

**A TOTAL CONVERTING AND BIOSAFE  
LIQUEFACTION  
COMPARTMENT FOR MELISSA**

**TECHNICAL NOTE: 86.1.3**

**Evaluation of potentially improved substrates**

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## **Introduction**

This technical note presents the current state of the MAP project “A Total Converting and Biosafe Liquefaction Compartment for MELISSA” on behalf of the Laboratory for Microbial Ecology and Technology at the University of Ghent.

This note describes the results from the mesophilic digestion of the raw substrate on the one hand in terms of mass balances and subsequent substrate distribution to the different partners. On the other hand, the returned substrates from the different partners have been evaluated in terms of biogas yields.

In particular, the overall C, N, P and S mass balances for the different substrates are given and the degradation of lignine, cellulose and hemicellulose are described. Finally, the actions to be taken in the nearby future concerning additional closed loop experiments are given.

## **Objectives and task description**

The results shown in this technical note are in accordance with the tasks described in WP 1.300. The task of this work package is two-fold. According to the progress meeting in Barcelona (3/12/2001), raw synthetic substrate was first mesophilically fermented and the fibrous residue was distributed among the partners for subsequent degradation in their respective unit. Second, the digested fibers were returned to partner 1 for a second mesophilic digestion. In this way, differences between the different substrates (and consequently between the different units) could be evaluated. Finally, the calculations with respect to the energy consumption of the mesophilic digestion as presented in TN2 were used to determine the energy requirements on behalf of the methanogenesis in an integrated concept as agreed upon in the progress meeting in Hamburg (4/07/02).

As shown in Figure 1, the latest reported conversion efficiency of the mesophilic digester accounted for 80-85% of the raw substrate into biogas, leaving 15-20% of mainly undigested fibrous residue. The further liquefaction (hydrolysis) of this residual fraction by the liquefaction units of the different partners was the main objective of this phase of the study.

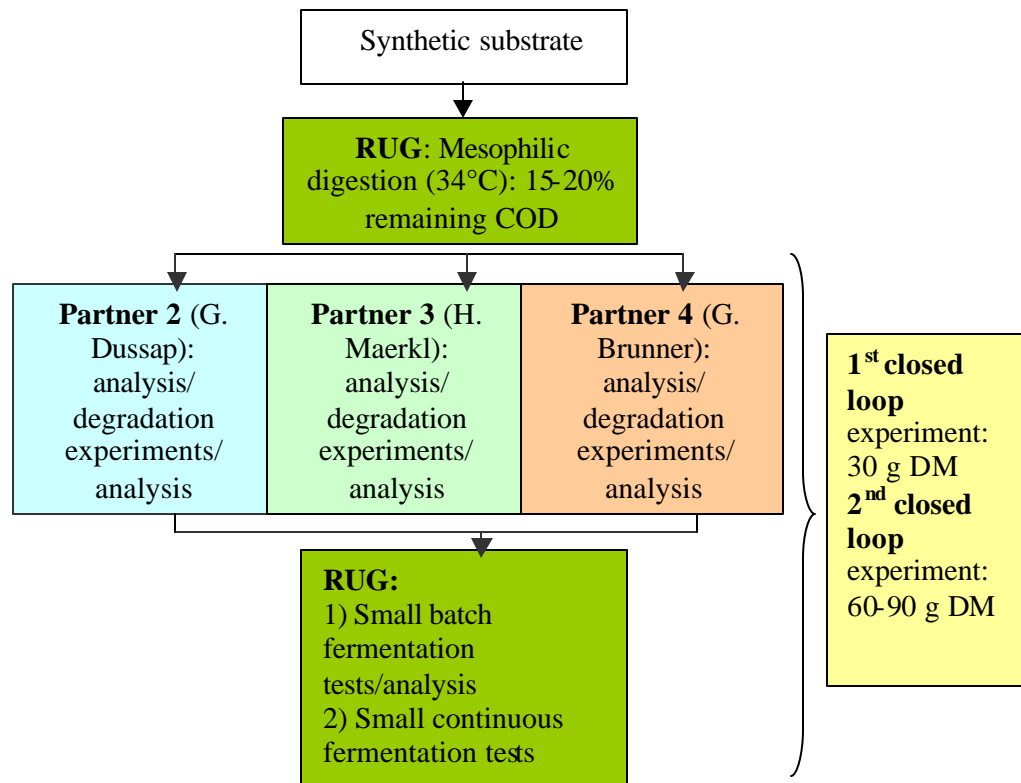


Figure 1: Closed loop experiments and main objectives of the 3<sup>rd</sup> phase of the study

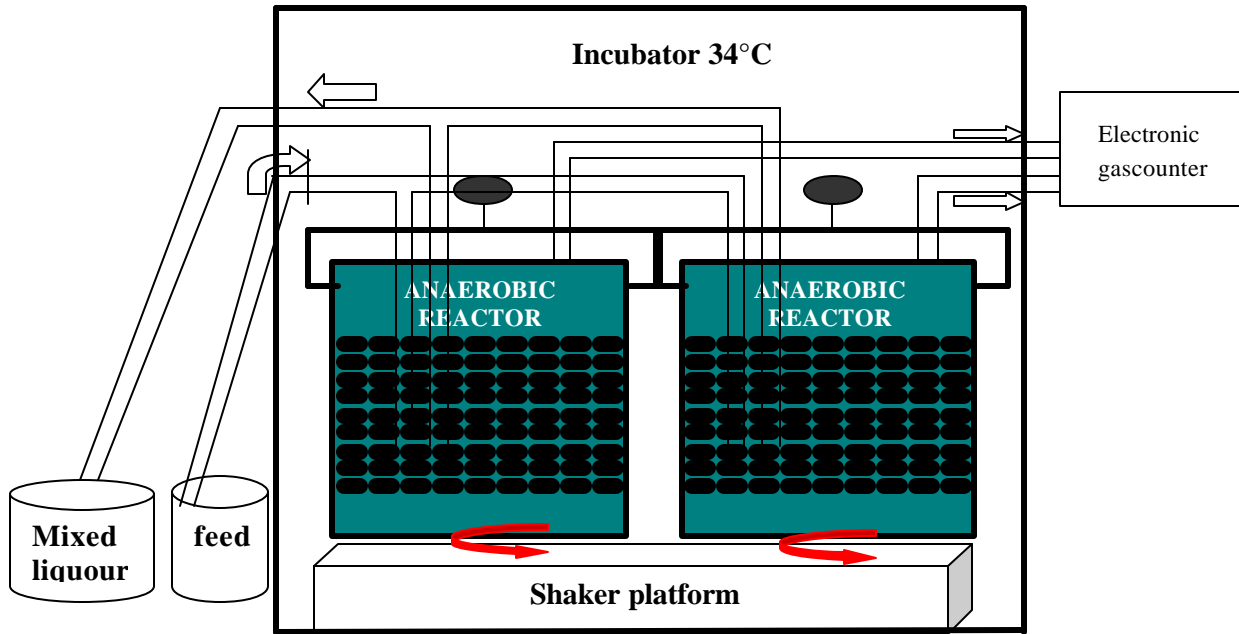
## Materials and Methods

### 1. Experimental set-up

#### 1.1 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 two minutes/hour on a shaker platform (INNOVA shaker) at a constant 90 rpm.

The feeding of 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.



**Figure 2: Scheme of the 2 mesophilic digesters for the fermentation and subsequent distribution of the synthetic substrate**

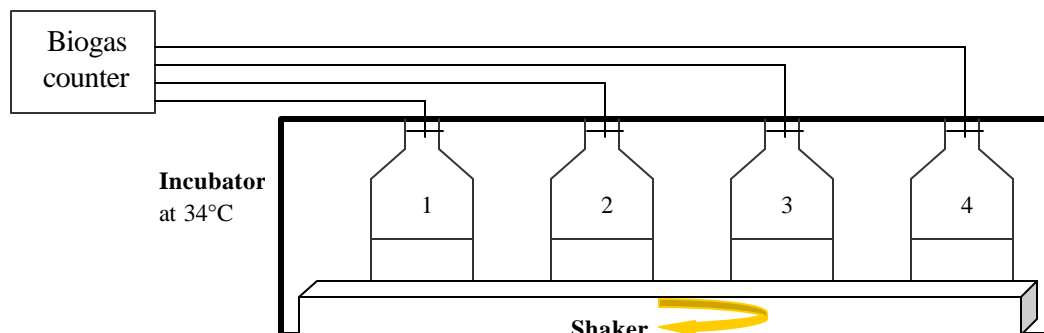
The volumetric loading rate of the mesophilic digester was held at 2.17 g COD/L.day over a period of 4 months in order to obtain the necessary amount of fibrous residue to distribute among the partners. Reactor performance was stable at the given volumetric loading rate. At higher volumetric loading rates ( $> 3$  g COD/L.day), the feed was pH-controlled (addition of sodium hydroxide) in order to prevent a fatty acid build-up in the reactor which was due to the acidification of the feed (pH 6) during storage.

In order to obtain the total amount of 270 g of dried digester particulates as agreed on the 2<sup>nd</sup> progress meeting in Barcelona, the operating parameters of the digester were changed. The volumetric loading rate was increased to 2.5 g COD/L. during the preparation of the fibrous residue for the 2<sup>nd</sup> closed loop experiment (60 g DM fibrous residue/partner). The dry matter content of the synthetic feed was strengthened up to 3.8% dry matter in order to meet the substrate criteria. The reactor was fed in quantities of 0.5 L feed/day. In order to maintain an hydraulic retention time of at least 15 days, the liquid reactor volume of both reactors was set at 7.5 L.

### 1.2 Batch fermentation tests

In order to evaluate the digestability of the different returned substrates, different experiments were set up with batch fermentation tests. All experiments were performed in 2 L bottles containing a fixed amount of mixed liquor from the two

main mesophilic reactors (as described in 1.1). The volume of mixed liquor present in each batch bottle varied from 100mL (small batch tests) until 750 mL (large batch tests) depending on the availability of returned substrate. The mixed liquor was taken from the main digester after shaking the reactor. Consequently, the mixed liquor contained a solid phase, existing both of granular sludge (methanogenic bacteria) and residual fibers from previous fermentations. The liquid phase consists mainly of soluble biopolymers. All experiments were performed at an hydraulic retention time varying between 15 and 20 days.



**Figure 3: Experimental set-up for batch fermentation tests**

Bottle 1 was used as control and therefore contained only mixed liquor from one of the main digesters. To bottle 2,3 and 4, different substrates were added from partner 2, 3 and 4 as described in the results section. The residues were added in amounts ranging from 50 mL till 300 mL representing a COD-content of 0.3 g till 2.7 g. The volume of biogas and pH was continuously measured for each bottle. All biogas yields have been calculated on COD-basis.

## **2. Substrate composition and preparation**

The composition of the 2.8% DM substrate was similar to the composition described in TN2:

90 g Spirulina (95%DM)	= 85.5 g DM = 2.85g/L = <b>10%</b>
210 g wheat straw (95%DM)	= 199.5 g DM = 6.65g/L = <b>24%</b>
2100 g fresh cabbage (9%DM)	= 189 g DM = 6.3g/L = <b>22.5%</b>
210 g soya (90%DM)	= 189 g DM = 6.3 g/L = <b>22.5 %</b>
1800 g faeces (10%DM)	= 180 g DM = 6g/L = <b>21.5 %</b>

Regarding the composition of the 3.8% DM substrate, the relative proportions of the different substrate components were kept similar.

With regard to the substrate preparation, *Spirulina* algae and soya were added to a small amount of tap water. Chopped straw was received from Partner 3. This straw was used for the digestion and preparation of 270 g of dried particulates which have been distributed in two phases (once in march and once in april). The cabbage and faeces were originally grinded with a kitchen mixer in a separate amount of water and then added to the algae, straw and water. At high volumetric loading rates, sodium hydroxide was added to the feed in order to increase the pH with 1.5 units. After a first fermentation, the effluent was centrifuged at 7000 g for 15 min leaving a fibrous residue and a supernatant phase. The fibrous residue

### **3. Influent and effluent analysis**

VFA-analysis (Volatile Fatty Acid), DM-content (dry matter), COD (chemical oxygen demand), Van Soest analysis (lignine, cellulose and hemicellulose analysis),  $\text{SO}_4^{2-}$ -S and  $\text{PO}_4^{3-}$ -P (ion-chromatography) were measured prior to and after fermentation. The concentrations after fermentation were calculated according to the dilution ratio of the mixed liquour and the added amount of fibrous residue.

The biogas composition was determined by means of gas chromatography. By including a septum between the digester and the gas meter device, gas sampling could be done on regular time intervals.

The phosphorous, sulfur and chloride concentrations present in the influent and effluent were determined by means of ion chromatography. Both influent- and effluent samples were diluted 100-fold after centrifugation and filtering over a 0.45  $\mu\text{m}$  filter. Peak identification and quantification of the components detected with this system were accomplished by internal standards, allowing to convert peak areas to concentration values.

Van Soest analysis was carried out according to the procedure provided by partner 2 (UBP) with some slight modifications. A first modification was that the samples were vacuum filtered with a Büchner filter and ordinary 40-90  $\mu\text{m}$  filter paper instead of using sintered glass. Secondly, the fibrous residues were dried at 80°C instead of 60°C for the same period of time.

## Results

### 1. Characterisation of the mixed liquour from the main digesters

In first instance, standard analysis was performed on the mixed liquour which was used for all batch fermentation tests. The results are shown in Table 1.

**Table 1: Composition of the mixed liquour**

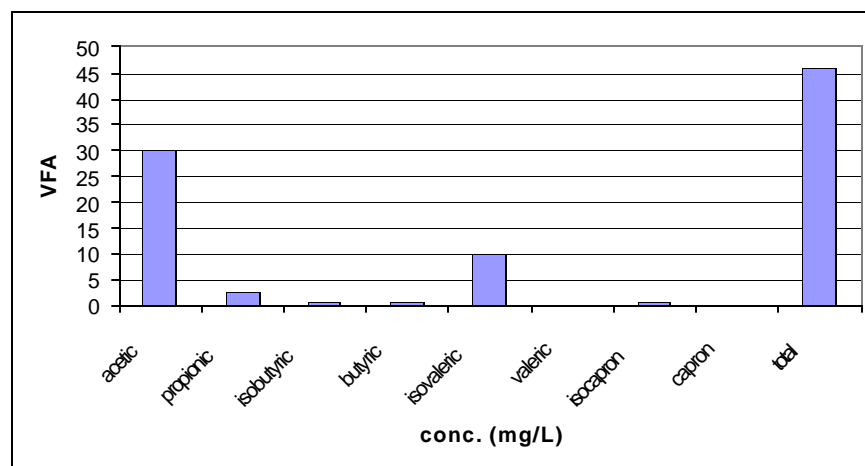
DM-content	COD	TAN	PO <sub>4</sub> <sup>3-</sup>	SO <sub>4</sub> <sup>2-</sup>	pH
2.4%	28 g/L	1.14 g/L	51 mg/L	58 mg/l	7.5

TAN (Total Ammonia Nitrogen) levels were found to be in the range according to the COD content. Phosphate and sulfate levels are rather low revealing the low P and S content of the feed and mixed liquour.

### 2. Biogas production of the main digesters

During the fermentation of the raw substrate, the biogas production was constantly monitored with an electronic gas metering device for both main digesters. The biogas production was found to be in accordance with the volumetric loading rate with on average a production of 0.4 L biogas/g COD or a biogas yield of 80%. The average methane content, measured over a 2 months period, accounted for 61% of the total biogas production.

The VFA profile of the reactor (Figure 4) shows a stabile performance with a total VFA concentration as low as 45 ppm. These results show that VFA's are consumed at a rapid pace by the methanogenic bacteria resulting in turn in a high biogas yield.



**Figure 4: VFA-profile of the mixed liquour from the main digesters**



Reactor performance was stable both at low (< 2.2 gCOD/L.d) and high (> 3 gCOD/L.d) volumetric loading rates. By controlling the pH of the feed, it could be shown that the H<sub>2</sub>S problem defined in the last TN (TN2) was not the cause for the reactor failure at high loading rates but the acidity of the influent (pH 6-6.5).

### 3. Results of the 1<sup>st</sup> closed loop experiment

#### 3.1 pH-effect

Four different batch fermentation experiments were conducted with the returned subcritical liquefaction effluent from Partner 4 (indicated as "P4" for the remainder of the document) as schematised in the Material and Methods section.

In a first series of experiments, it was noticed that the pH of the mixed liquor gradually decreased due to the relatively high acidity of the P4. This acidification starts at a dilution ratio (= volume of digestate over the volume of mixed liquor) of 1/6 with a significant pH decrease from a dilution ratio of 1/2 on (see Figure 5).

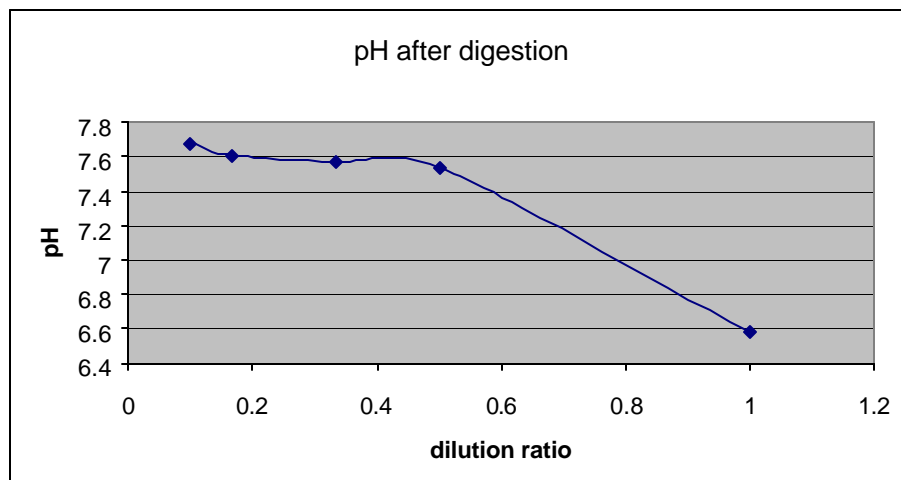


Figure 5: Gradual pH decrease during fermentation with increasing dilution ratio

It should be noticed that dilution ratios as much as 1/4 or higher might cause an acidification effect due to overloading of the methanogenic reactor. However, acidification (0.1-0.2 pH unit s) takes already place at a dilution ratio as low as 1/6. This ratio is similar when the 7.5 liter main digester is fed at a loading rate of 1L influent per two days or a B<sub>v</sub> of 1.86 g COD/L.day.

### 3.2 Biogas yield

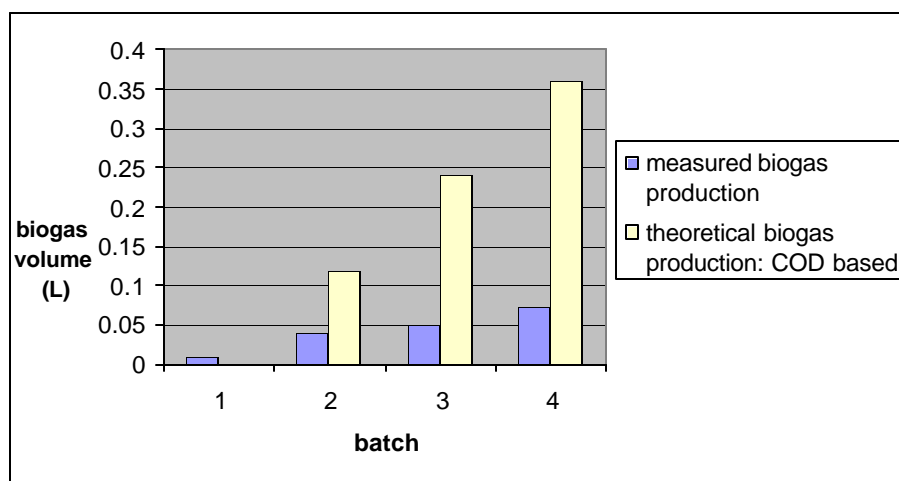
Assuming a theoretical biogas production of 0.5L per g COD, one can calculate the theoretical biogas production from the influent COD. Dividing the measured biogas production by the theoretical biogas production leads to the biogas yield.

From Table 2, it can be derived that the biogas yield roughly varies from 20-35% for the P4 with the highest biogas yield at the lowest dilution rates. This observation might be an indication for the presence of inhibitory compounds present in the subcritical liquefaction residue (P4). However, it should be mentioned that the methanogenic consortium hasn't got the time to adapt to P4 (see Discussion section). As a result, additional experiments with an adapted methanogenic consortium need to be performed in the nearby future.

**Table 2: COD balances and biogas yield with the liquefaction effluent at dilution ratio 1/6, 1/3 and 1/2**

mixed liq (mL)	P4 (mL)	pH after digestion	Meas. biogas (L)	IN DM (g)	IN COD (g)	Theoretical biogas (L)	COD yield (%)
600	0	7.68	0.01	0	0	0	0
600	100	7.61	0.04	0.1129	0.24	0.12	33
600	200	7.57	0.05	0.2258	0.48	0.24	21
600	300	7.54	0.075	0.3387	0.72	0.36	21

In Figure 6, the measured and theoretical biogas production are compared for the experiment described in Table 2.



**Figure 6: Measured and theoretical biogas production during fermentation of the subcritical liquefaction effluent (P4). Legend: 1 = control, 2 = 100 mL P4, 3 = 200 mL P4, 4 = 300 mL P4**

Despite the moderate biogas yields, all fermentation trials with P4 were characterised by a long lag phase. No significant differences in biogas yields could be noticed between the effluents V1, V2 and V3 (codes correspond to different operating parameters during the subcritical liquefaction, Partner 4). At high loading rates, the total VFA concentrations rised to a ten-fold higher concentration compared to the VFA content of the control (solely mixed liquour).

The VFA profiles of the 4 batches after fermentation ( $\Theta = 17d$ ) are shown in Figure 7. It can be derived that the VFA concentrations remain more or less constant except for the batch 4 (300 mL P4) where the final total VFA content was found to be ten-fold higher compared to the other three batches. The longer VFA's (e.g. capron acid) were mainly responsible for this VFA increase.

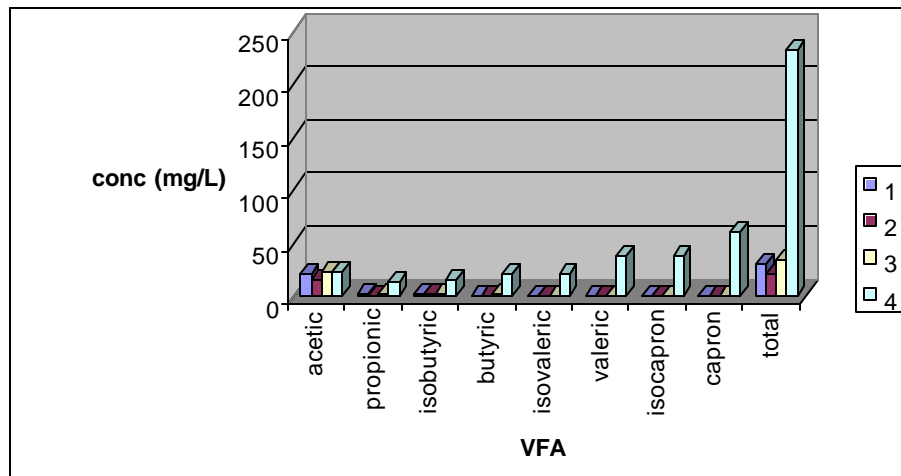


Figure 7: VFA profile after fermentation for the 4 batches (P4)

#### 4. Results of the 2<sup>nd</sup> closed loop experiment

##### 4.1 Biogas yields

During the 2<sup>nd</sup> closed loop experiment, 60 g of dried fermented fibrous residue was distributed among the other partners and returned for a second mesophilic digestion. A batch fermentation experiment was set up (as described in the Material and methods section) in which the biogas yields of the subcritical liquefaction residue (Partner 4, noted as "P4"), the hyperthermophilic residue (Partner 3, noted as "P3") and the *Fibrobacter* residue (Partner 2, noted as "P2") were compared.

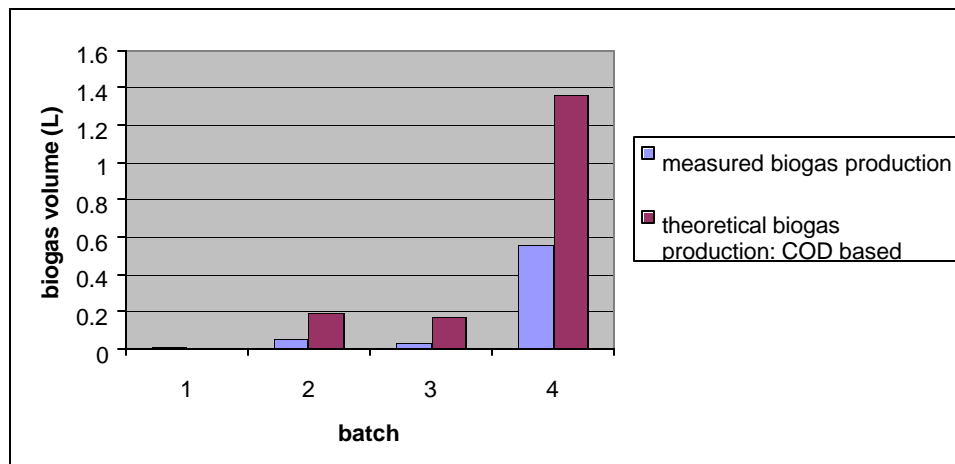
In Table 3, the results with regard to the biogas yields are shown. The biogas yields are clearly the highest for P2 (*Fibrobacter* residue) and P4 (Subcritical liquefaction),

with a significantly lower biogas yield for the P3 (Hyperthermophilic residue). The dry matter content and the COD content corresponded well for P2 and P3 but for P4, the COD-content was found to be appr. 2 times higher compared to the DM-content. This can indicate the presence of a large amount of volatile compounds present in P4, which are stripped off during the drying at 105°C for DM-analysis.

In Figure 8, the theoretical and measured biogas production are compared for the 3 hydrolysates (P2, P3 and P4) and a control. From this graph, it can be concluded that about up to 40% of the fermented fibrous residue can be converted further into biogas by mesophilic fermentation after hydrolysis by *Fibrobacter* or sub. liquefaction.

**Table 3: COD balances and biogas yield with the liquefaction effluent (line 2), hyperthermophilic residue (line 3) and *Fibrobacter* residue (line 4) at a dilution ratio of ¼ (control = line 1)**

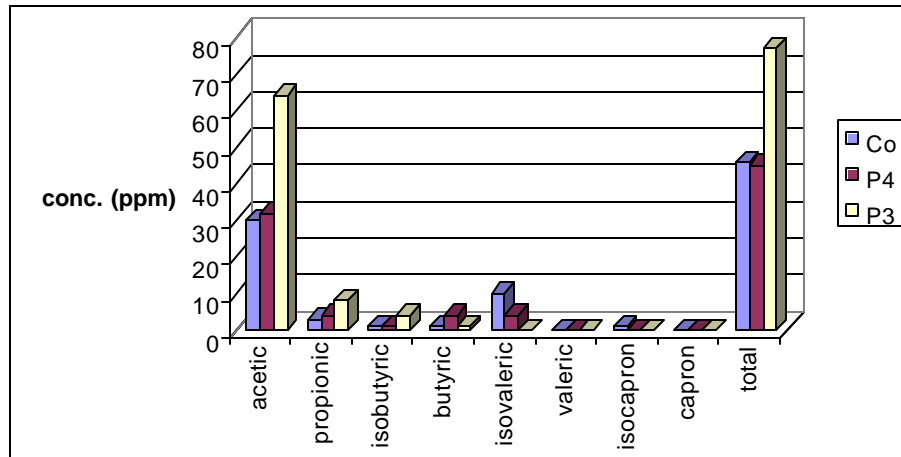
mixed liq (mL)	sample (mL)	pH sample after digestion	Meas. Biogas (L)	IN DM(g)	IN COD (g)	Theoretical biogas (L)	COD yield (%)
600	0	7.65	0.01	0	0	0	0
600	150	7.63	0.055	0.169	0.375	0.1875	29.3
600	150	7.58	0.035	0.339	0.34	0.17	20
600	150	7.63	0.56	2.73	2.75	1.375	40



**Figure 8: Measured and theoretical biogas production during fermentation of P2, P3 and P4. Legend: 1 = control, 2 = Subcritical liquefaction residue (P4), 3 = Hyperthermophilic residue (P3), 4 = *Fibrobacter* residue (P2)**

#### 4.2 VFA-profiling

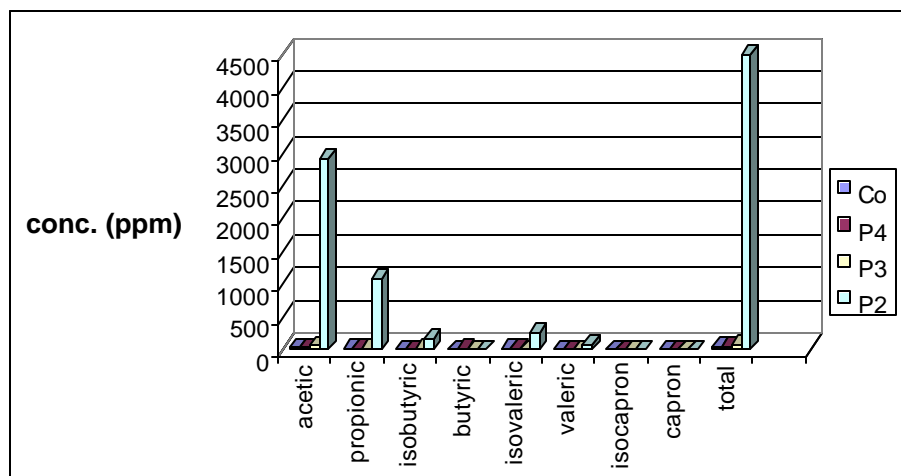
Firstly, VFA-analysis was performed on P2 (*Fibrobacter* residue), P3 (hyperthermophilic residue) and P4 (subcritical liquefaction residue) prior to batch fermentation. The results are summarized in Figure 9 and Figure 10.



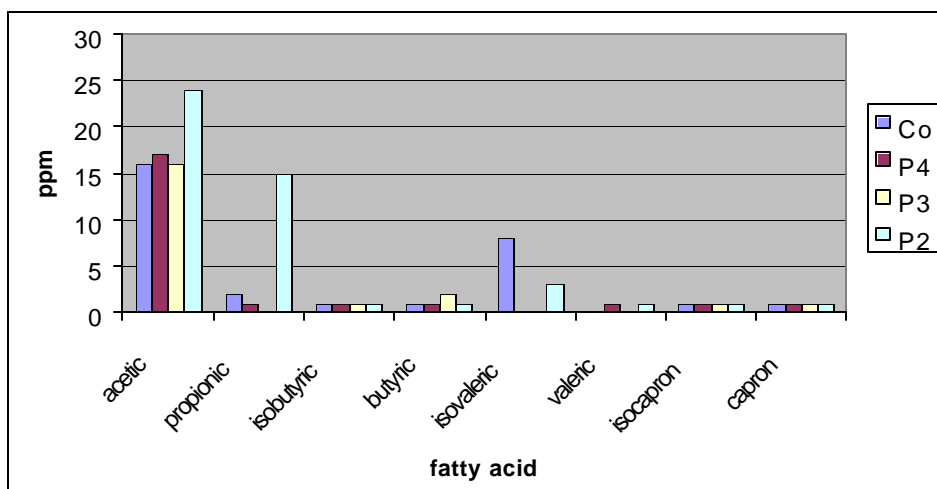
**Figure 9: VFA profile of, P3 and P4 before fermentation**

From Figure 9, it can be deduced that acetic acid is most commonly present in P3 and P4. The total VFA-concentrations are low compared to the *Fibrobacter* residue (P2). As a matter of fact, the total VFA concentration in P2 was found to be 100-fold higher compared to P3 and P4 (Figure 10). The major VFA's present in P2 were all found to be readily biodegradable and were mainly acetic acid and propionic acid.

Secondly, VFA-analysis was performed at the end of the batch fermentation tests. The results are depicted in Figure 11. The VFA-levels after digestion were comparable for the control, P3 and P4 and were somewhat higher for P2 (*Fibrobacter* residue).



**Figure 10: VFA-profile of P2, P3 and P4 before fermentation**



**Figure 11: VFA-profiles of P2, P3 and P4 after fermentation**

This indicates that the total VFA concentration for P2 was reduced at least 25-fold during mesophilic digestion (taking into account the dilution factor of 1/4).

#### 4.3 N, P and S profiling

TAN (Total Ammonia Nitrogen) were found to be low for the subcritical liquefaction effluent (Partner 4), in the range of 40-60 ppm. As can be seen in Table 4, TAN concentrations increased after mesophilic digestion with 16-96 ppm. These values are relatively low compared to the total TAN present in the batches. However, the increase might indicate the solubilisation of organically bound nitrogen into ammonia. A few Kj-N measurements will be made to confirm this statement.

**Table 4: TAN concentrations before (mixed liq.) and after (mixed liq. + digestate) fermentation for the subcritical liquefaction effluent coded as T1 and V1**

		T1 (750 mixed liq.)					V1 (600 mL mixed liq.)		
mL added	TAN (mg/L) initial	TAN (mg/L) after	increase	mL added	TAN (mg/L) initial	TAN (mg/L) after	increase		
0	1104	1100	-4	0	1110	1108	-2		
50	1034	1122	88	100	958	992	34		
100	976	1024	48	200	846	862	16		
150	926	1022	96	300	758	782	24		

With regard to the P and S balances, it can be derived from Table 5 that the P and S levels in the mesophilic digester (Control) are low compared to the carbon and nitrogen content. P and S content of P4 and P3 are even lower. These values are related to the high fiber content of the feed (high C/N, S, P ratio).

The P –and S values are 10-fold higher for P2. As discussed on the lastest progress meeting, these high values can be explained by the presence of a cysteine buffer (incl.  $\text{HPO}_4^{2-}$ ) which is used to initiate the fermentation by *Fibrobacter succinogenes*.

**Table 5: P and S contents of the mixed liquour (Co) and the pure digestates before fermentation (P2, P3, P4)**

sample	$\text{PO}_4^{3-}$ -P	$\text{SO}_4^{2-}$ -S
Co	51.6	58.6
P4	19.8	20
P3	10.4	7
P2	531.6	416.9

It can be derived that the C/N/P,S ratio of the mesophilic digester is in the range of 24/1,5/0,05 which makes this substrate very suitable for anaerobic digestion.

#### 4.4 Van Soest Analysis

The fiber content before and after digestion was determined for two fermentation tests with the *Fibrobacter succinogenes* effluent and with the subcritical liquefaction effluent. Van Soest analysis was not performed on the hyperthermophilic residue since no satisfactory liquefaction and biogasification could be reached in this way.

In first instance, the fiber fractions of the methanogenic reactor were determined. This was necessary since the reactor (mixed liquour) contains remaining fibrous particulates which have to be taken into account to evaluate the biogasification of the fibers present in the feed. The analysis was performed in duplicate (sample 1.1 and 1.2 in table 6) in order to check the experimental error. The results were found to differ little except for the lignine solid fraction which was hard to separate from the sulphuric acid (72%) fraction.

Sample 2 and 3(table 6) represent the first batch fermentation experiment. In this experiment, approximately 2 g of dried *Fibrobacter* fibers (only the solid residue) were gradually added to mixed liquour (500mL) from the methanogenic reactor. In a second batch, 250 mL of subcritical liquefaction effluent was added gradually to 500

mL of mixed liquor. After a residence time of 15 d, Van Soest analysis was performed on both batches.

With regard to the initial fiber content of the different residues, only the *Fibrobacter* residue could be analysed for its fiber content since no sufficient amount of particulates was available for the subcritical liquefaction effluent (see sample 4.1 and 4.2 in table 6).

Because no sufficient amount of particulates were available in the batch tests, returned effluents (*Fibrobacter* and subcritical liquefaction) were fed to two main methanogenic digesters (each with  $V = 5.5$  L) for a period of 1 week in a dilution ratio of 1/11 (0.5 L of sample) to exclude overloading. The fiber content of the reactor was determined after a residence time of 17 d for both residues (see sample 5 and 6 in table 6).

**Table 6: Cell wall quantification (Van Soest Analysis) on the solid fermentation residues**

Sample	Sample name	Total fibers (%)	Hemicellulose (%)	Cellulose (%)	Lignin (%)
1.1	Mixed liquor	49	15.4	24.6	9
1.2	Mixed liquor	43	20.7	16.3	6
2	Exp 1 (Fibro.)	47.3	14.2	29.1	4
3	Exp 1 (Subcr.)	52.3	13.7	33.6	5
4.1	<i>Fibrobacter</i>	22.2	16	2.2	4
4.2	<i>Fibrobacter</i>	22.2	14	5.2	3
5	Exp 2 (Fibro.)	47.85	18.25	23.6	6
6	Exp 2 (Subcr.)	39.77	19.73	13.04	7

From table 6, it can be concluded that the solid residues present in the methanogenic reactor consist of about 50% total fibers. From this 50%, about 15-20% represents hemicellulose and 20-25% is under the form of cellulose. The remainder is inert material such as lignin. It can also be deduced that the returned solid *Fibrobacter* residue only contained 22% fibers which confirms the high solubilisation power of *Fibrobacter* for cell wall constituents, particularly for cellulose. The results from batch experiment 1 (sample 2 and 3) show that the methanogenic reactor efficiently converts the released fiber from both *Fibrobacter* and the subcritical liquefaction effluent since the same fiber content was found as in the control (sample 1 and 2) at low dilution rates.

In the second experiment (with the main methanogenic reactors), the biogasification was also recorded on the side. Biogas production of the subcritical liquefaction effluent was found to be very efficient. As a matter of fact, approximately 2 g of COD



was inserted into the reactor with a measured biogas production of 1062 mL after 2 days (residence time of appr. 20 d). Theoretically, 2 g of COD would correspond to a biogas production of approximately 1 L. This would mean that the liquefied residue was very efficiently (close to 100%) converted into biogas. This value is significantly higher than the value found in the small batch fermentation tests. It should however be mentioned that no control was taken into consideration in this test and that there consequently might have been an additional contribution of biogas due to substrate already initially present in the reactor. A long duration fermentation test with the liquefaction residue (at least 1 month) will clarify this issue.

The biogasification of the *Fibrobacter* residue took longer, probably due to the presence of partly digested fibers which are less accessible for the methanogenic bacteria. After 2 days of fermentation ( $\theta = 15$  d), a biogas production of only 1.15 L was measured. This amount was found to be much lower than the theoretical biogas production being approximately 5 Liters ( $\text{COD}_{\text{feed}} = 10\text{g}$ ). This would mean that the *Fibrobacter* residue is fermented with a biogas yield of 23%.

## Discussion

From the presented results, it should be clear that the subcritical liquefaction and the *Fibrobacter* rumen bacteria are the most promising techniques for the hydrolysis of the fermented lignocellulolytic residue prior to a subsequent second methanogenesis. The discrepancy between the batch fermentation results and the fermentation results with the main methanogenic reactors are striking. With regard to the subcritical liquefaction effluent, the discrepancy can be explained on the basis of two (synergistic) effects. On the one hand, overloading during the batch fermentation tests can explain the relatively low biogas yields compared to the main fermentation tests. Indeed, in the latter, the dilution rate was found to be much lower compared to the batch tests. Secondly, the dilution effect is also responsible for the dilution of possible hazardous toxic compounds such as fermentation inhibitors which might be present in the liquefaction effluent.

With regard to the results presented for the *Fibrobacter* residue, the biogas yield was found to be significant lower in the main fermentation test compared to the small

batch tests. This discrepancy can partially be explained through the high experimental error in the batch test due to the presence of particulates in the *Fibrobacter* residue. Since the partially digested particulates contain the largest amount of COD, the COD value of the feed can be rather inaccurate. A longer main fermentation test will therefore also be set up with the *Fibrobacter* residue.

In the rumen, *Fibrobacter* species and methanogenic bacteria occur together and mostly compete for commonly available substrates such as hydrogen gas and formate (Asanuma et al., 1999). As a result, it can be stated that the proposed loop as agreed upon on the progress meeting in Hamburg is to a large extent the simulation of the rumen system. On the one hand, the subcritical liquefaction should be seen as a means to achieve the highest liquefaction and biogasification possible. On the other hand, the thermal treatment guarantees complete sterilisation of the residue (Schieder, 2000);(Bonmati *et al.*, 2001).

## Conclusions

- The subcritical liquefaction and the *Fibrobacter* rumen bacteria are clearly the most promising techniques for the hydrolysis of the fermented lignocellulolytic residue prior to second methanogenesis. Liquefaction and biogasification with the hyperthermophilic residue is minor.
- From batch fermentation tests, it can be concluded that the biogas yields for the fermented fibrous residue are in the order of 40% for both the rumen bacterium *Fibrobacter* and the subcritical liquefaction. From a main fermentation test however, the fermentation potential of the subcritical liquefaction effluent was found significantly higher (up to 90%) probably due to a much lower dilution ratio (neither overloading nor inhibition). As a result, at least 90% of the influent COD can be converted into biogas by means of the loop methanogenesis-*Fibrobacter* / subcritical liquefaction-methanogenesis.
- It could be shown that inhibition of methanogenesis occurred from a dilution ratio of 1/6 on with the subcritical liquefaction effluent. This effect is beside the possible presence of inhibitory substances primarily due to overloading and secondly to the shock-effect on the methanogenic consortium (no adaption).

- Liquefaction of cellulose is most easiest to achieve (results *Fibrobacter*). Due to intense cross-linking of hemicellulose with lignin, the liquefaction of hemicellulose is most challenging

## Outlook

- A long fermentation experiment (at least 1 month) with both the subcritical liquefaction effluent and the *Fibrobacter* effluent is planned in the near-future of which the results will be added in TN4. Firstly, this experiment should give more insight into 1) hemicellulose, cellulose and lignin degradation, 2) inhibitory effects and 3) biogas yields. Secondly, in this way, the methanogenic consortium will be conditioned (adapted) towards the both effluents.
- The two available methanogenic reactors will continue fermenting raw synthetic substrate which will be distributed after sterilisation to Partner 2 (UBP) for subsequent degradation.
- A third methanogenic reactor will be started up for the direct fermentation of the liquid returned from Partner 2. Due to the much shorter retention times, a sludge withdrawal system will have to be implemented into the reactor to prevent sludge wash-out.
- Finally, the subcritical digestate will be reintroduced in the main methanogenic reactor closing the loop.

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