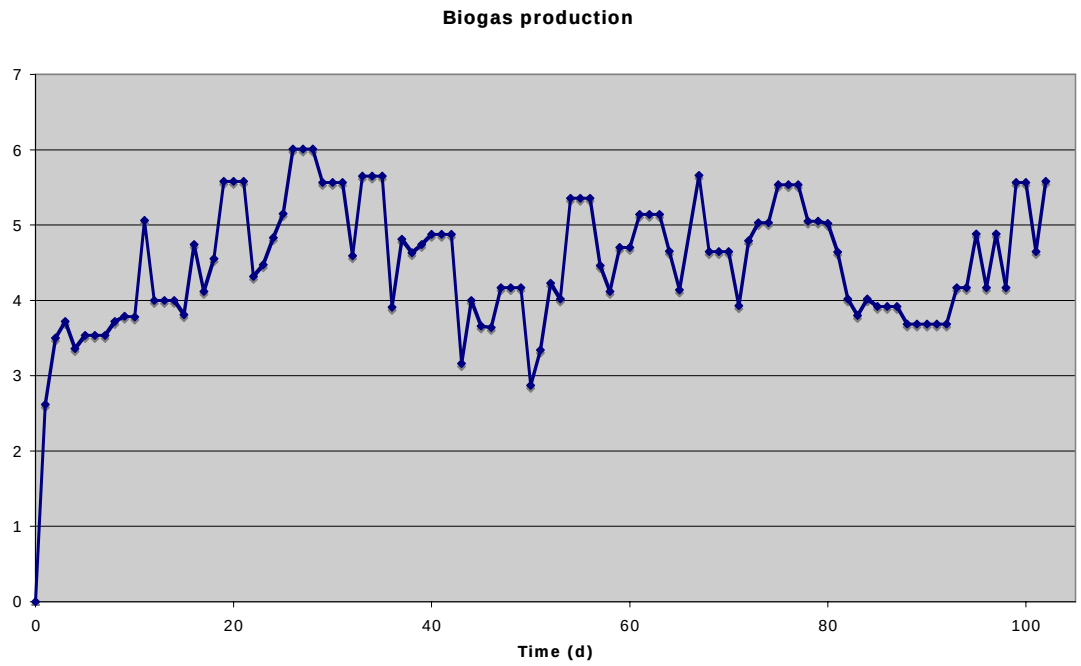

Figure 4 Daily biogas production of the high load methanogenic digestor

On average 4.67 ± 0.8 L of biogas was produced per day (data from day 20 to day 100). This production was found to be in accordance with the volumetric loading rate with on average a production of 0.44 L biogas/g COD or a biogas yield of 87.7%. The average methane content measured over a 2 months period, accounted for 62.8 ± 1.8% of the total biogas production.

CHARACTERISATION OF THE EFFLUENT

In first instance, standard analysis was performed on the reactor effluent. Average results are shown in Table 2.

Table 2 Effluent characterization

DM-content	COD	TAN	Kj-N	VS	ash-content
0.48%	7.07 g/L	0.71 g/L	1.0 g/L	3.75 g/l	1.1 g/l

COD profile

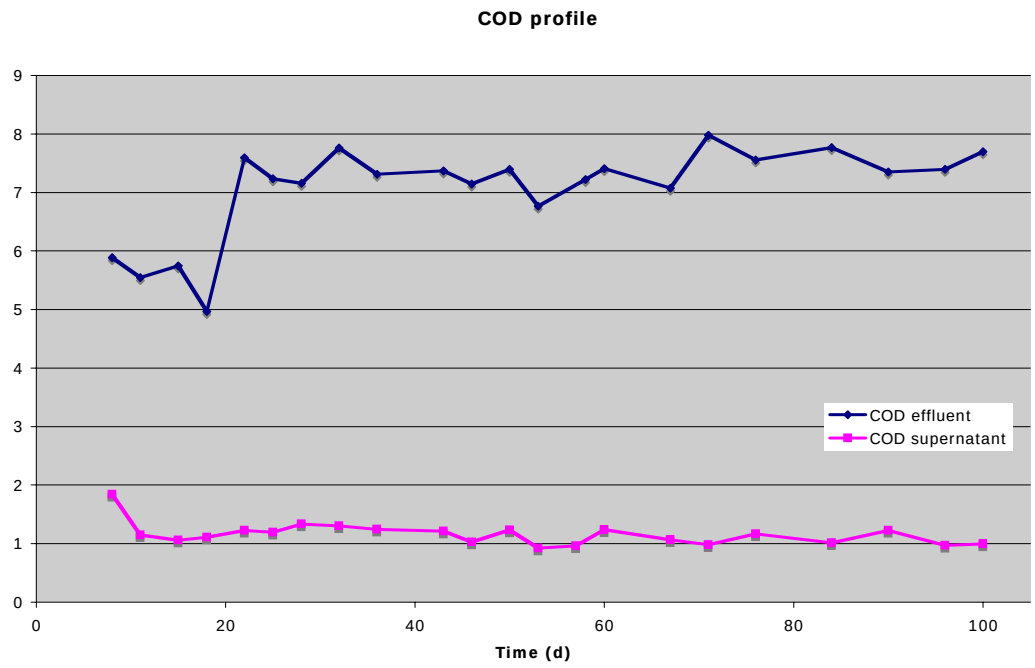

Figure 5 Profile of the COD of the effluent and its supernatant

As can be seen from Figure 5, the COD of the reactor effluent reaches a stable value of about 7 g/L after a start-up period of 20 days. This value remains stable over the course of the operation of the reactor, indicating stabile performance. Only about 1 g/L can be attributed to the COD content of the supernatant of this effluent, which indicates that most COD is comprised in the biomass and fibrous particulates leaving the reactor.

Nitrogen profile

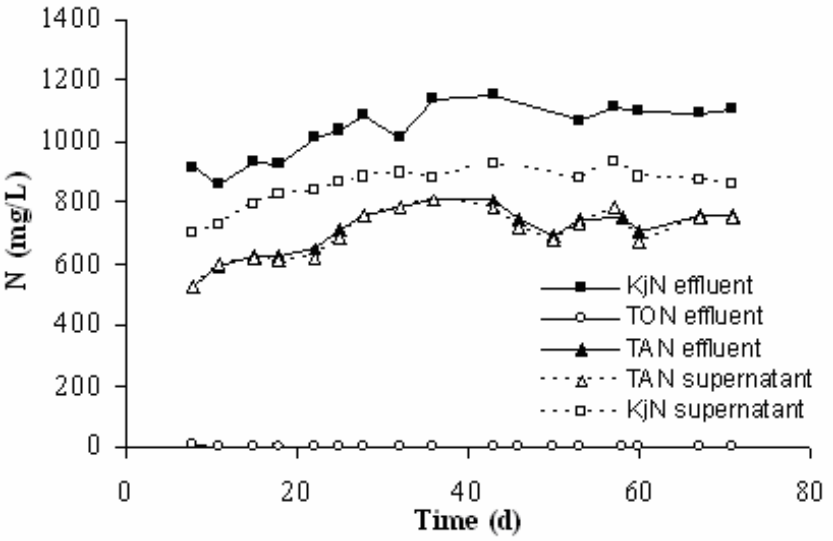

Figure 6 Profile of the KjN, TAN and TON of the effluent and its supernatant

From Figure 6, it is clear that the organic bound nitrogen is converted during anaerobic digestion into solubilized ammonia. There is no oxidized nitrogen present (TON level is zero). All ammonia of the effluent is in the soluble phase (supernatant) since the TAN-level of the supernatant was found to be similar to the TAN-level of the total effluent. Some nitrogen is still organic bound (part of KjN not attributed to TAN or TON), comprised in the biomass and fibrous particulates leaving the reactor.

Volatile Fatty Acids

Volatile fatty acids where extracted from the digester effluent with diethylether and analyzed with GC-FID (with internal standard). The following fatty acids have been determined: acetic acid (2.7 mg/L), propionic acid (0.5 mg/L), and traces of isobutyric acid, isovaleric acid and iso capric acid. It can be concluded that all VFA-concentrations were found to be very low (< 5 mg/L). This clearly indicates the high organic carbon removal and the stability of the digester.

3.2 High-load methanogenesis CSTR – Hydrothermolysis – Low-load methanogenesis upflow biofilm reactor

This paragraph describes the treatment sequence:

Influent => High-load methanogenesis CSTR => Oxidative Hydrothermal treatment => low-load methanogenesis upflow biofilm reactor

Results summarizing the above-mentioned sequence are shown in table 3.

Table 3: High load methanogenic CSTR => Oxidative Hydrothermolysis => Low load methanogenesis up flow biofilm reactor sequence results

High Load methanogenic CSTR		Oxidative Hydrothermolysis	Low load methanogenesis up flow biofilm reactor	
Influent		Pressure: 250 BAR Temperature: > 300°C 4s < τ < 17s stoichiometric H_2O_2 excess ≈ 2 (1)	**Influent** (= effluent of oxidative hydrothermolysis)	
Concentration	21 gCOD/L 24 g/L VSS 4.4 g/L ash		Concentration	0.23 g COD/L (all in liquid phase)
Reactor volume	7.5L		Reactor volume	1L
Volumetric loading rate	1.4g COD/L.d		Loading	0.23 g COD/L (i.e. volumetric loading rate at day 0, 1L, recirculating)
VFA	0		VFA per L	164 mg HAc, 3mg propionate
Nitrogen	0.41 g/L ammonia 1.2 g/L KjN		Nitrogen	71.5 mg KjN 58.8 mg ammonia (all in liquid phase)
Biomass concentration	0		Biomass concentration	0
Effluent			**Effluent**	
Concentration	5.73 g CODs/L effluent 1.18 g COD/L clear effluent		Concentration	0.10 g COD/L (all in liquid phase)
HRT	15d		HRT	7d
SRT	40d			
Biogas	4.7 L/d 62.8% CH_4		Biogas	60 mL/7d (i.e. 60 mL per 0.23 g COD)
VFA in liquid phase	2.7 mg HAc		VFA per L	45 mg HAc; 14 mg propionate; 3

per L	0.5 mg propionic acid			mg butyrate; 1mg isovalerianate
Nitrogen per L	717mg ammonia in liquid, 454 mg ammonia in solids, 844 mg KjN in liquid; 528 mg KjN in solids		Nitrogen per L	98.7 mg KjN; 71.3 mg ammonia
Biomass concentration	1.83 g/L effluent		Biomass concentration	0 (fixed bed)
% degradation based on biogas production	87.6%		% degradation based on biogas production	52.2%

(1)

The reacting conditions of the oxidative hydrothermolysis were chosen based on the results presented in TN's 4.6 and 4.7. Stoichiometric excess of a factor 2 based on the carbon content of the effluent were required to attain complete liquefaction of the influent at temperatures higher than 300°C and a pressure of 250 BAR. Pump speeds of the hydrothermal unit were chosen to attain a hydraulic residence time of about 10 seconds in the reactor.

Prior to hydrothermal treatment, the particulate size of the high load methanogenic CSTR effluent was reduced to < 180 μm using an IKA mixer.

3.3 *Comparison between two treatment sequences – 7th closed loop*

This paragraph compares the performance of two treatment sequences:

A: High load methanogenic CSTR => Fibrobacter succinogenes treatment => Oxidative Hydrothermolysis => Low load methanogenic upflow biofilm reactor

B: High load methanogenic CSTR => Oxidative Hydrothermolysis => Low load methanogenic upflow biofilm reactor

As in previous closed loop experiments, solid digester residue from the high load methanogenic CSTR was sent to Partner 2, Laboratoire de Génie chimique et biochimique, Université Blaise Pascal, Clermont Ferrand, France. The CSTR solid digester residue was treated with the fiber degrading *Fibrobacter succinogenes* to allow for a further degradation of the recalcitrant fibrous fraction of the solid digester residue.
Following the *F. succinogenes* treatment, the sample was sent to LabMET, Ghent University, Belgium for treatment with the Hydrothermolysis unit at optimized conditions. Table 4 below shows the resulting data for treatment sequence A, Table 5 contains data from treatment sequence B.

The data clearly shows that *F. succinogenes* treatment has a very small impact in the total liquefaction capacity of the proposed treatment sequences. Given that in the B sequence mixed liquor was treated, the higher effluent COD after the oxidative hydrothermolysis can be explained by the presence of soluble recalcitrant compounds in the liquid fraction of the treated influent which were not present in the A sequence.
The oxidative hydrothermolysis is capable of 'cracking' these recalcitrant compounds to a very high degree, shown by the final COD values of the low load methanogenic upflow biofilm reactor effluents. Given this data, the superiority of hydrothermolysis at oxidative conditions becomes clear, and total liquefaction and near-total biodegradation of the standardized MAP waste is a fact.

Table 4: results of treatment sequence A

***Fibrobacter succinogenes* treatment**	
In: CSTR solid digester residue	
Out	6% Dry Weight (DW) 23 g COD/L
Oxidative hydrothermolysis	
In	10 fold dilution of *F. succinogenes effluent* stoichiometric x2 H_2O_2 dosage P: 240 BAR

	T: 350°C Q_{out}: 21 mL/min
Out	0.04% DW 0.417 g COD/L 0.123 g KjN/L 0.037 g TAN/L 0 g TON/L pH 6.6
Low load methanogenic upflow biofilm reactor	
In	Effluent of oxidative hydrothermolysis corrected to pH 7.2 using NaOH
Out	Cumulative biogasproduction: 570 mL 69% methane, 31% CO_2 0.01% DW 0.092 g COD/L 0.162 g KjN/L 0.052 g TAN/L 0 g TON/L

Table 5: Results of treatment sequence B

High load methanogenic CSTR	
In	Standardized feed as described above
Out	0.5% DW 7.07 g COD/L 1.09 g KjN/L 0.76 g TAN/L 0 g TON/L
Oxidative hydrothermolysis	
In	No dilution required Stoichiometric x2 H_2O_2 dosage P: 240 BAR T: 350°C Q_{out}: 23 mL/min
Out	0.06% DW 0.721 g COD/L 0.201 g KjN/L 0.072 g TAN/L 0 g TON/L

Low load methanogenic upflow biofilm reactor	
In	Oxidative hydrothermolysis effluent, pH corrected to 7.1
Out	Cumulative biogas production: 1040 mL 72% methane, 28% CO_2 0.012% DW 0.14 g COD/L 0.189 g KjN/L 0.091 g TAN/L 0 g TON/L

The effluents from the (oxidative) hydrothermolysis were subjected to microbial plate count techniques using selective media for typical indicator organisms in drinking water.
Prior to testing, the HT unit was rinsed with at ambient temperature and atmospheric pressure using 70% ethanol in water, and subsequently rinsed with 3 total reactor volumes of water to eliminate potential contamination from bacteria in the HT unit.

A 1L sample of mixed liquor from the high load methanogenic CSTR reactor, operating at 34°C, was treated in the HT unit. The effluent from one batch HT treatment at 250 BAR and 350°C, in absence of hydrogen peroxide, was collected and 100 microliter samples were plated on the following media:

- Mac Conkey agar: selective medium for coliform bacteria, indicating faecal pollution
- PCA: plate count agar, a general purpose agar to allow a total count

Incubation at 37°C for 24h failed to show any growth, demonstrating the effective sterilization of all HT influent.

3.4 Ammonium, nitrate and nitrite conversion in the hydrothermolysis unit

The goal of this experiment is to assess the conversion of NH_4^+, NO_3^- and NO_2^- in the hydrothermolysis reaction. For this experiment, the HT unit was run at a pressure of 200 BAR and 180°C (Table 6) in a first series of experiments, and at 200 BAR and 240°C (Table 7) in a second series of experiments.

Table 6: Nitrogen interconversions at P: 200 BAR and T: 180°C

Influent 1 (NH_4NO_3), HRT 40s			
	Before treatment	After treatment	Removal efficiency
mg N(NH_4^+)/L	250	139	44,5%
mg N(NO_3^-)/L	250	132	47%
mg N(NO_2^-)/L	0	17	
Influent 2 (NH_4NO_2), HRT 64s			
	Before treatment	After treatment	Removal efficiency
mg N(NH_4^+)/L	500	155	69%
mg N(NO_3^-)/L	0	3	
mg N(NO_2^-)/L	500	209	58%

The removal efficiency in this first series of experiments was low for all N species. At higher hydraulic retention times, an increase in the removal efficiency was seen. In the second series of experiments, temperature and HRT were increased.

Table 7: Nitrogen interconversions at P: 200 BAR and T: 210°C

Influent 1 (NH_4NO_3), HRT 214s			
	Before treatment	After treatment	Removal efficiency
mg N(NH_4^+)/L	500	9	98%
mg N(NO_3^-)/L	500	12	97,6%
mg N(NO_2^-)/L	0	0	
Influent 2 (NH_4NO_2), HRT 231s			
	Before treatment	After treatment	Removal efficiency
mg N(NH_4^+)/L	670	156	76,8
mg N(NO_3^-)/L	0	11	
mg N(NO_2^-)/L	500	1,5	99,7

Removal efficiencies at these operating conditions were much higher than in the previous set of experiments.

3.5 Single cell protein

Methane occurs in nature as part of the built-up of the atmosphere, and –together with carbondioxide- contributes highly to the greenhouse effect. The current concentration of methane in the troposphere is 1.8 μL per liter of air, and increases by about 1% yearly.
About half of the methane in the atmosphere is antropogenic, important contributions are the production and consumption of fossil fuels, rice cultivation, live stock and waste treatment.

Several studies showed that biological methane oxidation is very sensitive to environmental disruptions. The usage of certain types of herbicides and fertilizers can greatly reduce the methane oxidizing capacity of a soil. When organic fertilization is used, the methane oxidation capacity may increase with 200 to 300%. A sensible soilmanagement can thus contribute to attaining the Kyoto norms (Seghers et al., 2003).

In nature, the vast majority of the methane in the atmosphere is abiologically oxidized (470 Tg per year). Microbial methane oxidation takes care of about 30 Tg per year). The methanotrophic bacteria are unique in their capacity to use methane as the sole carbon and energy source, and can play an important role in the global methane cycles. The amount of methane that is emitted towards the atmosphere is the net result of the methane produced by various sources and the amount of methane consumed by aerobic and anaerobic methane oxidizing bacteria. Methanotophs with a low affinity towards methane can be found close to methane sources (e.g. tundra, swamps, ricepaddies), whereas methanotrophs with a high affinity for methane use atmospheric methane.

The most-studied methane oxidizing bacteria are obligate aerobic, gram negative bacteria that belong to the α- or γ- proteobacteria (Hanson & Hanson, 1996). So far, the only pure methanotrophic cultures studied are methanotrophs with a low affinity towards methane. The seemingly impossibility to culture these bacteria using classical culturing techniques results in the scarceness of information on aerobic methanotrophs with a high affinity to methane.

Almost all methane that is produced in anaerobic environments is oxidized before it reaches the atmosphere. Anaerobic methane oxidation (AMO) is largely responsible for this. For example, 75% of the marine methane is oxidized by anaerobic methanotrophs. Several studies showed syntrophic consortia of archaea and bacteria responsible for AMO in marine and sweet water sediments. The archaea are members of distinct fylogenetic groups and use reverse-methanogenesis to oxidize methane. The bacterial partners are commonly sulfate reducing organisms related to Desulfosarcinales (Strous & Jetten, 2004) or denitrifying species belonging to a new, uncultivated fylum. It is not clear yet why such consortia occur and what chemicals are exchanged.

Some calculations on methanotrophic growth were made:

$Y \approx 0.10$ g SCP . (g CH_4 – COD)$^{-1}$

With Y the cell yield calculated from the amount of COD required to utilise CH_4 aerobically as carbon source.

From the reaction…

$CH_4 + 2\ O_2 \rightarrow CO_2 + 2\ H_2O$

… it follows that 1 mol of methane requires 2 mol oxygen to be oxidised, or also:
16g of CH_4 requires 32g O_2.

Based on the consumption of methane, it follows that

$Y \approx 0.05$ g SCP . (g CH_4)$^{-1}$

For each gram of methane consumed, two grams of oxygen are required, to result in 0.05g of single cell protein. The rest of the methane that is not built into biomass is required to sustain the bacterial homeostasis.
This low value for the Y of methanotrophs is in line with what is known about cell yields in slow-growing organisms in nitrifying- and methanogenic consortia.

In the case of methanogens, their cell yield is greatly influenced – and reduced - by the myriad of small variances in the organism's environment destabilising their performance, making them very sensitive to even the smallest disruptions in their ecosystem - even at stable reactor performances.
As the methane fixing pathway in methanotrophs is described in literature as the reverse-methanogenesis, it stands to logic that the methanotrophs biochemistry is equally sensitive, resulting in similarly low cell yields. The sensitive nature of methanotrophic soil bacteria is used as a biomarker to determine fertilisation regimes (chemical vs. natural), and the slow recovery of soils depleted of methanotrophs, supports this thesis.

In all, it can be stated that the direct usage of methane to form SCP might not be advisable.

ICI chemicals investigated –but publicised very little scientific data – the use of methane as a C-source for the production of SCP. Their final reactor design firstly used a catalytic conversion of methane into methanol, and the subsequent production of SCP from methanol. As the organism involved in SCP production is no longer methanotrophic, cell yields much higher than for methanotrophic SCP production are obtainable.

4 CONCLUSIONS

Prolonged running of the high load methanogenic CSTR reactor, the MAP study's main digester, at high loading and high solid retention time, showed stable and reliable performance resulting in a conversion of >80% into biogas.

The two treatment sequences, where *Fibrobacter* was either placed in between methanogenesis and hydrothermal destruction, or left out of the methanogenesis => hydrothermolysis => methanogenesis showed again the power of the hydrothermal treatment.
The role of the *F. succinogenes* treatment in this MAP study, and subsequently in MELiSSA, is unclear, as it's presence in the treatment sequence failed to to be a great added value to the overall efficiency of the MAP concept.

It is clear now that the strong physico-chemical conditions in the HT-unit are capable of providing a biosafe barrier that cracks down the recalcitrant fractions and fibrous materials, partly gasifying them to carbon dioxide, partly converting them into dissolved organic carbon compounds (DOC).

This remaining DOC was shown to be degradable using a low load methanogenic biofilm upflow reactor, capable of using low-COD influents. No adverse effects caused by byproducts of the HT-treatment were observed in this second methanogenesis, showing that the HT-unit can be implemented into MELiSSA's existing C1 concept without great risks.

Hydrothermal treatment at subcritical conditions may greatly influence the interconversion of nitrogen species and should be further investigated in the scope of incorporating an HT-like treatment in MELiSSA's C1.

Finally, the role of methane in the MELiSSA concept is a difficult one. The explosive nature and the very low nutritional value (only to methanotrophs) are certainly supporting the case against it's usage. However, for terrestrial applications the conversion of methane to methanol, and the subsequent production of single cell protein is feasible and received great interest in the 1970's. It's popularity decreased with the coming of the oil crisis and alternative sources of nutrients, but it remains an interesting means to provide nutrients to for animal feed.