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OPTIMIZATION OF THE COULPED THERMOPHILIC LIQUEFACTION UNIT

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1 ___ INTRODUCTION

The aim of this TN according to the scheduled plan was the investigation of a coupled hyperthermophilic liquefaction and a moderate thermophilic methanogenic unit. Previous results and discussions with other partners made us to change the field of experiments. Instead of focusing on the production of biogas which has to be taken care of in future applications the circumvention of methane production and direct production of carbon dioxide and volatile fatty acids was pursued.
Therefore an anaerobic hyperthermophilic reactor was set up and coupled with a dialysis membrane. The membrane was used to remove inhibitory substances and volatile fatty acids. This leads to a significant acceleration of the degradation process. Fibrous matter was degraded with turnovers between 57 and 75%. In previous reports (TN 3.5) we described the liquefaction process in a lab scale membrane fermentor with a specific membrane area of 30m^2/m^3.
To prove the scale up feasibility a different set up was tested. Instead of membrane sheets hollow fibre membrane modules were used in this report.
Also the microbial characterization and identification of the hyperthermophilic consortium was pursued.

2 MATERIAL AND METHODS

2.1 DOC

The DOC and TIC value of the samples was determined with TOC + TN_b from Analytic Jena. Part of the sample is burnt at 800°C in a pure oxygen atmosphere in a column filled with carriers of Cer catalyst. The CO_2 content in the off-gas is integrated and the total carbon (TC) determined. The Total Inorganic Carbon (TIC) is determined by acidifying the sample to pH 2 and flushing out the dissolved CO_2

2.2 TN, Ammonia

Total nitrogen and Ammonia was detected by using test kits form Dr. Lange (TN 20-100mg/L LCK 3338, NH_3 / NH_4^+ 2-47mg/L LCK 303)

2.3 VFA

VFA are determined with a headspace gas chromatograph Chrompack CP9001. A 30mx0.32mm Nukol capillary from Supelco is used. Carrier gas is nitrogen. The samples are acidified with 2% H_3PO_4. The column is heated to 60°C, after tree minutes the temperature rises with 10°C/min to 200°C. The detector is a FID working at 220°C. The following VFA can be measured: C2, C3, i-C4, n-C4, i-C5, n-C5, n-C6, n-C7

2.4 Microbial characterization and isolation of pure cultures

Microbial characterization of the hyperthermophilic consortium was done with LB-Medium (5g yeast extract, 10g tryptone, 10g NaCl per liter) and ESA-substrate supernatant. For the latter medium 3.7g/L Spirulina platensis 8.9g/L ground wheat straw and 9.4g/L ground soy-pellets were adjusted to the desired pH with HCl or NaOH and autoclaved at 120°C for 20 minutes. The medium was centrifuged three hours at 7500rpm and centrifuged. The supernatant was taken as growth medium. The supernatant contained approximately $1x10^6$ dead cells per mL (cells were not stained).
Liquid culture experiments were done in 100mL anaerobic culture vials. The medium was boiled for 1 minute, cooled below boiling temperature. 1g/L Na_2CO_3 and 0.5g/L L-Cysteine x HCl were added to the hot, but not boiling liquid. pH was adjusted with HCl or NaOH to the desired value. 50mL of medium were then filled into 100mL cultures vials. The vials were gassed with nitrogen for two minutes and then sealed with butyl rubber seals. Liquid cultures were inoculated with 10% pre culture (TN3.1).

Serial dilution technique was used for isolation experiments. 5 mL mixed culture were inoculated into a 100mL vial. The vial was shaken and 5mL were taken out for the inoculation of the next vial. The procedure was repeated 8 times to a dilution of 10^{-8}.
Plating experiments were done with LB and ESA-medium containing 1% Agar or 2% Gelrite and 0.2M $MgCl_2$. The plates were reduced in an anerobic chamber with 100µL 5% Na_2S solution or 1% L-Cysteine and inoculated with 10µL culture. The following cultures were sampled: fermentor effluent, liquid culture in LB-medium, liquid culture in ESA-medium. The plates were inoculated at 70°C (agar) and 75 and 80°C (gelrite).
Characterization cultures were done with the consortium. Vials were prepared with LB-medium, at pH 5, 6, 7 and 8. Cultivation temperatures were 70, 80, 90, 95, and 100°C. Samples were taken every day for 5 days. The vials were reduced with 1g/L L-cysteine, so the DO was zero. Anaerobic conditions were maintained in all vials, since the resazurin oxygen indicator did not cause a color change.
Another set of vials were prepared with ESA-substrate, at a pH of 5, 6, 7, and 8. The vials were inoculated and incubated at 70, 80, 90, 95, and 100°C. Growth was monitored for 24h.

2.5 Fermentor set up

The 6L Dialysis membrane reactor was used for further experiments. The fermentor was modified to install a submersed hollow fiber membrane module. Instead of the culture chamber and the dialysate membrane sheet the membrane module was set into the fermentor. Fresh dialysate was pumped through the hollow fibers of the module and was loaded with small molecules.
The bottom part and lid were manufactured from PEEK (poly-ether-ether-ketone). In these plates two holes were made for the connection to the dialysis module (Fresenius medical care, Hemoflow highflux HF 80). Another set of connections were manufactured to use a microfiltration module (Microdyn, MD 070 FP 1N). This module uses PP as membrane material, which has to be hydrophilized with 70% ethanol before usage. The module was stored for 24h in 70% ethanol. This was recommended by the manufacturer. During the hydrophilization ethanol adsorbs at the hydrophobic membrane material with its hydrophobic side ($-CH_3$). The hydrophilic side (-OH) shows to the outside and makes the membrane hydrophilic. The hydrophilization lasts, until the membrane dries completely; then it has to be repeated. After hydrophilization, the non adsorbed ethanol is washed away with water. The binding of ethanol onto the PP-membrane is due to hydrophobic-hydrophobic interaction.
The hollow fibers are gathered in a matrix made from PU, which is not thermostable. To prevent thermal degradation of the PU cooled dialysate was pumped back onto the top section of the dialysate module to lower the dialysate effluent temperature.
The set up is displayed in figure 1 (FMC-Blood dialyzer) and figure 2 (Microdyn MF module). The fermentor was heated to 90-92°C by pumping hot water trough the cooling facility of the fermentor. With this set up a stable and continuous heat dissipation was possible. Earlier experiments with an electrical heater lead to boiling water near the heater because of the good insulation characteristics of sedimented solids.

The feed and effluent pump were triggered by a timer and pumped for 2 minutes every 3 hours. Prior to pumping the stirrer was switched off for one minute to allow big particles to sedimentate. Dialysate was exchanged continuously. When using the microfiltration membrane two pumps, one at the dialysate inlet and one at the outlet were necessary to prevent a convective volume flow over the porous membrane. The dialysate was stripped with 5L/h nitrogen and reduced with 0.5g/L Na_2S x $7H_2O$. Nothing else was added to the dialysate. pH was titrated by addition of 4M NaOH. Redox potential was measured on line.
A sample was taken every day from the effluent and dialysate. DOC, VFA and TS were measured every day, nitrogen, ammonia, proteins and carbohydrates every second day.

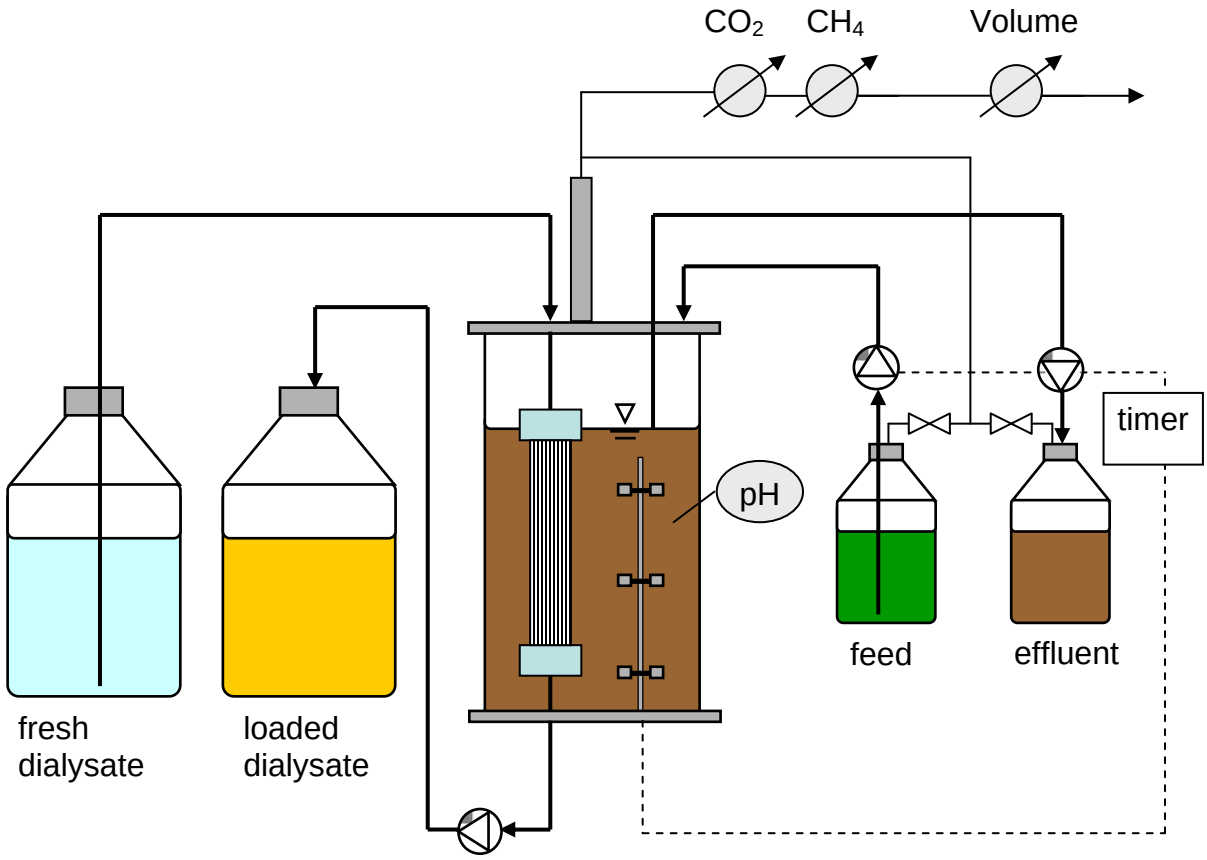

figure 1 Fermentor set up for the submersed dialysis module FMC Hemoflow HF80. For further details see text.

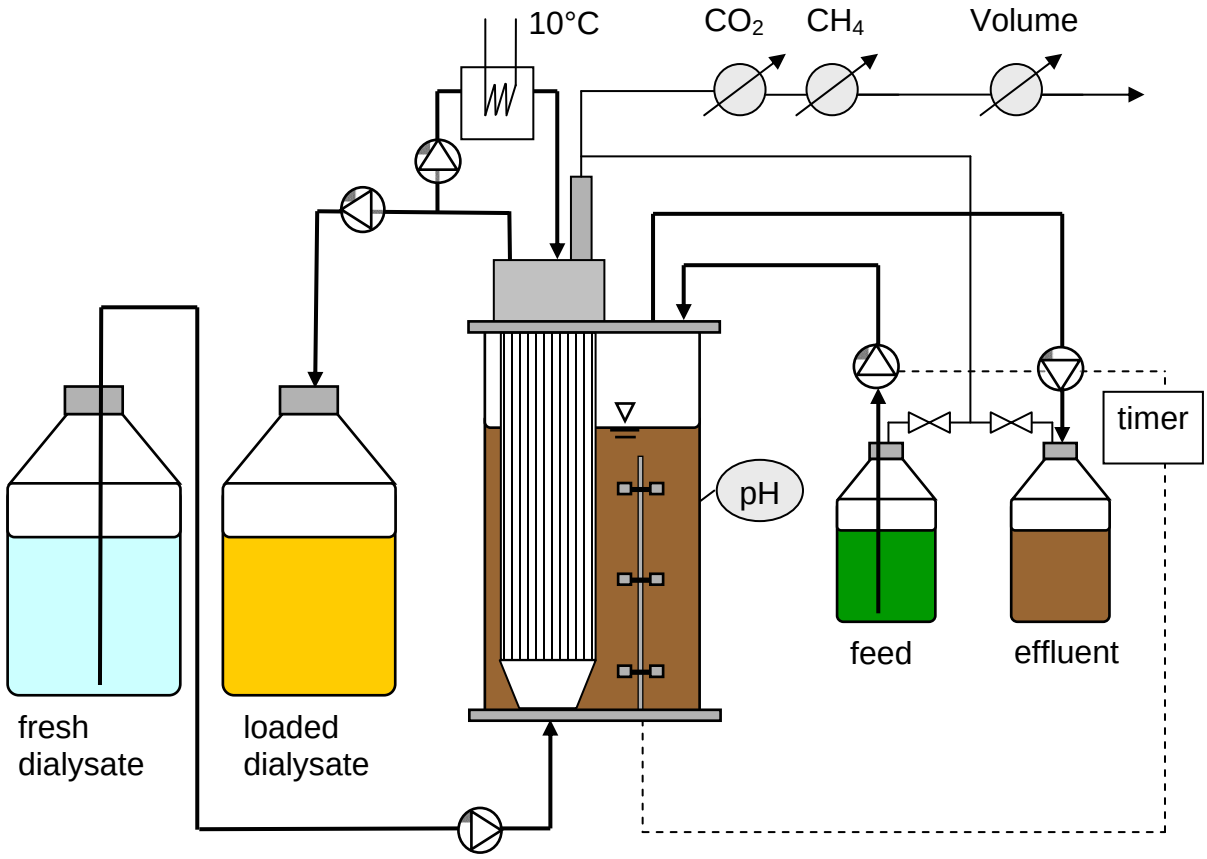

figure 2 Fermentor set up for the submersed dialysis module Microdyn, MD 070 FP 1N. Used dialysate was cooled in a heat exchanger and given to the dialysate outlet stream to cool down the PU collector of hollow fibers. For further details see text.

2.6 16S rDNA DGGE

DNA was isolated with Quiagen Genomic DNA Buffer kit and amplified with PCR, using the 907 Reverse primer (5`CCG TCA ATT CMT TTG AGT TT) and GM5 clamp primer (5`CGC CCG CCG CGC CCC GCG CCC GTC CCG CCG CCC CCG CCC CCC TAC GGG AGG CAG CAG). The PCR products was put on a GDDE Agarose gel and run at 55°C and 200V for 5h. The gel was stained with Ethidiumbromide and photographed under UV-light.

The DGGE gel had the following composition:

80% denaturing acryalmide solution (6% acryl amide)

- 22.5 mL acrylamide (40%)
- 3 mL 50x TAE buffer
- 48 mL Formamide
- 50.4g urea (predissolved in 30 mL water)
- Fill to final 150 mL water

0% denaturing acrylamide solution

- 22.5 mL acrylamide (40%)
- 3 mL 50x TAE buffer
- Fill to final 150 mL water

3 RESULTS

3.1 Growth of microbial consortium

Two media for characterization were used. On LB-medium the consortium showed poor but detectable growth within 4 days. The temperature range from 70 to 95°C and pH from 5 to 8 was screened. Best growth was detected at pH 8; the best growth temperature was 50 and 95°C. (data not shown)
To check whether the consortium is of marine origin the impact of NaCl concentration was evaluated. Vials with 1, 2 and 3% NaCl were inoculated and growth was monitored for 4 days. The result is shown in figure 3. Cell count was not determined at the beginning of the experiment.

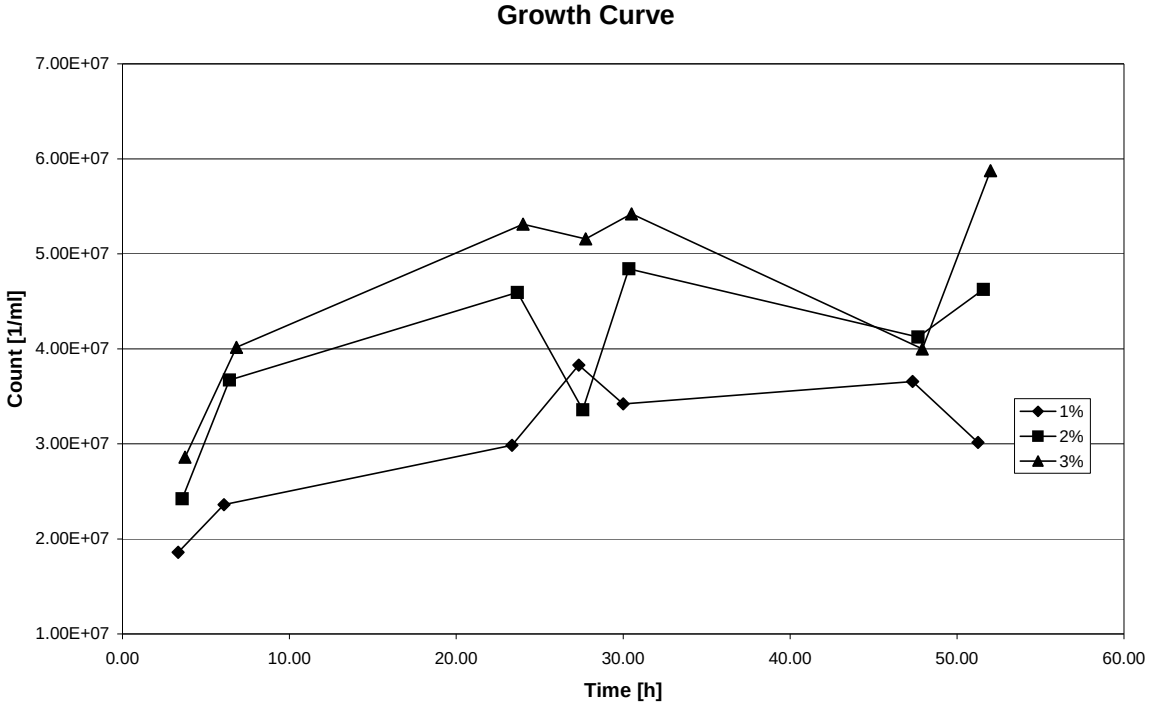

figure 3 impact of NaCl concentration on growth of the anaerobic and hyperthermophilic consortium at pH 8 and 90°C

Best growth was monitored at 3% NaCl indicating a marine origin of the consortium. Nevertheless growth was also monitored on ESA-medium without salt addition.

On the modified particle free ESA-substrate growth was approximately ten times better than on LB-medium (see figure 4), so this medium was used for further characterization and isolation experiments. No growth was visible on LB-Medium at 100°C

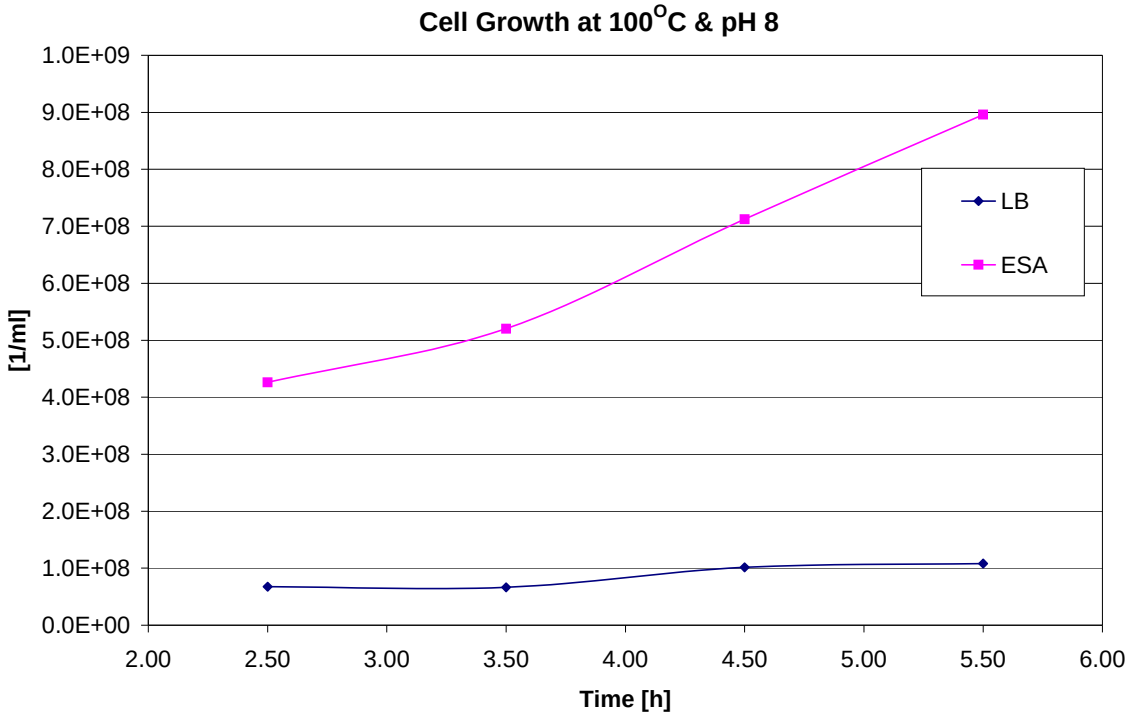

figure 4 growth of consortium at pH 8 and 100°C on particle free ESA-substrate and LB-medium

After these results isolation experiments were started. LB-Medium and ESA-substrate were solidified with agar and gelrite. The plates were reduced with 100µl 5% Na_2S and inoculated with 100µL pre culture. The culture was anaerobic, the DO was zero. The plates were incubated at 70° and 75°C. Higher temperatures were not possible, since the agar melds at 70°C and gelrite at 75°C. No growth was visible on the plates after one week. Control plates with resazurin indicated no presence of oxygen. Obviously the consortium is not able to grow on solid media, so liquid medium was used for further isolation experiments. The serial dilution technique uses liquid media for isolation. The disadvantage of this method is that only one pure strain can be isolated with this technique. Pre culture was diluted 1:10 with fresh medium. This step is repeated ten times to a maximal dilution to 10^{-10}. Vials were incubated at 90°C. After three days growth was visible to a dilution of 10^{-8}. This dilution was used as next pre culture and the serial dilution was repeated twice.
The serial dilution yielded a non spore forming coccus which is sent for 16S rDNA identification to the DSMZ. The strain is probably an archaeon, since the only two hyperthermophilic bacterial stains (*Thermotoga* and *Aquifex*) are both rods. Also DGGE-experiments using bacterial primers failed. The DGGE method encountered several

difficulties. At first it was hard to open the cells to get access to the DNA. We tried protocols for bacteria (lysozyme lysis, Miller et al. 1999) and archaea (proteinase K, freezing cells, Zhou et al. 1996, Sugano et al. 2005). Secondly the DNA-yield was very low. Hardly any DNA could be detected on the gels, even when we overloaded the gel. Thirdly, the cells showed no good growth anymore. The cultures we used had an OD600 of approx. 0.2-0.3, together with a background noise of cell debris (ESA-Medium supernatant) or crystals (probably carbonate crystals on LB). Fourthly, the extraction of DNA from archaea seems to be a difficult process. (Casamayor 2001) Even for well established strains like *Pyrococcus furiosus* and *Piccrophilius torridus* the extraction often fails, so there are probably also other reasons. All microbial experiments were done at the technical microbiology lab of the TUHH (Prof. Antranikian) under the guidance of experienced co-workers in this field.
DSMZ identification has to be repeated. Currently no well growing cultures are available for identification. However, our lab is greatly interested in these results and we will pursue the identification further; but in the moment no more results are available.

3.2 Continuous dialysis fermentation with membrane modules

Two different membrane modules were used for continuous experiments. The FMC Hemoflow HF 80 module and the Microdyn MD 070 FP 1N module display some main differences. While the FMC module is designed for medical application for the dialysis of blood and parental feeding, the Microdyn module can be used for different applications, such as microfiltration of bacteria or pigments. The FMC module is cast into a plastic housing. This housing was removed before inserting the module into the fermentor. During this procedure several hollow fibers were damaged, so a convective flow from dialysate to reactor and vice versa was possible. This was circumvented by sucking the dialysate through the module. Solid particles closed the holes in the damaged membrane and the convective flow through the membrane was successfully reduced. The Microdyn MF-module did not have to be modified. It was directly set into the fermentor. Due to the high ultrafiltration coefficient a huge convective flow was visible in first test runs leading to fluctuating reactor volumes. The problem was solved by using two pumps for the dialysate. One pump was used to pump the dialysate into the module and another pump controlled the outgoing flow of the dialysate. Both pumps pumped the same volume flow, so the net convective flow over the membrane was minimized.

Table 1 Membrane modules used in this project

	FMC Hemoflow HF 80	Microdyn MD 070 FP 1N
Material	PS Polysolphone	PP Polypropylene
Hydrophobicity	Hydrophilic	hydrophobic
Type of membrane	Dialysis	Mircofiltration
Cut off	30kD non porous	0.1µm porous
Membrane thickness	~10µm	~100µm
Membrane area	1.8m²	1.1m²
Lumen diameter	200µm	600µm
Number of hollow fibres	13000	2700

convective flow [L/h bar]	30.4	~250
connection	Luer lock	O-ring

Both membranes were compared in two identical experiments. The experimental parameters were: B_V=5g/(L d), V_D=20L/d, T=90°C, pH=7.
The experiment with the FMC dialysis membrane was terminated after 14d because of breakage of the collector housing. The experiment with the MF-module was terminated after 35d by changing the fermentation conditions.
The results from the FMC dialysis membrane and Microdyn MF experiments are shown in figure 5 and 6 respectively.

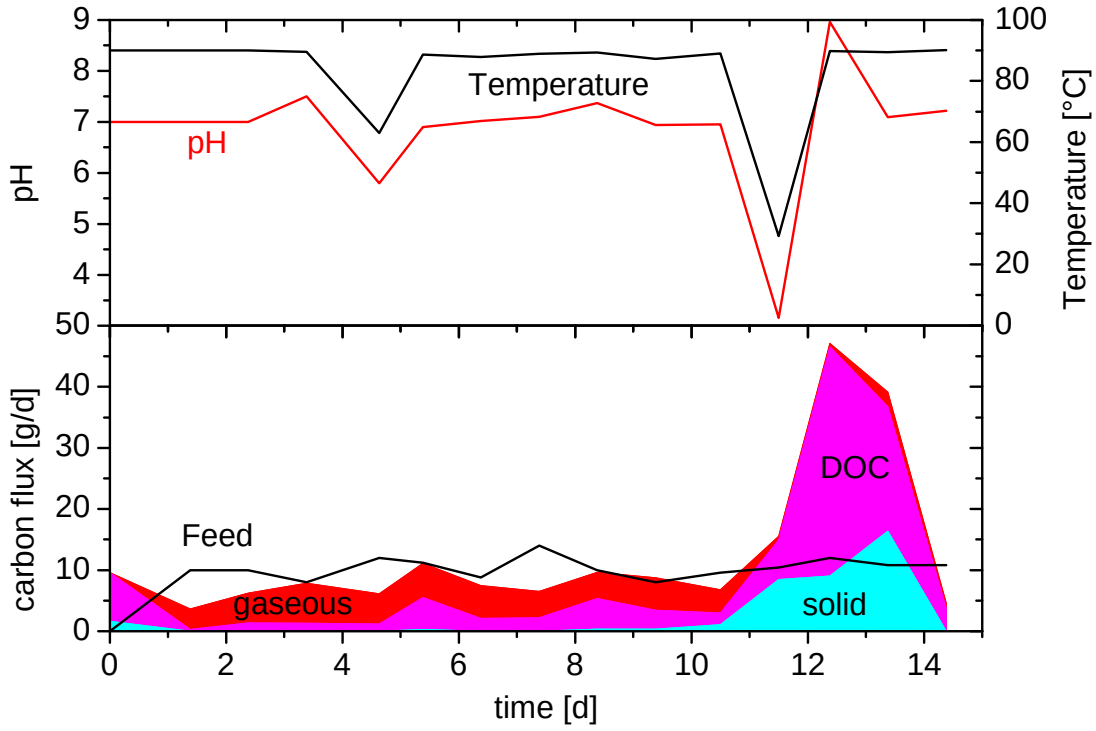

figure 5 Temperature, pH and carbon fluxes for the continuous dialysis experiment B_v=5g/(L d), V_D=20L/D using the FMC hemoflow HF80 module.

At the beginning of these experiments often problems with the temperature controller occurred. These problems were solved later on and did not occur in following experiments. The malfunction of the temperature controller also led to a change in pH. Interestingly this caused a higher liquefaction of organic material. Gas production was very high. No gas analyzer was connected to the gas outlet at the beginning of the experiment, so the gas composition is unknown. After the second temperature disturbance the gas production stopped. Three days later the membrane housing broke and the experiment was aborted.
After this failure we looked for a more stable solution and made a market survey on membrane modules. Most suppliers only offer dead end filtration modules with asymmetric membranes.

From the first experiment we learned that a high pressure drop over the capillaries is not favorable, since the pressure drop cause a pressure gradient over the membrane which induced a convective flow over the membrane. We therefore looked for membranes with broader capillary diameter. The Mircrodyn MF-module offers 600µm capillary diameter instead of the normal 200 or 220µm of a UF- or dialysis module. So we chose this module type. A special adapter was constructed to set the module into the fermentor and first test runs with water were carried out. The very high convective flow required the use of an additional pump to ensure constant in and out going dialysate volume flows.
The system was then started and run successful for 35 days without any greater failure. After this period of time the fermentation conditions were changed to pH8 and the experiment is still running at the moment after total 60 days without any problem. Carbon fluxes, temperature and pH are displayed in figure 6.

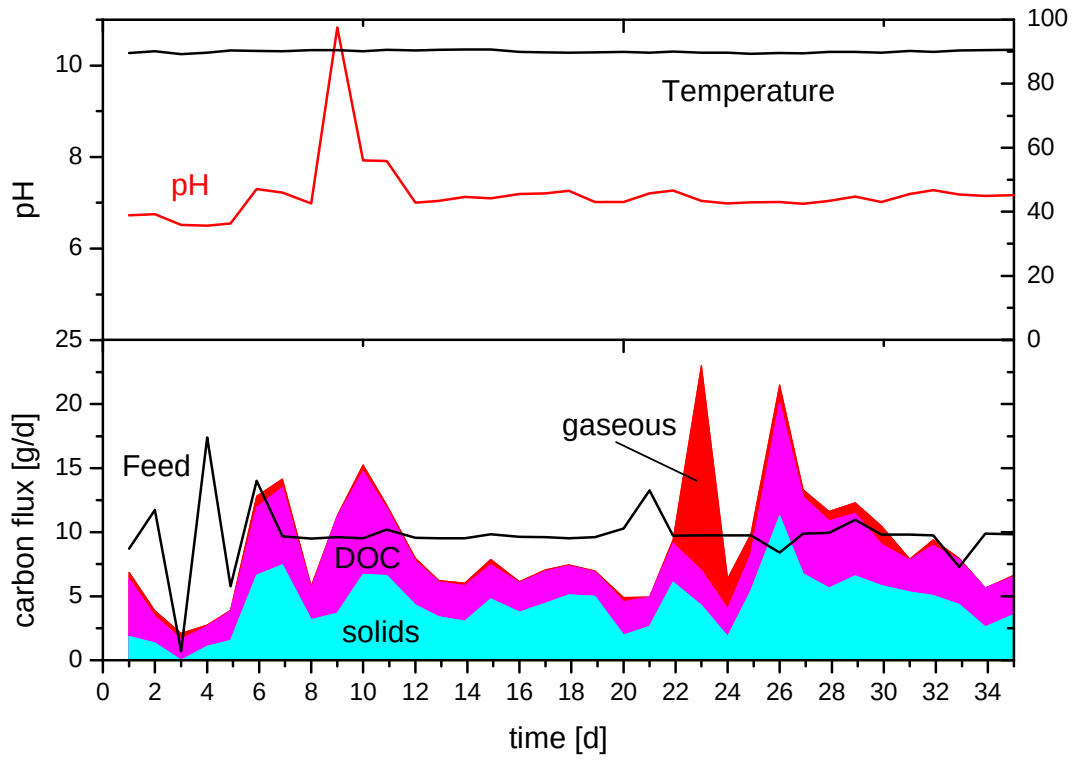

figure 6 Temperature, pH and carbon fluxes for the continuous dialysis experiment B_v=5g/(L d), V_D=20L/D using the Microdyn MF-module

The comparison of reactor performance is shown in figure 7. The gaseous phase was assumed to have 1 atom carbon per molecule (CO_2, CH_4). MS-analysis revealed, that the major component of the gas phase was CO_2, hydrogen was found in concentrations several orders of magnitude lower. The solid degradation performance was better with the FMC-dialysis module (62%) than with the MF-module (55%). The liquefaction performance was in similar regions for both set ups (34 and 37%). With the FMC-module a very high gas production was

measured. This was probably caused by two major changes in temperature which have a great impact on the density of the gaseous phase.

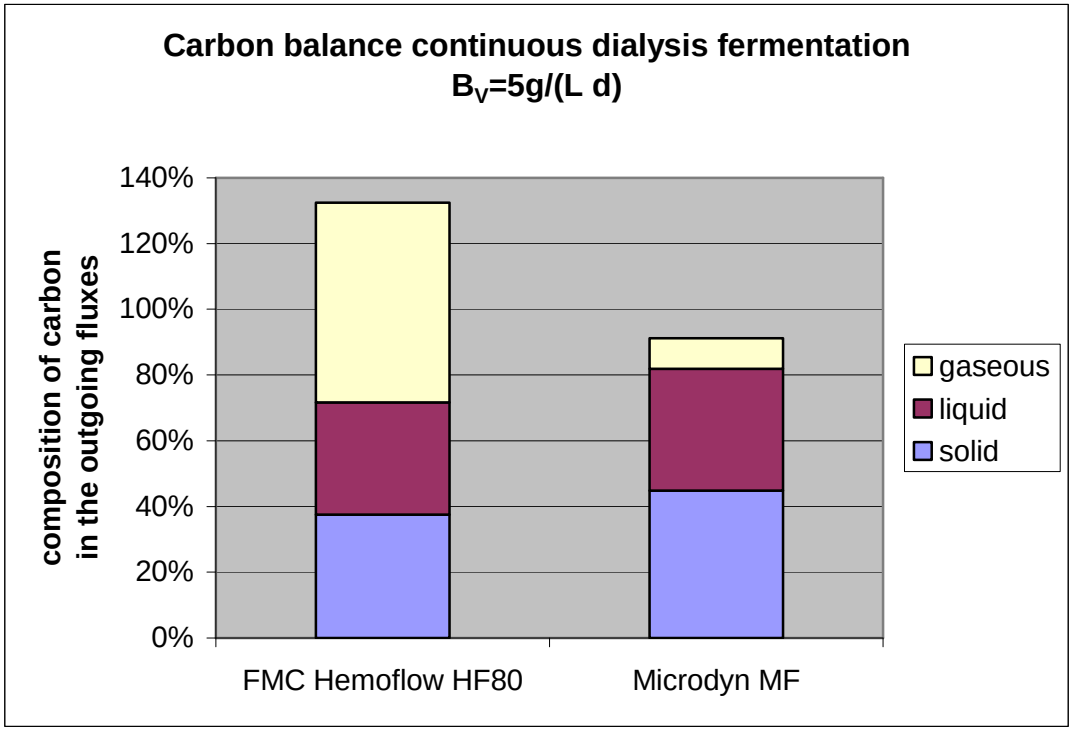

figure 7 distribution of carbon in solid, liquid and gaseous phases in the outgoing fluxes from the continuous dialysis fermentor. Carbon content of the gaseous phase was set to 100% (v/v)

To evaluate the effective membrane which participates on the mass transport the acetic acid gradients were investigated.
With the FMC HF80 Module the average acetic acid concentration in the dialysate is 45% of the effluent concentration. During the usage of the Microdyn MF module the average gradient was 18%. From this gradient the mass transport resistance can be calculated from an acetic acid balance. (eq.1) The balance also is true if acetic acid is produced in the feed stock.

$$\dot{n}_{HAc,P} = \dot{V}_D \cdot c_{HAc,D} + \dot{V}_E \cdot c_{HAc,E} \qquad \text{(eq.1)}$$

where $\dot{n}_{HAc,P}$ is the acetic acid production term, V_R is the reactor volume, $\dot{V}_D$ is the dialysate stream and $\dot{V}_E$ is the effluent stream. $c_{HAc,D}$ and $c_{HAc,E}$ are the acetic acid concentrations in the dialysate and effluent respectively. The mass transport is described by eq.2

$$\left.\frac{dn_{HAc}}{dt}\right|_{membrane} = P \cdot A \cdot \Delta c_{HAc} \qquad \text{(eq.2)}$$

The concentration profile over the membrane is displayed in figure 8. The mean logarithmic concentration difference is used. In this special case it can be written as in eq.3

$$\Delta c_{HAc} = \frac{c_D}{\ln\left(\frac{c_E}{c_E - c_D}\right)} \qquad \text{(eq.3)}$$

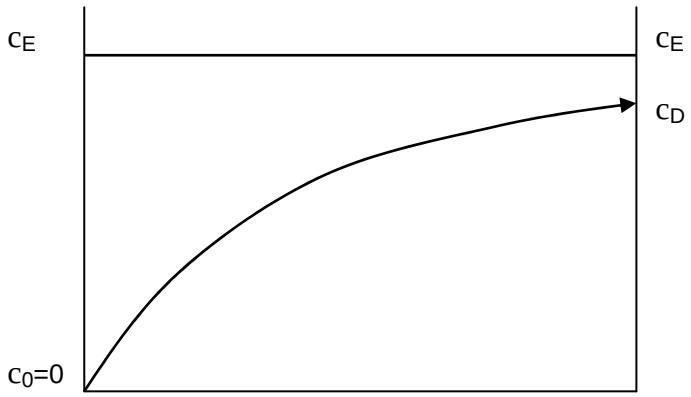

figure 8 concentration profile in the membrane module. Outside the membrane the concentration does not depend on the position in the vessel, since CSTR conditions are assumed. The concentration of the unloaded dialysate is zero.

An acetic acid balance over the reactor at stationary conditions yields eq.4:

$$\frac{dc_{HAc}}{dt} = 0 = \left(\dot{n}_P - \dot{n}_{mem}\right) \cdot V_R - \dot{V}_E \cdot c_E \qquad \text{(eq.4)}$$

Inserting eq.1-3 into eq.4 yields eq. 5.

$$P \cdot A = \frac{\left(\dot{V}_D \cdot c_D + \dot{V}_E \cdot c_E\right) - \dfrac{\dot{V}_E \cdot c_E}{V_R}}{\dfrac{c_D}{\ln\left(\dfrac{c_E}{c_E - c_D}\right)}} \qquad \text{(eq.5)}$$

The mass transport coefficient PA is calculated with eq.5 to 0.55L/h for the FMC-hemoflow module and to 0.21L/h for the Microdyn MF-module. For PS the permeability for acetic acid was determined to $2x10^{-2}$ cm/min. With the known membrane area of the modules and assuming that the permeability is the same for the thicker PP-Membrane inside the Microdyn MF-module it can be calculated that only 2.6% (FMC hemoflow) and 1.6% (Microdyn MF) of the membrane area participate on the mass transport.

This can be explained with the unfavorable flow regime inside the hollow fiber bundle. The diameter of the hollow fiber bundle is 60mm (Microdyn MF) and 50mm (FMC HF80). From the percentace of active membrane area the thickness of the active layer on the bundle can be calculated. The thickness is 240µm for the FMC-hemoflow module and 320µm for the Microdyn MF module, corresponding to the dimensions of one hollow fiber (FMC HF80) or even a half hollow fiber (Microdyn MF).

4 CONCLUSIONS

Two different membrane types were compared in a hyperthermophilic dialysis fermentor, a thin PS dialysis membrane and a thicker PP MF-membrane which was used in dialysis mode. Better results were achieved with the dialysis membrane, though the system was unstable and the membrane housing broke after two weeks at 90°C. The MF-membrane is still running after 60 consecutive days at 90°C without any integrity problems. However for both membranes can be stated that only a small percentage of the total membrane area participates on the mass transport. Further experiments will focus on this problem to increase the percentage of active membrane area.
The characterization of the microbial hyperthermophilic consortium revealed that optimal growth condition were at pH 8 and temperatures above 90°C. Pure cultures were produced and send in for 16S-RNA identification. Also the operation of the continuous dialysis reactor at optimal pH conditions is currently under investigation.

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