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TESTING OF A COUPLED THERMOPHILIC-METHANOGENIC REACTOR

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1. INTRODUCTION

Throughout the first phase of the MAP – project "A total converting and biosafe liquefaction compartment for MELiSSA" a thermophilic liquefaction step was examined. The general results of the studies were that liquefaction was quick but incomplete at elevated temperatures.
From our long experience with other thermophilic bacteria and archaea we know that dialysis technique has a strong positive impact on the performance. We therefore decided to concentrate on continuous dialysis experiments. The continuous nature of the culture is also important, considering the potential application later on. Especially long-term stability is of vital importance in space applications.
For the prolongation of the project the research will focus on the coupling of a hyperthermophilic continuous liquefaction unit with a mesophilic biogas reactor trying to increase the volumetric degradation rate. As benchmark we use the liquefaction performance of the original MELiSSA thermophilic liquefaction or the mesophilic methanogenesis reactor of partner 1. Both fermentors achieve a degradation performance of approximately 75% and a volumetric loading rate of 2g/(l d). In our studies we could realize much higher volumetric loading rates of 13 g/(l d) but at a lower liquefaction performance. The same liquefaction performance as reached at a loading rate of 2.5 g/(l d).
Instead of the coupling with a methanogenic reactor to regenerate the dialysate a concentration step can be thought of. The concentrated dialysate can be directly passed to the phototrophic compartment. The liquefaction compartment can be down scaled severely. No biogas will be produced in this arrangement, reducing the volume of gas tanks and piping.

2. MATERIAL AND METHODS

2.1 SUBSTRATE

2.1.1 ESA-SUBSTRATE

The ESA-Substrate consists of wheat straw, soy pellets, cabbage, algae, and faecal matter. To draw balances, the carbon- and COD-content of each constituent was determined.
The COD-values and TC-values of the ESA-substrate were calculated to COD_{feed}= 19.44g_{COD}/l, TC_{feed}=6.00g_C/l, and TN_{feed}=0,53g_N/l (without faeces, TS=1.5%). The C/N ratio is calculated to 11.9.
For continuous dialysis fermentations a mixture of 17.7% algae and 41.1% of straw and Soy beans was used. All carbon balances were done with an average carbon content of 40% carbon per dry biomass.

2.1.2 SUBSTRATE FOR METHANOGENIC FERMENTOR

Caldocellulosiruptor medium was used without carbon source. The medium was buffered to pH7 with a 6mM phosphate buffer. Acetic acid was used as carbon source in concentrations ranging from 5 to 20mM. No organic nitrogen source was added.

2.2 DETERMINATION OF TOTAL SOLIDS (TS)

Two samples of 10 ml each are filled in previously dried and weighed ceramic beakers. The samples are weighed again. Then the beakers are dried at 105°C for 48 hours. After this the beakers are cooled down and weighed again. The TS-value is calculated by dividing the mass difference after drying by the mass difference before drying. The arithmetic average of the two measurements is determined.

2.3 MINIMAL MEDIA

For Fermentation of pure cultures the following media were used

2.3.1 *CALDOCELLULOSIRUPTOR LACTOACETICUS*

Medium 671 from DSMZ was used. The medium contains the following substances [mg/l]: 1000 NH4Cl, 100 NaCl, 100 MgCl2x6H2O, 50 CaCl2x6H2O, 400 K2HPO4x3H2O, 2600 NaHCO2, 750 Yeast extract, 500 Cysteine, 10ml Trace element solution from DSMZ Medium 141, 1ml vitamin Solution from *Pyrococcus furiosus* medium, 1ml Resazurin solution.
For pre-cultures 2g/l cellulose was used as main carbon source.

2.3.3 *ANAEROCELLUM THERMOPHILUM*

Medium 516 from DSMZ was used. The medium contains the following substances [mg/l]: 330 NH4Cl, 330 KH2PO4, 330 KCl, 330MgCl2x6H2O, 330 CaCl2, 500 Yeast extract, 1500

NaHCO3, 500 Cysteine, 1ml Resazurin Solution, 10ml Trace element solution, 1 ml vitamin solution.
For pre-cultures 5g/l cellobiose was used as main carbon source.

2.4 MICROORGANISMS

2.4.1 *CALDOCELLULOSIRUPTOR LACTOACETICUS*

The extremthermophilic obligate anaerobic Bacterium *C. lactoaceticus* (DSMZ9545) was isolated by Mladenovska et al. from alkaline hot springs in Iceland. The bacterium is not spore forming. The optimal growing conditions are 70°C and pH7. *C. lactoaceticus* is able to break down cellulose and degrade it to CO2 (8%), acetic acid (18%), lactate (52%), ethanol (1%), hydrogen (1%) and glucose (16%). The turnover was 95% after 3 days.
C. lactoaceticus is also able to degrade pectin and xylose.

2.4.2 *ANAEROCELLUM THERMOPHILUM*

The extremophilic obligate anaerobic bacterium *A. thermophilum* (DSMZ6527) was isolated by Svetlichny et al. [1990] from geysers in Kamchatka, Russland. The bacterium is not spore forming. The optimal growing conditions are 75°C and pH7.2. *A. thermophilum* degrades Cellulose to lactate and acetic acid, CO2 and H2. *A. thermophilum* uses a wide range of carbon sources, such as glucose, mannose, glycogen, fructose, maltose, starch, and sucrose.

2.4.3 CONSORTIUM FROM THE AZORES

The hyperthermophilic anaerobic consortium was isolated from hot sources at the Azores, Portugal. Fermentation of ESA-Substrate showed a liquefaction of 40%. Lactate was only produced in traces. The main metabolic products are acetate, propionate, butyrate and valerianate

2.4.4 MESOPHILIC ANAEROBIC CONSORTIUM

Non sterile ESA-substrate was inoculated with 1ml of sewage sludge samples from different wastewater treatment plants and filled into a 1000ml bottle and stirred for 1 week at 37°C. The mesophilic consortium showed a broad variety of different single cell organisms. Cocci, rods and spore forming cells have been monitored. No sign of fungi cells or yeasts could be seen.

2.4.6 BIOMASS FOR BIOGAS EXPERIMENTS

Biomass was taken from the anaerobic sludge fouling of a local wastewater treatment plant. The biogas reactor was run at 37°C.

2.5 FERMENTATION SETUP

2.5.1 DIALYSIS EXPERIMENTS

A Bioengineering Visual Safety Membrane Reactor (VSR) was used for dialysis experiments with a total volume of 8 litres. The reaction volume is 1.5 litres, the dialysate volume 4.5 litres. A PES 300kDa UF-membrane [Micro PES-1F/PH 0.1µm Membrana GmbH, Wuppertal, Germany] was used as dialysis membrane. The membrane was glued with a scotch tape [Scotch 3M VHB 9469 19mm x 55m, Firma Bossert, Hamburg, Germany]. pH was maintained at 7.0 by titrating with 2M NaOH. Dialysate was added and withdrawn by peristaltic pumps [Watson-Marlow, 101 U/R]. Effluent and influent pumps [Watson-Marlow, 501U] were triggered by a timer for two minutes every 3 hours. Before pumping the inner stirrer was switched off for one minute. One minute after the pumping ended the stirrer was switched on again. Different dilution rates were achieved by a change in the inlet pump rate. Both chambers were gased with 10l/h Nitrogen. Figure 1 shows the fermentor.

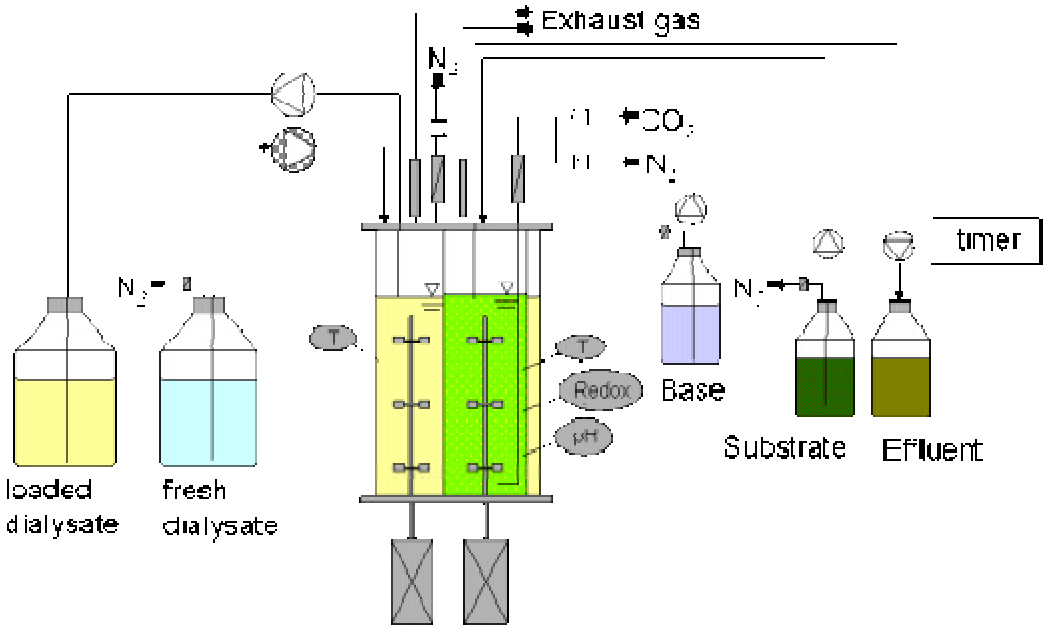

Figure 1 Piping and instrumentation of the continuous dialysis liquefaction reactor

2.5.2 FIXED BED BIOGAS REACTOR

A 5l radial flow fermentor was set up for biogas experiments. The fixed bed had a total volume of 3.12 l and was filled with Biolox 10 carriers [Rauschert, a=640 1/m, e=82%]. The maximal superficial velocity was set to 1 mm/s with a circulation pump. The reactor was heated via a heating jacket to 37°C. pH was measured online. The biogas fermentor is shown in Figure 2

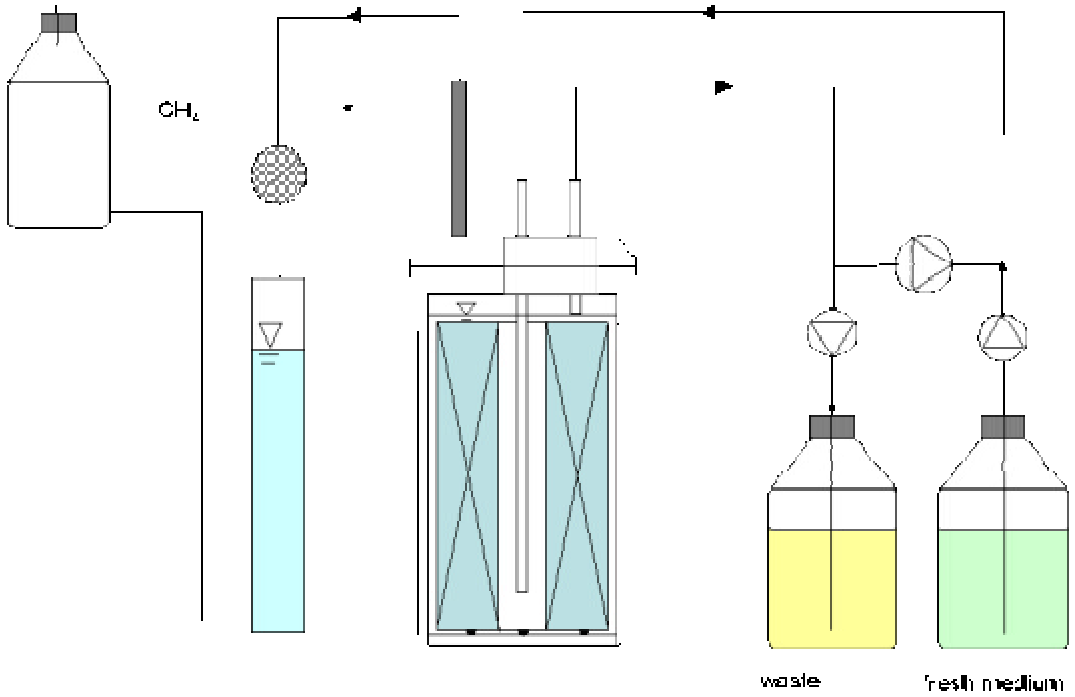

Figure 2 Fixed bed biogas fermentor for low retention times

2.6 CHEMICAL OXYGEN DEMAND (COD)

For the determination of the COD the tests LCK 414, LCK014, LCK154 from Dr. Lange with a sample volume of 200 and 1000µl were used.

2.7 DISSOLVED ORGANIC CARBON (DOC), TOTAL INORGANIC CARBON (TIC)

The DOC and TIC value of the samples was determined with TOC + TN_b from Analytic Jena.

2.8 TOTAL CARBON (TC)

The TC value of the samples were determined with Leco CNS 2000 Analyser

2.9 LACTATE AND GLUCOSE ANALYSIS

Lactate and Glucose are determined simultaneously with YSI 2700 select, YSI inc. Yellowsprings USA. The measurement is done enzymaticly.

2.10 VOLATILE FATTY ACIDS DETERMINATION

VFA are determined with a headspace gas chromatograph Chrompack CP9001. A 30mx0.32mm Nukol capillary from Supelco is used. The samples are acidified with 2% H_3PO_4. The detector is a FID.

3. OPTIMAL TEMPERATURE FOR DIALYSIS LIQUEFACTION - COMPARING ANAEROBIC AND AEROBIC CONDITIONS

Alternative to a hyperthermophilic consortium a mesophilic consortium was tested. The advantage of a mesophilic consortium is that it will probably show greater stability towards changes in growth conditions. A mesophilic consortium will be constantly reinoculated by bacteria from higher plant material . On the other hand this is also a severe disadvantage regarding the biosafety of the loop. Pathogenic microorganisms can accumulate in the dialysis reactor under mesophilic conditions.
Three different growth conditions were tested. The substrate concentration in the feed was set to 1% (10g/l). Hydraulic retention time of the reaction chamber was 4 days, the dialysate retention time was 1day. The reference was the hyperthermophilic consortium gathered from different hot springs from the Azores. The consortium is anaerobic and shows best degradation performance at 90°C. The results are shown in Figure 3. The reactor was loaded with 2% solid concentration at the beginning of the experiment. A washout of carbon occurred therefore in the first days of the experiment.

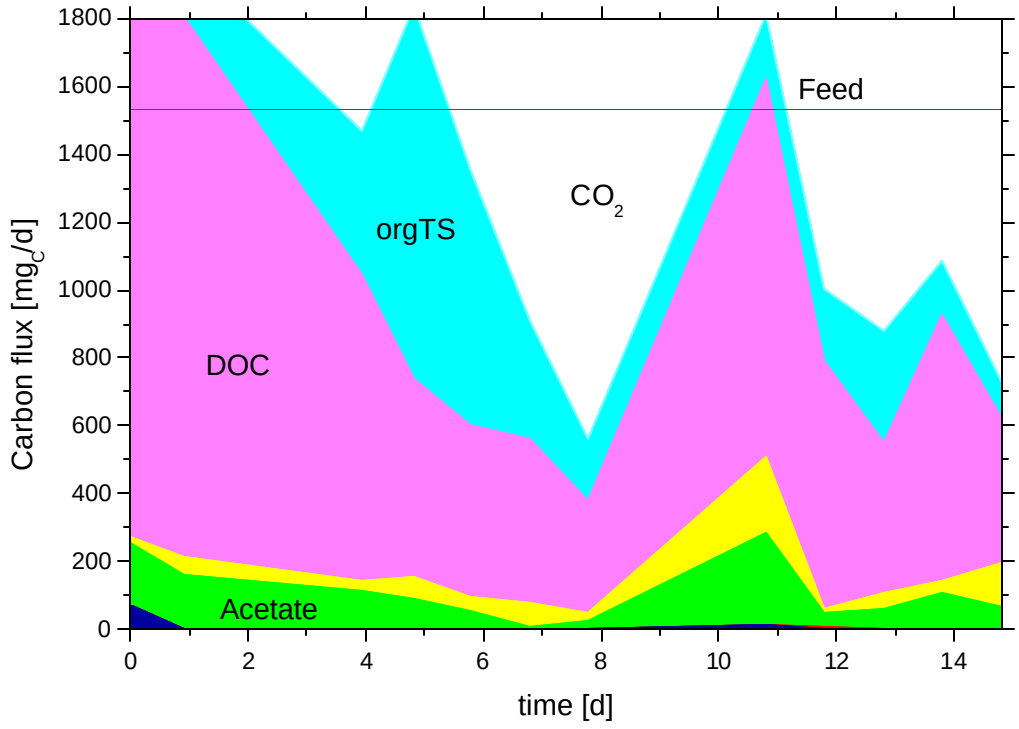

Figure 3 Anaerobic, hyperthermophilic liquefaction and degradation of 1% ESA substrate in a continuous dialysate reactor. t_R=4d, t_D=1d, 90°C, Consortium from the Azores. The fermentor was started with 2% substrate. Cyan: organic total solids, magenta DOC, yellow VFA ranging from C3 to C7, green Acetic acid, blue lactate, red glucose. The feed line displays the daily carbon flow into the system. The colored areas show the composition of the effluent and dialysate leaving the system.

The second experiment was run at 70°C with a mixed culture of *Anaerocellum thermophilum* and *Caldocellulosiruptor lactoaceticus* both obligate anaerobic non spore forming bacteria. The result of the second experiment is displayed in Figure 4.

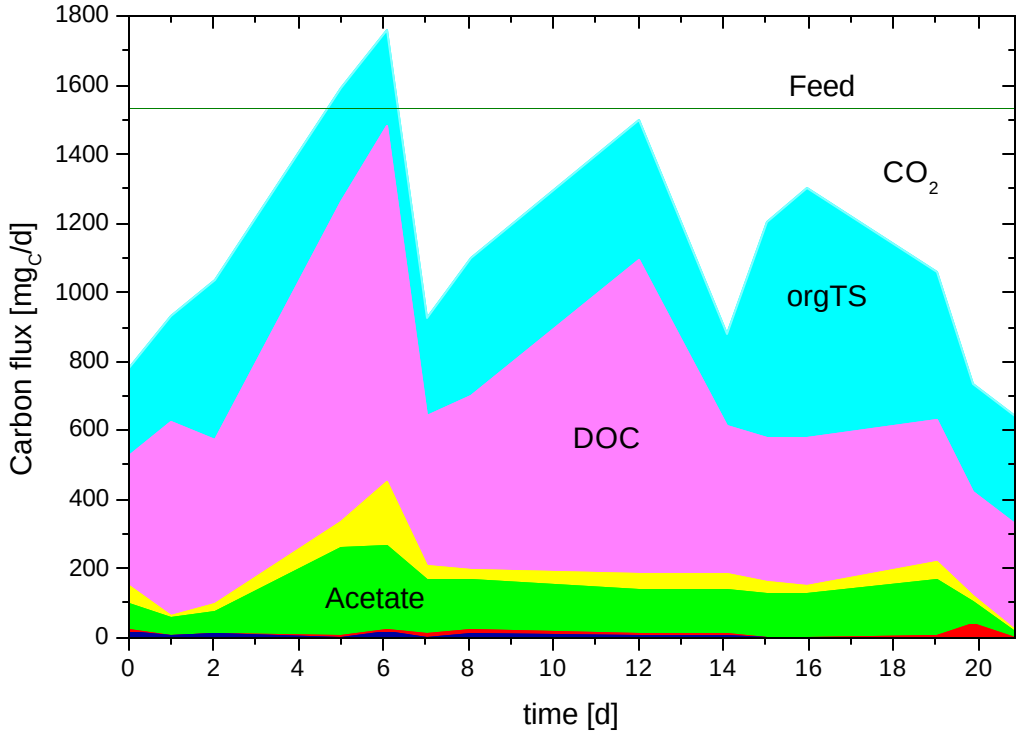

Figure 4 Anaerobic, extreme thermophilic liquefaction and degradation of 1% ESA substrate in a continuous dialysate reactor. t_R**=4d,** t_D**=1d, 70°C,** ***Anaerocellum thermophilum, Caldocellulosiruptor lactoaceticus*** **The fermentor was started with 1% substrate. The feed line displays the daily carbon flow into the system. The colored areas show the composition of the effluent and dialysate leaving the system.**

The temperature of the third experiment was set to 37°C. A consortium for mesophilic conditions was prepared from tree different samples of anaerobic sludge. A one liter pre culture was inoculated with 1% each and stirred for one week at 37°C under anaerobic conditions. The mesophilic culture showed strong gas production. Methanogenesis was prevented due to the low pH (pH4) at the end of the cultivation.
Fermentative bacteria are often facultative anaerobic, so microaerophilic growth conditions (10%air, 90% N_2) were adjusted after 20days of anaerobic cultivation. This did not show any change in reactor performance. The liquefaction and degradation performance is exhibited in Figure 5.

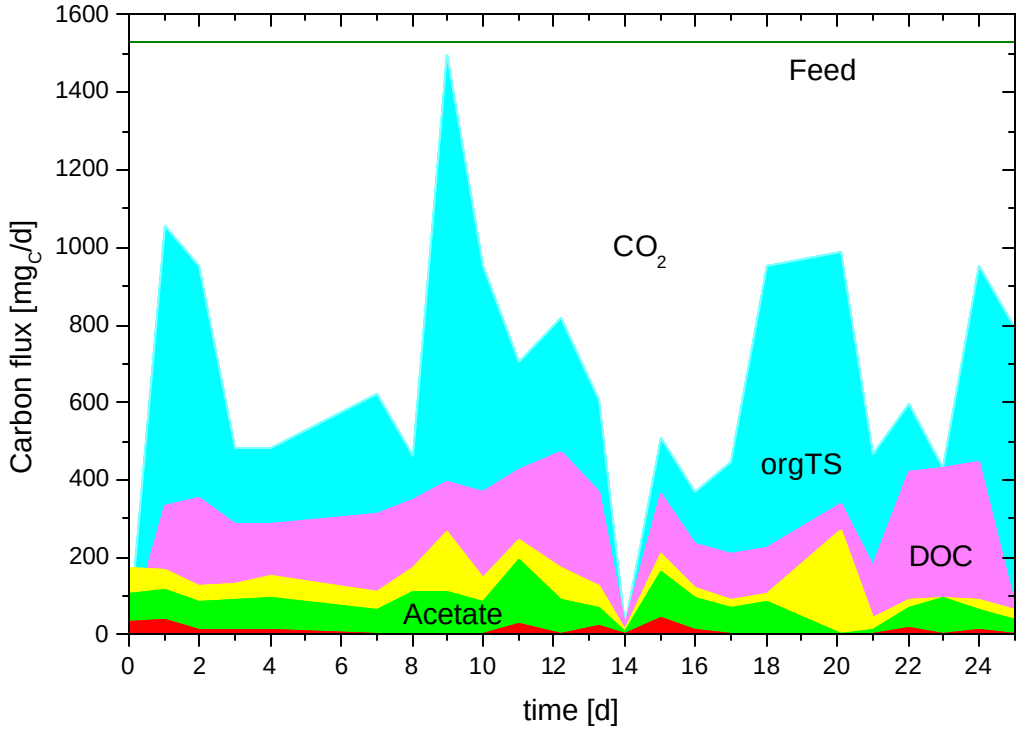

Figure 5 Anaerobic, mesophilic liquefaction and degradation of 1% ESA substrate in a continuous dialysate reactor. t_R=4d, t_D=1d, 37°C, mesophilic consortium. The fermentor was started with 1% substrate. The feed line displays the daily carbon flow into the system. The colored areas show the composition of the effluent and dialysate leaving the system.

Comparing the three experiments shows interesting features of the dialysis liquefaction. Figure 6 displays the liquefaction efficiency in steady state after two weeks at mesophilic (37°C), extreme thermophilic (70°C) and hyperthermophilic (90°C) conditions.

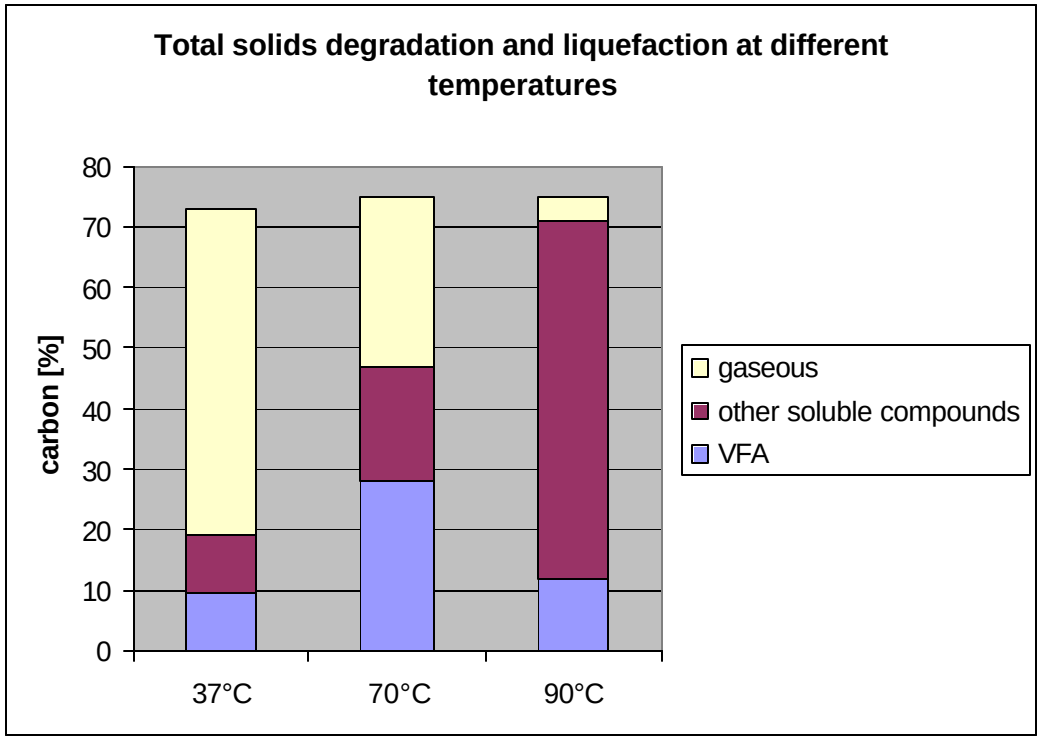

Figure 6 Liquefaction on 1% ESA-Substrate under anaerobic conditions in a dialysis reactor with a hydraulic retention time of 4 days and a dialysis retention time of 12hours. The volumetric loading rate was 2.5g$_{TS}$/(l d).

The comparison shows that the TS-degradation in a dialysis reactor is not depending on the temperature. Degradation efficiencies of approximately 75% were achieved in all experiments. The best liquefaction performance was reached at 90°C. The best gasification performance could be detected at 37°C. The highest volatile fatty acid production was exhibited at 70°C. The composition of the liquid effluent is not known in detail. It will be composed of dissolved polysaccharides, proteins and peptides, fatty acids,. Its color is comparable to tee or coffee.

The dialysis system shows good degradation efficiency. Even at low substrate concentrations of 1% the volumetric loading rate of the original MELiSSA cycle was toped by 25%. So further experiments were done, trying to optimize the dialysis liquefaction unit.

The composition of the VFA is shown in the Appendix (Figure 13).

4. CONTINUOUS HYPERTHERMOPHILIC DIALYSIS LIQUEFACTION – IMPACT OF RETENTION TIME AND SUBSTRATE CONCENTRATION

The hyperthermophilic consortium showed the highest liquefaction rates as stated in the previous chapter. The next experiments dealt with the impact of the dialysate exchange and the volumetric loading rate.
Dialysate retention time was kept constant. The substrate concentration was maintained at 1%. The hydraulic retention time of the culture chamber was reduced from 4 days to 2.25 days. After 20 days the retention time was again lowered to 1.5 days. Form the analysis of all data points it becomes evident, that the fermentor was not in a steady state after 20 days. The reactor was run for additional 12 days with reduced retention time of 1.5 d. After that the outer foil of the laboratory fermentor was damaged and the experiment had to be stopped. The reactor performance was almost stable at this time. Figure 7 shows the liquefaction and degradation performance.

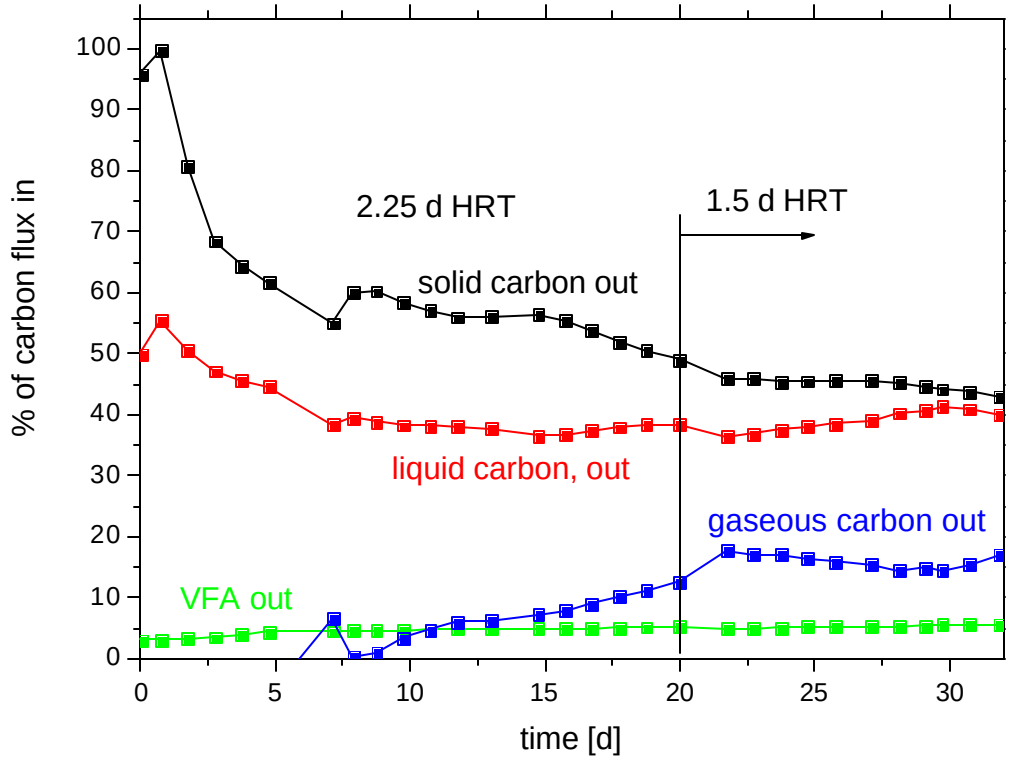

Figure 7 Liquefaction and degradation performance of a hyperthermophilic continuous dialysis fermentor with the consortium from the Azores. The ESA-substrate concentration was 1% The dialysate retention time was 1 day. The hydraulic retention time of the culture chamber was 2.25days for the first 20 days. After that the retention time was lowered to 1.5 days. The lines display the integrated degradation and liquefaction values.

Compared to the higher retention time of 4 days the degradation performance decreased. The volumetric loading rate increased from 2.5 g/(l d) to 6.7 g/(l d). A further way to increase the volumetric loading rate is to increase the substrate concentration in the feed. The concentration of the substrate in the feed was reduced to 1% in all previous experiments to prevent clogging of the tubes and pumps. The following experiment was done with 2% ESA-substrate concentration and 1.5 days hydraulic retention time. A volumetric loading rate of 13.3 g/(l d) is reached.
The experiment is shown in Figure 8.

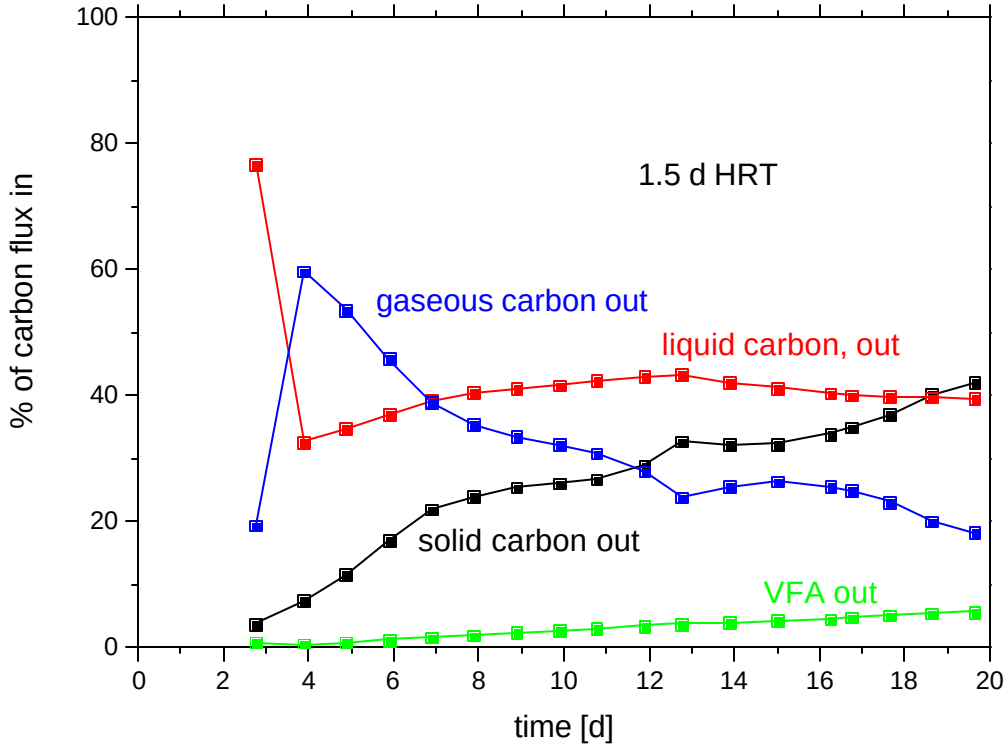

Figure 8 Liquefaction and degradation performance of a hyperthermophilic continuous dialysis fermentor with the consortium from the Azores. The ESA-substrate concentration was 2% The dialysate retention time was 1 day. The hydraulic retention time of the culture chamber was 1.5days. The lines display the integrated degradation and liquefaction values.

Comparing the later two experiments with the 1% ESA-substrate and 4 days retention time experiment shown in Figure 3, a decrease in degradation performance becomes visible with lower retention times. The concentration of the substrate seems to have no effect on the degradation performance. The comparison is listed in Figure 9.

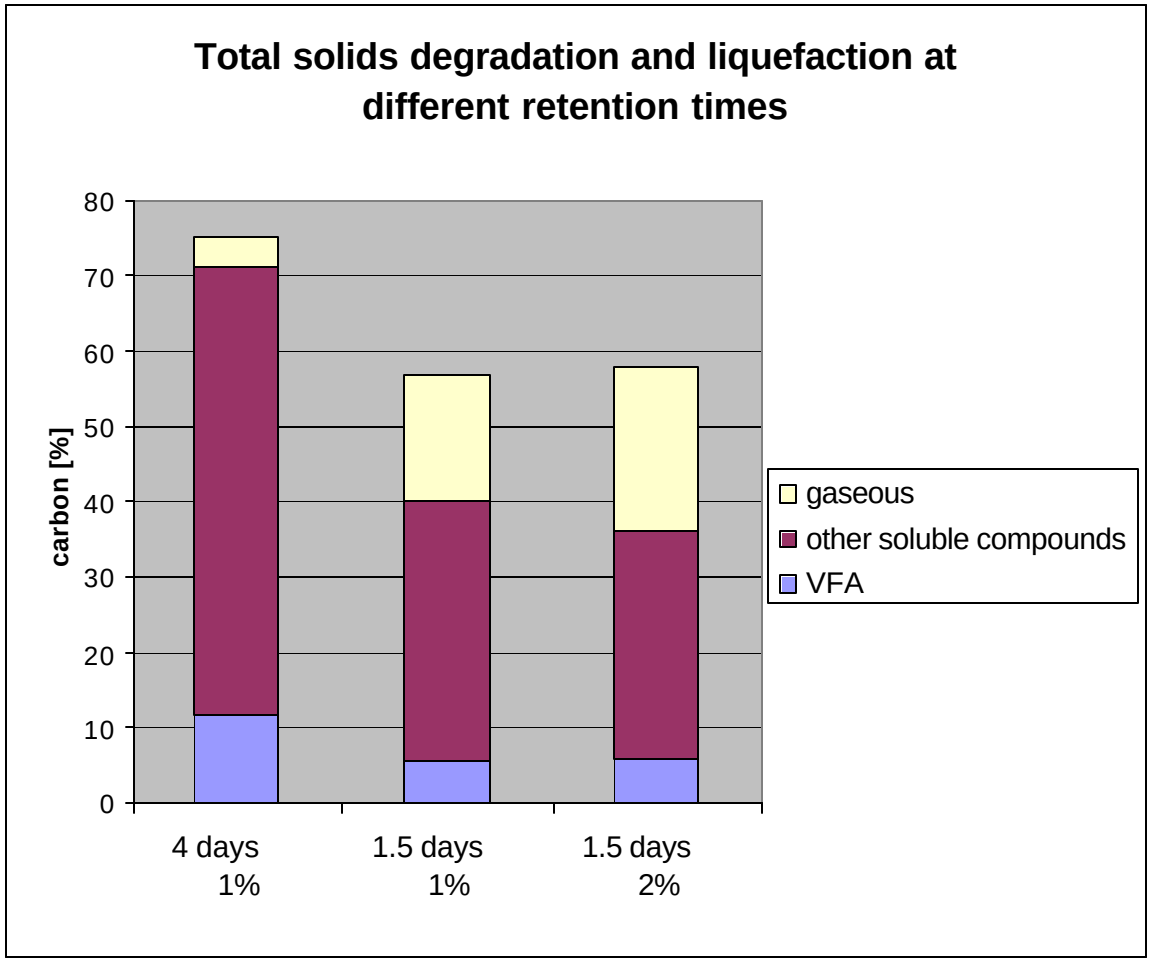

Figure 9 Degradation and liquefaction performance of a hyperthermophilic anaerobic continuous dialysis reactor with the consortium from the Azores at different retention times and substrate concentrations. The volumetric loading rates are 2.5 g/(l d), 6.7 g/(l d) and 13.3 g/(l d).

5. METHANOGENIC FIXED BED REACTOR

One way for the regeneration of the loaded dialysate could be the coupling to a methanogenic reactor. Because of the relative low concentrations of COD and DOC and the good biodegradability of the dialysate a radial flow fixed bed reactor is an efficient solution for this problem. In a fixed bed reactor the methanogenic microorganisms are immobilized on carrier materials and thus cannot leave the reactor. Compared to a CSTR without any biomass retention system lower hydraulic retention times than the generation time of the slowest growing organism can be realized. A typical generation time of methanogenic organisms is 3-5d (70-130h). To increase the volumetric loading rate lower retention times have to be used. A 5 Liter fixed bed reactor was constructed and set up as described in 2.5.2. Preliminary experiments were done with a modified *C. lactoaceticus* medium (see 2.1.2) with acetic acid as sole carbon source. The medium was strongly buffered to pH7. Because of the degradation of the acetic acid the pH in the fermentor increased to 7.5. A hundred days experiment with an initial acetic acid concentration of 5mM in the feed can be seen in Figure 10.

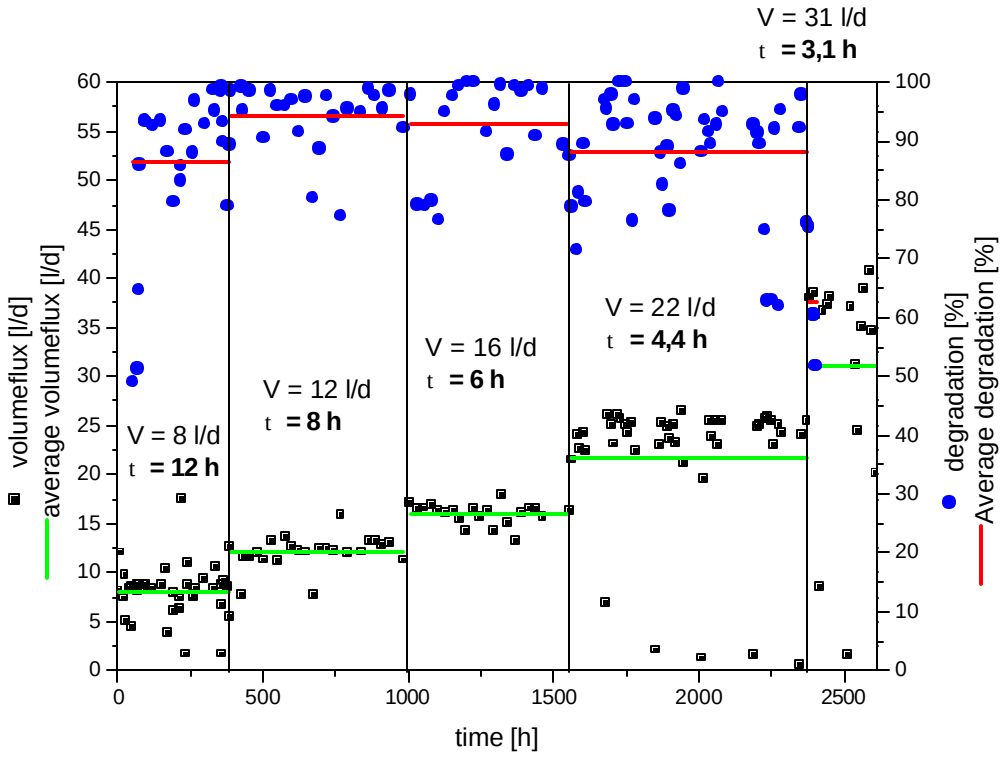

Figure 10 Acetic acid degradation and volume flux for a 5 Litre radial flow fixed bed reactor. 5mM acetic acid was the sole carbon source. Degradation rates of the acetic acids were higher than 90% for retention times > 4.4h

The highest possible loading rate with a degradation of 90% was 1.3g/(l d) at a retention time of 4.4 hours. Higher volumetric loading rates can be achieved at higher acetic acid concentrations in the feed as shown in Figure 11.

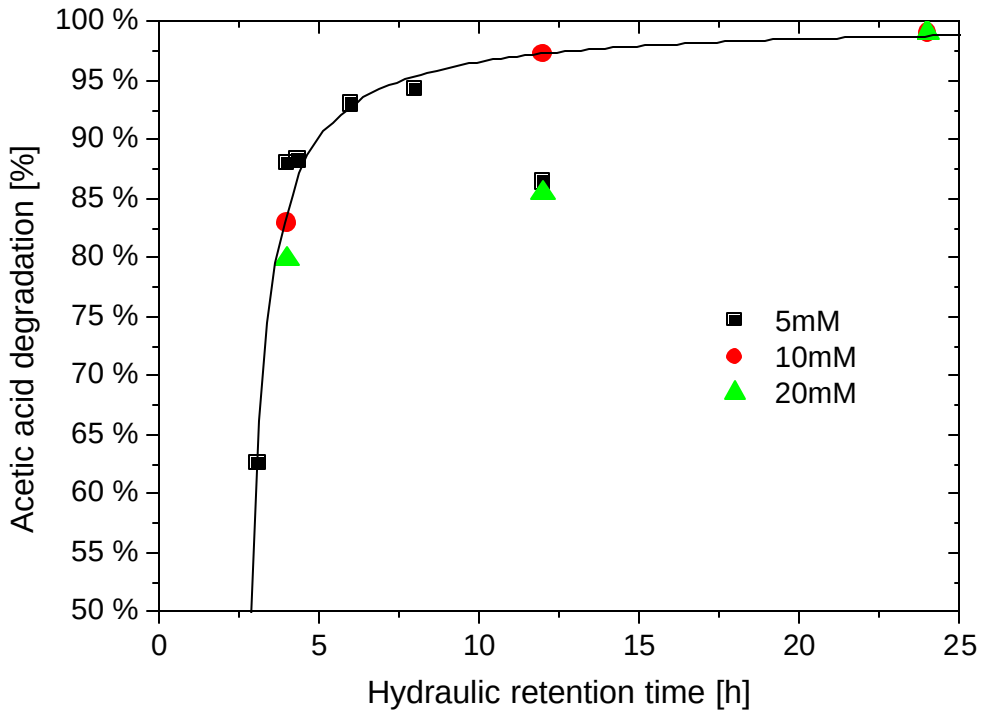

Figure 11 Degradation efficiency and volumetric loading rates of a radial flow fixed bed fermentor at different retention times and acetic acid concentrations. The two points at 85% degradation and 12h retention time both originated from a start up phase.

Further scale up can be calculated with the model of Witty and Märkl [1986]. The growth rate of methanogenic organisms on acetic acid can be calculated by eq 1.

$$\frac{m}{m_{\max}} = \frac{c_{(HAc)}}{c_{(HAc)} + 0.07\,mM} \qquad \text{(eq.1)}$$

The methanogenic microorganisms can only use the non dissociated part of the acetic acid (eq. 2).

$$c_{(Ac)} = c_{(HAc)} + c_{(Ac^-)} \qquad \text{(eq.2)}$$

The equation is graphical displayed in Figure 12. At the given conditions (pH7.5, 5mM HAc in feed, 90% degradation) an average concentration of non dissociated acetic acid can be calculated with eq. 3 to 0.7µM resulting in a growth rate of 1% of the maximal growth rate. The activity coefficient γ is 0.73 for monovalent ions. The dissociation constant is 1.17×10^{-5} mol/l.

$$c_{(HAc)} = \frac{c_{(H^+)} \cdot g^2}{K_D + c_{(H^+)} \cdot g^2} \cdot c_{(HAc)} \qquad \text{(eq.3)}$$

Thus a concentration step is reasonable to increase the concentration in the feed and the degradation rate in the fixed bed reactor.

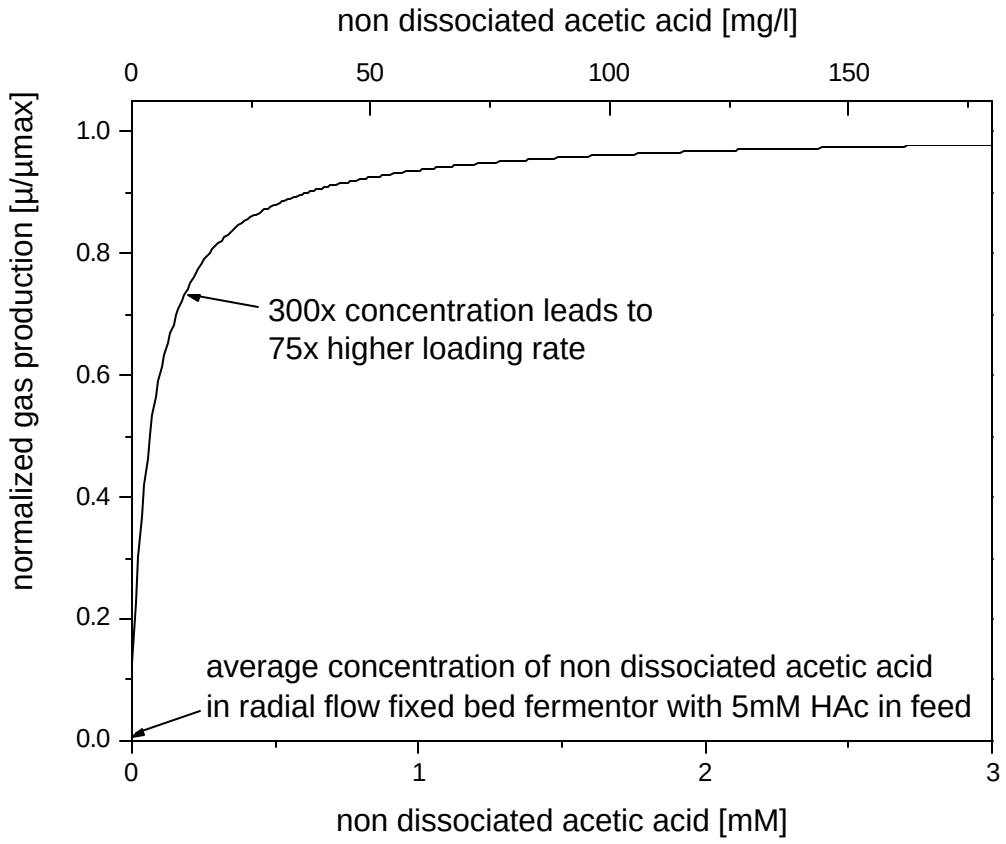

Figure 12 kinetics of a methanogenic culture in dependence of non dissociated acetic acid. A concentration of the 5mM feed will lead to a higher gas production.

Also for biosafety a methanogenic reactor can be important. A large number of fixed-bed applications are known dealing with the removal of toxic by-products, such as cyanide [Fallon et al. 1990], chlorobenzoate [Bae et al. 2004], p-nitrophenol [Swaminathan K, Subrahmanyam PV. 2002] trichloroethylene, tetrachloroethylene and 1,2-dichlorethane [Wild et al. 1995], dichloromethane [Stromeyer et al. 1991] or terephthalate [Kleerebezem et al. 1999]. This type of fermentor is very resistant against occasional overloading with toxic compounds.

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Appendix:

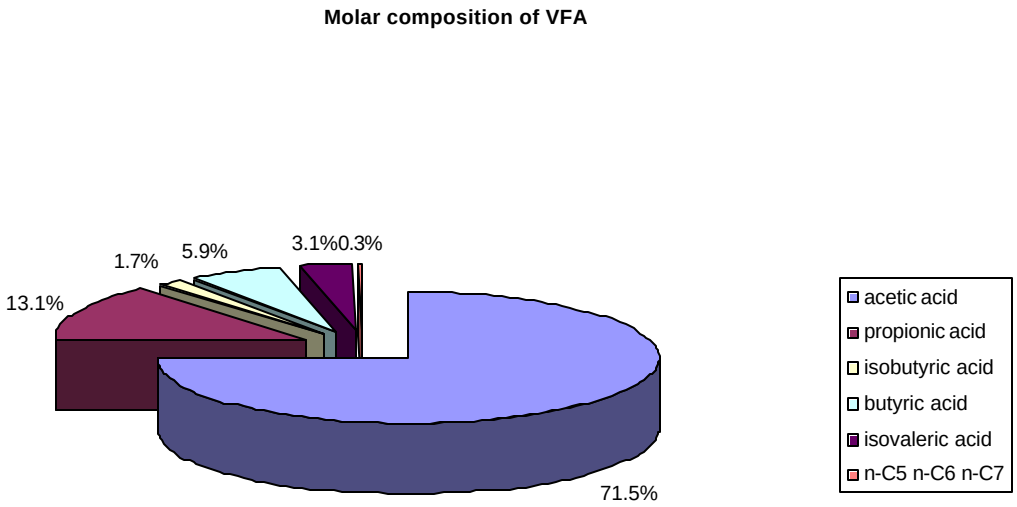

Figure 13 Molar composition of VFA in Dialysate at 90°C t=4d, 1%TS measured with GC -FID