Technische Universität Hamburg-Harburg

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on the MAP-Project
"A Total Converting and Biosafe Liquefaction Compartment for MELISSA"

Work Package 3.200: Optimisation of Hyperthermophilic Liquefaction

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1. Introduction

The hyperthermophilic liquefaction is the breakdown of long biopolymeres by microorganisms able to live at temperatures higher than 60°C. Sugars and starch for example are hydrolysed to glucose and further digested to volatile organic acids.

In spring 2001 a new anaerobic, hyperthermophilic consortium was isolated in hot springs at the Azores. First results showed, that this consortium performs a better liquefaction than a single culture of *Pyrococcus furiosus*. Even a COD-reduction was detected. More experiments have to proof whether the consortium is able to digest all parts of the ESA-substrate. The consortium should be analysed and the strains are going to be identified and described.

The objectives of work package 3.200 are the optimisation of the hyperthermophilic pretreatment in terms of pH-Value and stirrer-speed. Carbon and nitrogen balances have to be drawn for continuos fermentation. The COD-reduction efficiency in the hyperthermophilic pretreatment has to be determined. To evaluate the hyperthermophilic pretreatment, the energy consumption was calculated for a continuous fermentation.

The carbon, nitrogen and COD-balances were generated for a continuous fermentation at 90°C, pH 7, 500rpm, 0.05 vvm nitrogen gassing and HRT=2.6d. Batch fermentations with different pH-Values and stirrer speeds were done.

2. Material and Methods

2.1 Substrate

2.1.1 ESA-Substrate

The ESA-Substrate consists of wheat straw, soya pellets, cabbage, algae, and faecal matter. To draw balances, the carbon- and COD-content of each constituent was determinated. Due to its inhomogenities faeces were left out in continuous fermentation. So the dry matter content of the substrate was reduced to 1.6%. The TC-value of cabbage was estimated to 0.4. Table 1 shows the properties of the ESA-substrate.

Table 1 Characterisation of the ESA-Substrate. Content of COD, carbon, and nitrogen

	Mass-%	Dry matter content	COD of dry matter	TC of dry matter	TN of dry matter
	[g_{DM}/g_{DM}]	[g_{DM}/g]	[g_{COD}/g_{DM}]	[g_C/g_{DM}]	[g_N/g_{DM}]
straw	0.23	0.93	1.30	0.39	0,0087
soja	0.23	0.88	1.21	0.39	0,0166
cabbage	0.23	0.08	1.26	---	0,0450
algae	0.10	0.95	1.49	0.42	0,1027
faeces	0.20	0.20	---	---	---

From this table 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.

2.1.2 Minimal medium

The minimal medium was the same as used for *Pyrococcus furiosus* fermentations. The salt content was lowered to 1%. Also the sulphate concentration was decreased. A trace element solution was used described by Belay (1984). The amino-acid cocktail was described by Raven and Sharp (1992). Table 2 shows the minimal medium.

Table 2 The minimal medium used for stirrer speed optimisation and maintenance.

Chemicals	[ml/l]
Saltstock	33
Sulfatestock	33
$NaSeO_3$*5H_2O (5mmol)	1
Na_2WO_4*2H_2O (3,3g/l)	1
$FeSO_4$*7H_2O (35g/l)	1
Belay solution	10
Amino acid cocktail	50
Vitaminecocktail	1
Cystein HCl*H_2O	0,5g

Saltstock:	[g/l]
NaCl	300
$CaCl_2$ X 2H_2O	0,66
KH_2PO_4	14
Sulfatestock:	
$(NH_4)_2SO_4$	13
$MgSO_4$ X 7H_2O	2,5

Vitamincocktail	[mg/l]
D-Biotin	20
Cyanobalamin	1
Folic acid	20
Lipoic acid	50
Nicotinic acid	50
DL-Calcium Phantothenate	50
Pyridoxine HCl	100
Riboflavin	50
Thiamine HCl	50

Belay- trace element solution	[g/l]
$CoCl_2$x6H_2O	0,20
$MnCl_2$x4H_2O	0,10
$NiCl_2$x6H_2O	0,10
$ZnCl_2$	0,10
$CaCl_2$x2H_2O	0,05
$CuSO_2$x2H_2O	0,05
Na_2MoO_4x2H_2O	0,05

Amino acid	[mg/l]
L-Alanine	600
L-Arginine HCl	1000
L-Asparagine H_2O	800
L-Aspartate	400
L-Glutamat Na^+	1600
L-Glutamin	400
Glycin	1600
L-Histidin	800
L-Isoleucin	800
L-Leucin	800
L-Lysin HCl	800
L-Methionine	600
L-Phenylalanine	600
L-Prolin	1000
L-Serin	600
L-Threonine	800
L-Tryptophan	600
L-Tyrosine	800
L-Valine	400

The carbon and energy source was soluble starch at a concentration of 10g/l.

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 Fermentation setup

For the fermentation a 2l Visual Safety Reactor from Bioengineering (Wald, Switzerland) was used [Biller et al. (2001); Krahe (1998)]. The fermentor was equipped with temperature and pH-control. The titration was done with 2M NaOH. The fermentation parameters were: T=90°C, stirrer speed=500rpm, pH on-line 7.0, fermentation volume 1.8 litre, if not otherwise mentioned. The fermentor set up is shown in figure 1.

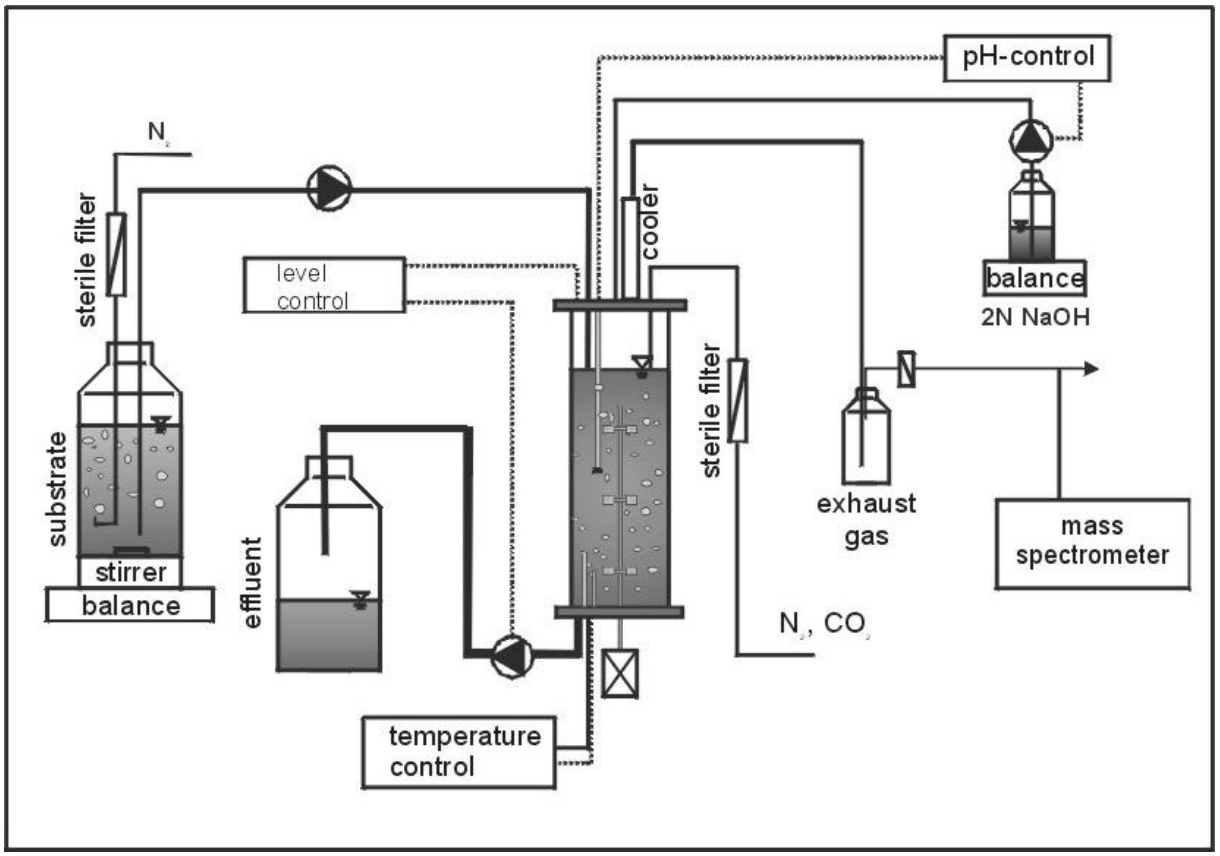

figure 1 Hyperthermophilic pretreatment of fibrous material, experimental set up

Dialysis fermentations were performed in a 10l dialysis reactor. The principle of dialysis is shown in figure 2.

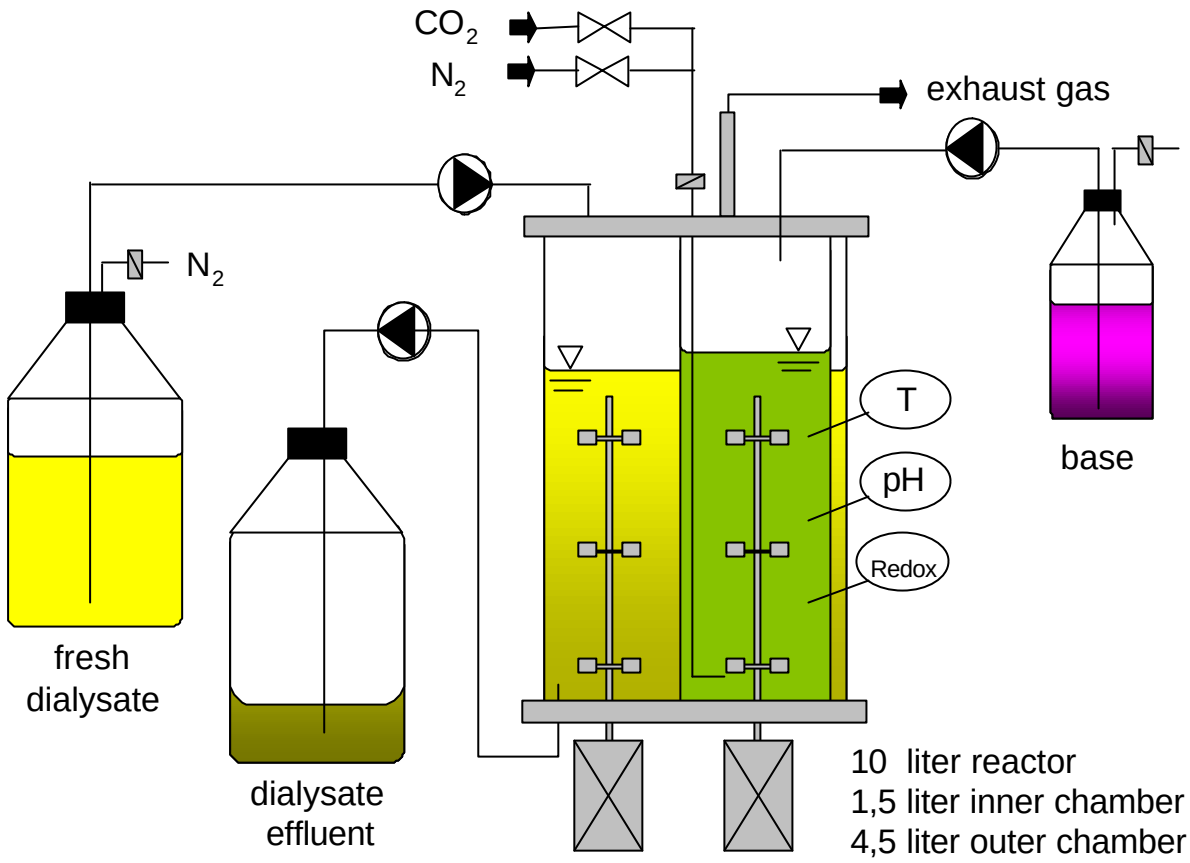

figure 2 reactor scheme of a dialysis culture.

2.4 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.

2.5 Dissolved Organic Carbon (DOC)

The DOC value of the samples were determinated with TOC + TN_b from Elementar.

2.6 Total Carbon (TC)

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

2.7 Analyses of exhaust gas

The fermentor's exhaust gas is analysed qualitatively with a mass spectrometer (Omni Star, Blazers Instuments).

3. COD-Reduction potentials in pretreatment

The main task of the hyperthermophilic pretreatment is the liquefaction of organic matter. Besides this a COD-reduction in the pretreatment step is desirable. Under strict anaerobic conditions, the COD-term can be balanced. So a COD-reduction in the suspended phase is an indicator for COD-increase in the gas-phase (fig.3).

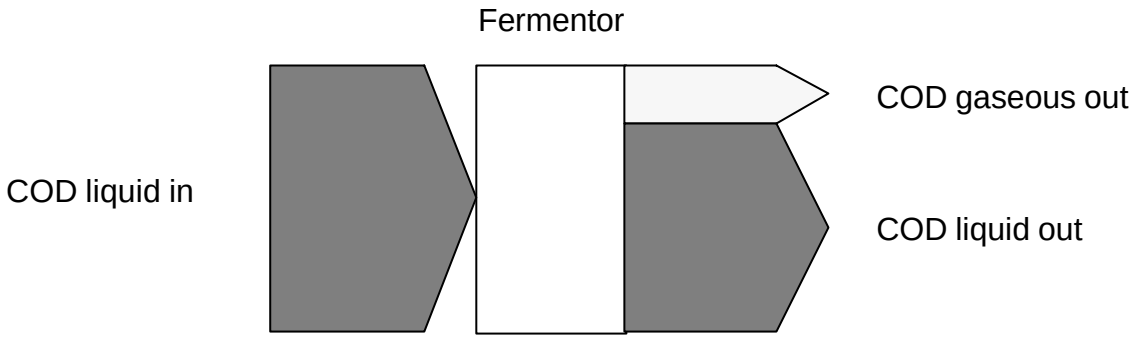

figure 3 qualitative COD-Balance of the hyperthermophilic pretreatment under anaerobic conditions.

The formed gas must not only consist of CO_2, but also has to be composed of COD-rich gases, like CH_4, H_2, NH_3, H_2S or even N_2 or acetic acid vapour. An anaerobic pretreatment with only CO_2-forming microorganisms like *Pyrococcus furiosus* shows no COD-reduction.

The newly isolated consortium from the Azores is able to decrease the COD-value of the ESA-Substrate. In technical note 3.100 a COD-reduction is presented for a batch fermentation of algae. Within one week the COD-value was reduced by 45%.

Because solved algae are a homogeneous system, the COD-time-plot could be presented. The suspended ESA-substrate, consisting of straw, crushed soya, cabbage and human faeces is a heterogeneous system. For the COD-samples have the same size as the inhomogeneities of the ESA-substrate, no total COD could be measured. The COD-reduction is determined by means of balances. Figure 4 shows the COD-balance for a continuos fermentation.

The sample volume for a COD-Test is 200-1000µl. The particle size of the ground solids must not be smaller than 3mm according to the meeting in Gent 2001. So a COD-measurement of o total sample will lead to greater errors than COD measurement of the liquid and solid phase.

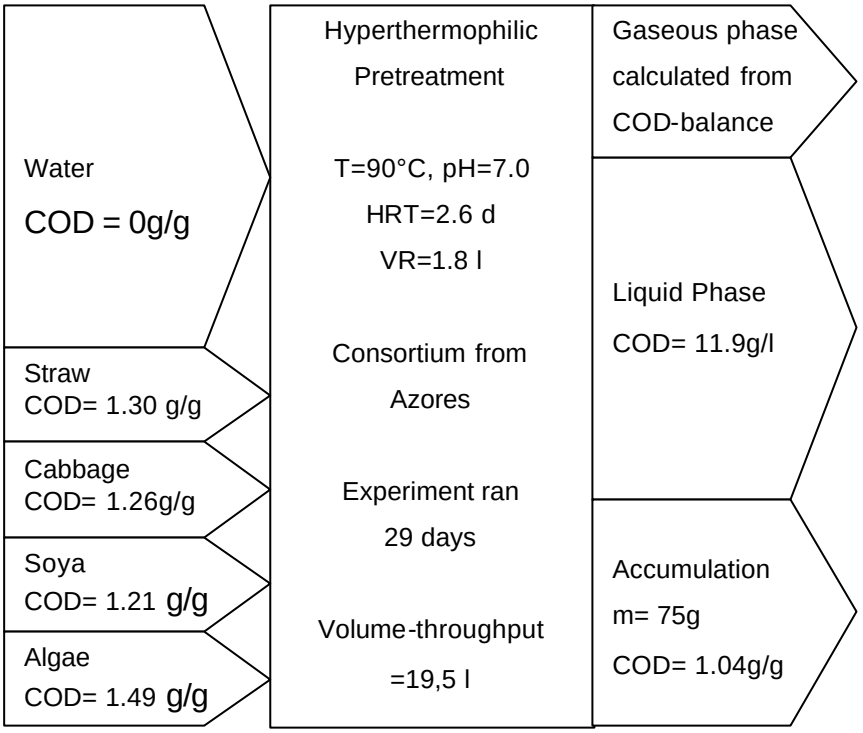

figure 4 COD-balance. The COD-value can be determined for either pure liquid phases or pure solid phases. The COD-value of the gaseous phase can not be measured. To determine the COD-reduction of heterogeneous systems each term is measured separately. The COD-reduction is determined by dividing the total gaseous COD through the total COD-input.

The COD-balance is shown in eq. 1.

$$COD_{feed} \cdot \left(m_0 + \int \dot{m}\, dt\right) = COD_{reactor} \cdot m_{reaktor} + COD_{gas} \cdot m_{gas} + \int \dot{m} \cdot COD_{effluent}\, dt \qquad (1)$$

$$19.44 \tfrac{g_{COD}}{kg} \cdot (1.8kg + 19.5kg) = 1.04 \tfrac{g_{COD}}{g_{DM}} \cdot 75 g_{DM} + COD_{gas} \cdot m_{gas} + 232 g_{COD}$$

$$414 g_{COD} = 310 g_{COD} + COD_{gas} \cdot m_{gas}$$

$$COD_{gas} \cdot m_{gas} = 104 g_{COD}$$

The COD-reduction is calculated with eq. 2.

$$\eta_{COD} = \frac{COD_{gas} \cdot m_{gas}}{CODfeed \cdot \left(m0 + \int \dot{m}\, dt\right)} \qquad (2)$$

$$\eta_{COD} = 0{,}25$$

During continuos fermentation of the ESA-Substrate a COD-reduction of 25% was achieved. Due to gassing with nitrogen no produced gases could be detected with the mass spectrometer.

Further experiments are planned to examine the composition of the produced gas. No gas samples were taken. So the gaseous phase cannot be analysed afterwards.

4. Carbon balance

A carbon balance was drawn for a continuous fermentation with a hydraulic residence time of 2.6d. A second fermentation with a hydraulic retention time of 4.8d was ended due to technical problems. The DOC level was measured continuously to determine the amount of carbon which is stripped out of the reactor. $DOC_{ù}$ indicates the DOC-level during the last day of the fermentation.

Eq. 3 shows the carbon balance

$$TC_{feed} \cdot \left(m_0 + \int \dot{m}\, dt\right) = \tag{3}$$
$$TC_{reactor} \cdot m_{reactor} + DOC_w \cdot V_{reactor} + TC_{gas} \cdot m_{gas} + TC_{out} \int TS_{out} \cdot \dot{m}\, dt + \int DOC_{out} \cdot \frac{\dot{m}}{r_{H_2O}} dt$$

Solving the carbon balance leads to

$$0{,}399 \tfrac{g_C}{g_{TS}} \cdot 15 \tfrac{g_{TS}}{l} \cdot 1{,}8l + 0{,}399 \tfrac{g_C}{g_{TS}} \cdot 15 \tfrac{g_{TS}}{l} \cdot 19{,}5l =$$
$$0{,}387 \tfrac{g_C}{g_{TS}} \cdot 75 g_{TS} + 0{,}518 \tfrac{g_C}{l} \cdot 1{,}8l + TC_{Gas} \cdot m_{Gas} + 0{,}397 \tfrac{g_C}{g_{TS}} \cdot 161 g_{TS} + 22{,}91 g_C$$
$$127{,}5 g_C = 116{,}8 g_C + TC_{Gas} \cdot m_{Gas}$$

The term $TC_{Gas} \bullet m_{Gas}$ is computed to 10.7g. This means that 8.4% of the incoming carbon leaves the fermentor in a gaseous state. From the incoming carbon 27% is liquefied. Another 50% leaves the fermentor in suspended state with particle sizes smaller than 350µm. Figure 4a shows the carbon balance. The gaseous carbon can consist of CO2, CH4, EtOH, acetic acid vapour, acetic aldehyde or other volatile carbon products.

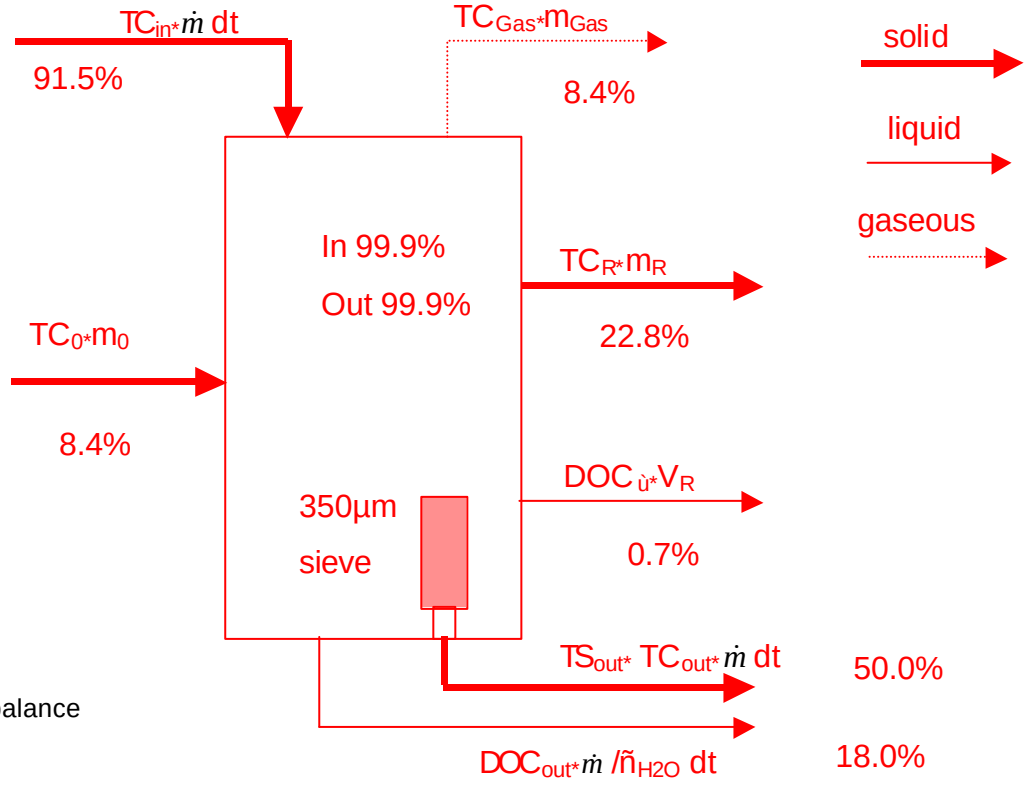

Figure 4a C-balance

5. Nitrogen balance

In the nitrogen balance all streams are considered which may include chemical bound nitrogen. Because the reactor was gassed with nitrogen, no gaseous streams are considered. The amount of physical dissolved nitrogen in the fermentation broth can not be neglected though it was not measured. Here it is assumed that dissolved nitrogen is in equilibrium with the gaseous phase. The nitrogen balance is given in eq. 4

$$TN_{feed} \cdot \left(\int \dot{m}\, dt + m_0\right) = \int \left(TN_{reactor} - c_{N_2}^{equ.}\right) \cdot \dot{m}\, dt + V_R \cdot \boldsymbol{r}_R \cdot TN_w + m_R \cdot TN_R \qquad (4)$$

The dissolved nitrogen is calculated with Henry's law (eq.5).

$$x_{N_2}^{gas} \cdot p = x_{N_2}^{liq} \cdot H_{N_2,H_2O} \qquad (5)$$

The Henry-coefficient for nitrogen in water at 90°C is $H_{N2,H2O}$= 17 µmol/mol•bar. With a nitrogen fraction of the gas phase of 99% and a slight overpressure of 20 mbar in the reactor the equilibrium concentration is calculated to 0,92 mmol/l or 26 mg/l. Solving the nitrogen balance leads to:

$$0.534\tfrac{g_N}{kg} \cdot \left(19.5kg + 1.8kg\right) = 4.15g_N + 1.8l \cdot 0.196\tfrac{g_N}{l} + m_R \cdot TN_R$$

$$11.37g_N = 4.50g_N + m_R \cdot TN_R$$

$$m_R \cdot TN_R = 6.87g_N$$

Forty percent of the incoming nitrogen is liquefied. The amount of ammonium is determined to 20% of the effluent's nitrogen. The rest could consist of nitrate, nitrite, solved peptides or amino acids. Special tests are bought to analyse the effluent and to complete the nitrogen balances. These tests need fresh samples, so no analysis afterwards could be done.

6. Optimisation of the hyperthermophilic liquefaction unit

There are many parameters for an optimisation of the new consortia. The parameters are temperature, pH, stirrer speed, and for continuous fermentation the hydraulic residence time.

As the temperature, which was adjusted to 90°C during the experiments corespondents to the temperature of the consortium's natural environment (i.e. the hot springs at the Azores) no temperature optimisation was done.

The pH-value of the sources varied from slightly acidic to neutral. So the normal pH-value during the fermentation was 7. Many cellulases have pH-optima from 5 to 6. An experiment was done without adjusting the pH-value with NaOH. The pH-Value dropped rapidly to 5.3 and the cell density decreased exponentially.

A dialysis-batch experiment with a fermentation volume of 1.5l and a dialysate volume of 4.5l was done. The substrate was the ESA-substrate, the dialysate was the minimal medium as described above with additional 2g/l yeast extract.
Figure 5 shows the DOC-time plot for a fermentation with pH7, pH5.3, and dialysis pH7.

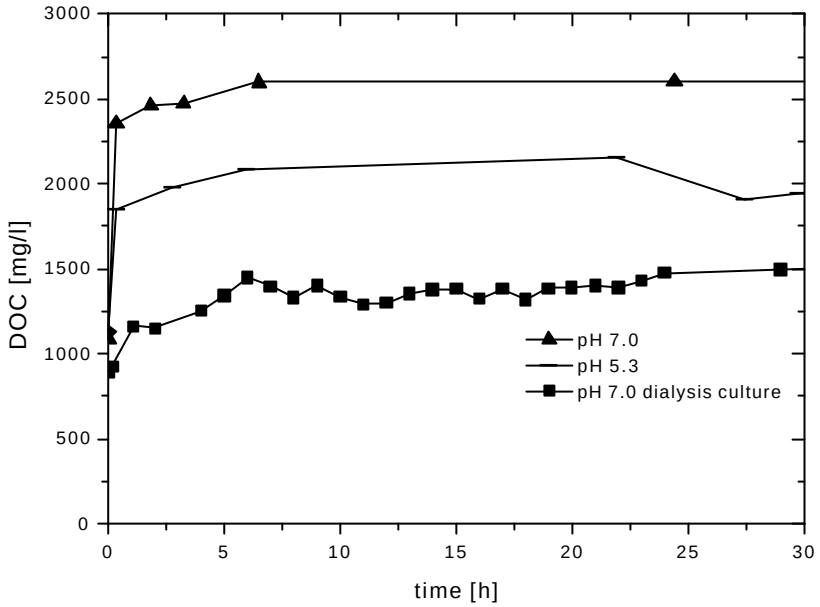

figure 5 optimisation of pH-value on ESA-substrate. The stirrer speed was adjusted to 100rpm

The carbon liquefaction in batch fermentation was 43% for pH 7, and 36% for pH5.3. A dialysis-batch culture showed the maximum liquefaction of 66% after one day.
Adjusting the stirrer speed has two different effects. At higher stirrer speeds inhibiting gasses are removed quicker. Thus higher cell densities can be monitored. But at a certain speed an increase in stirrer speed leads to a strong decrease in cell density. The cells are disrupted by shear stress. Krahe [1998] did a stirrer speed optimisation for *Pyrococcus furiosus*. The results are shown in figure 6.

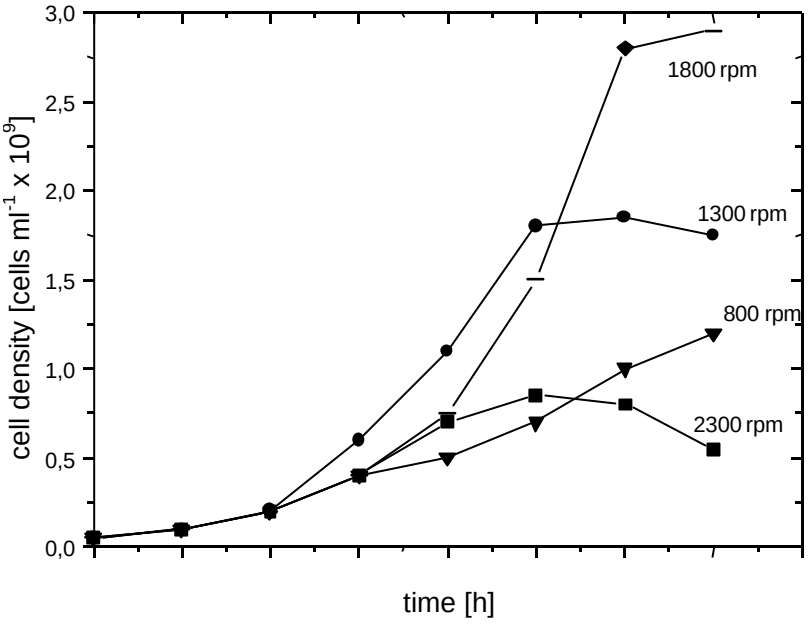

figure 6 stirrer speed optimisation for *Pyrococcus furiosus*. fermented on maltose [Krahe 1998]

The optimal stirrer speed for Pyrococcus furiosus is 1800rpm. At higher stirrer speeds (2300rpm) lysis is monitored.

The stirrer speed optimisation for the new consortium from the Azores was done in minimal media. A 2 liter reactor was inocculated. After 12 hours the reactor was cooled down, drained to 200ml, refilled with fresh medium, and heated up again. This procedure was done to remove dead mesophilic microorganisms from the fermentation broth. Now the hyperthermophilic cells could be counted easily. Figure 7 shows the cell density plot over time after refilling.

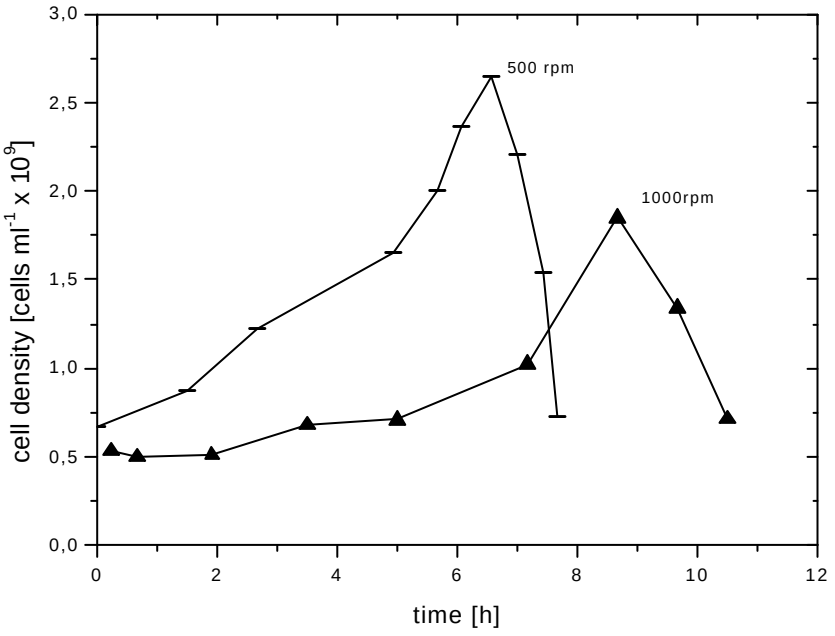

figure 7 stirrer speed optimisation for consortium from the Azores on 10g/l starch

Further experiments with lower stirrer speeds failed, because the amino-acid cocktail spoiled. But even with this two plots the tendency is obvious. Either no inhibiting gases have to be removed from the liquid phase, or the consortium is extremely sensible to mechanical stress. In both cases a lower stirrer speed is preferable.

7. Energy consumption

To evaluate the energy consumption of the hyperthermophilic pretreatment the assumptions have to be considered very carefully. The flow chart of the hyperthermophilic reactor is presented in figure 8

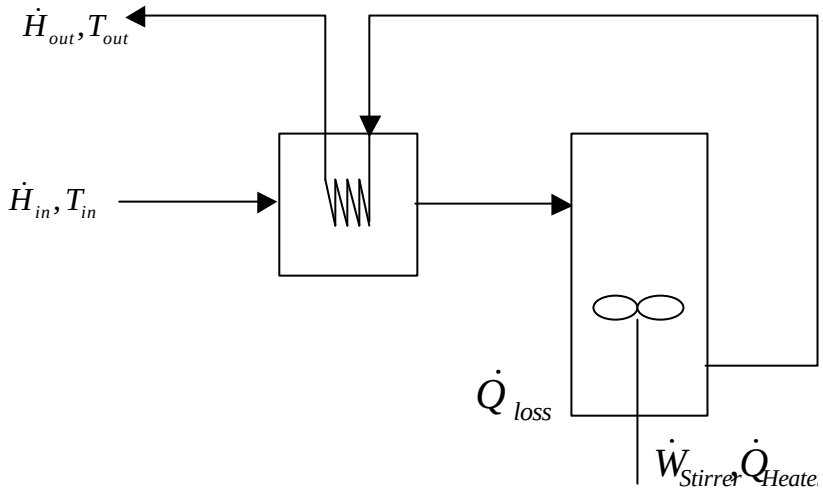

figure 8 reactor scheme of the hyperthermophilic pretreatment

The energy balance over the whole Process unit is written in eq. 6.

$$\dot{H}_{in} + \dot{W}_{Stirrer} + \dot{Q}_{Heater} = \dot{Q}_{loss} + \dot{H}_{out} \qquad (6)$$

For the estimation of the energy consumption a volumetric flow of 1200 l/d through the hyperthermophilic pre-treatment is assumed. Further assumptions are:

T_{in}=20°C, T_{out}=37°C, $T_{reactor}$=90°C,

$\dot{W}_{Stirrer}$=25W/l, HRT=7d $\dot{V}$ =1m³/d H/D=3

The mass flow has got the same physical properties as water (c_p=4.183 kJ/(kg*K), ñ=1000 kg/m³). The surrounding gas is air (T_{air}=350K, α=30•10^6 m²/s, *í*=52.7•10^6 m²/s, k=3•10² W/(m K), Pr=0.7). The reactor is not insulated, $\dot{Q}_{loss}$ appears only due to free convection and radiation. The temperature of the environment is T =20°C. No heat of reaction appears (worst case scenario).

The heat-loss consists of a convection term and a radiation term (eq.7).

$$\dot{Q}_{loss} = \dot{Q}_{conv} + \dot{Q}_{rad} \qquad (7)$$
$$\dot{Q}_{loss} = \bar{h} \cdot A \cdot \left(T_{reactor} - T_{\infty}\right) + \boldsymbol{e} \cdot \boldsymbol{s} \cdot A \cdot \left(T^4{}_{reactor} - T^4{}_{\infty}\right)$$

The convectional heat transport coefficient $\bar{h}$ is calculated with eq. 8.

$$\bar{h} = \frac{k}{H} \cdot \overline{Nu} \qquad (8)$$

Churchill and Chu [1975] found an empirical correlation for the Nusselt number on vertical plates (eq. 9)

$$\overline{Nu} = \left(0{,}825 + \frac{0{,}387 \cdot \overline{Ra}^{\frac{1}{6}}}{\left(1 + \left(\frac{0{,}492}{\mathrm{Pr}}\right)^{\frac{9}{16}}\right)^{\frac{8}{27}}} \right)^2 \qquad (9)$$

both valid for laminar and turbulent flow. The Rayleigh Number $\overline{Ra}$ is calculated with eq. 10.

$$\overline{Ra} = Gr \cdot \mathrm{Pr} = \frac{g \cdot \boldsymbol{b} \cdot \left(T_{reactor} - T_{\infty}\right) \cdot H^3}{\boldsymbol{a} \cdot \boldsymbol{n}} \qquad (10)$$

to $\overline{Ra}$ =1.1•10^{11}. The gravity is assumed to be 9.81 m/s², the thermal expansion coefficient is 1/T (ideal gas). The Nusselt number is calculated with eq.9 to $\overline{Nu}$ =537. By inserting eq.8 into eq. 7 the total heat-loss is computed to

$$\dot{Q}_{loss} = -4.9kW - 2.3kW = -7.2kW$$

The convectional heat loss of the top and bottom plate was neglected because the thermal boundary layer of the shell will have a strong influence on the heat-transfer over the top and bottom and will spoil the calculation. In the radiation term the top and bottom was considered.

The energy consumption of the stirrer is calculated with the upscale formula eq.11 starting with a 300l fermentor with a stirrer diameter of 100mm and 0.06W/l dissipated power. The stirrer of the 8400l fermentor should have a diameter of 300mm.

$$\frac{\dot{W}}{V} \sim n^3 D^2{}_{Stirrer} \tag{11}$$

$$\frac{\dot{W}}{V \cdot n^3 D^2{}_{Stirrer}} = const.$$

$$\frac{0.06 \frac{w}{l}}{(100rpm)^3 \cdot (100mm)^2} = \frac{\dot{W}}{8400l \cdot (100rpm)^3 \cdot (300mm)^2}$$

$$\dot{W} = 4.5kW$$

The total energy balance can be solved now.

$$\dot{Q}_{Heater} = \dot{Q}_{loss} - \dot{W}_{Stirrer} + (\dot{H}_{out} - \dot{H}_{in})$$

$$\dot{Q}_{Heater} = 7.2kW - 4.5kW + \dot{V} \cdot c_p \cdot (T_{out} - T_{in})$$

$$\dot{Q}_{Heater} = 7.2kW - 4.5kW + 1.2 \frac{m^3}{d} \cdot 1000 \frac{kg}{m^3} \cdot 4.183 \frac{kJ}{kg \cdot K} \cdot (90°C - 78°C) \cdot \frac{d}{24h} \cdot \frac{h}{3600s}$$

$$\dot{Q}_{Heater} = 7.2kW - 4.5kW + 0,7kW$$

$$\dot{Q}_{Heater} = 3.4kW$$

The total energy consumption of a hyperthermophilic pretreatment with a hydraulic residence time of 7 days and a daily volumetric load of 1000l would be 7.9kW.The energy demand can be segregated into 3.4kW thermal energy and 4.5kW electrical energy. In the case of startup additional 3.4kW would be needed to heat up the feed from 20°C.

Figure 9 shows the specific heat demand of the hyperthermophilic pretreatment for different hydraulic residence times and different volume-flows through the process unit.

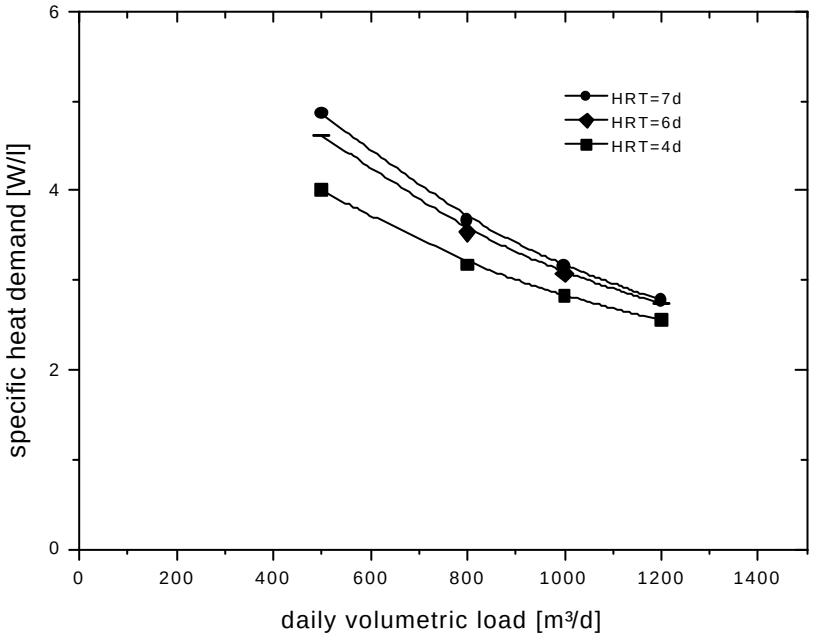

figure 9 specific heat demand as a function of daily volumetric load.

A linear dependence could be monitored between the specific energy demand and the hydraulic residence time (figure 10).

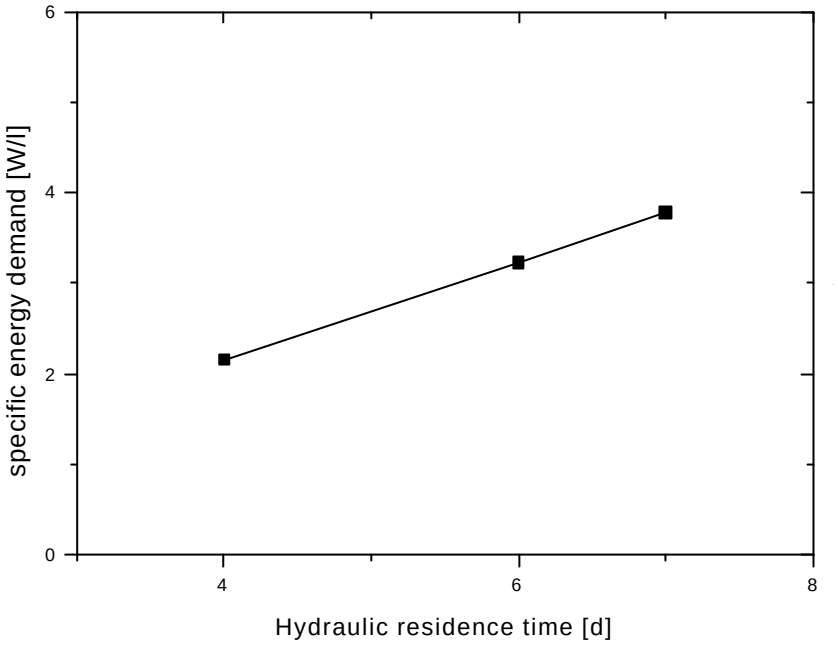

figure 10 specific energy demand as a function of the hydraulic residence time. The stirrer has always the same size (Ø300mm).

There is no dependence visible between the specific energy demand and the daily volumetric load.

8. Discussion

A continuous fermentation was done. A method to balance heterogeneous systems by determining the terms of balance for the liquid and solid phase separately was used. The COD-balance showed a COD-reduction of 25% for continuos fermentation. Though a COD-reduction is not the mayor task of the hyperthermophilic pretreatment it is a favourable feature. In comparison to the biogas process with a typical COD-reduction of 75-95% the turnover ratios were lower.

The carbon-balance showed that a quarter of the carbon is liquefied. Greater particles were broken down and suspended. A residue of anaerobic non degradable carbon remained inside the fermentor. Non degradable matter is for example lignin [Scherer 2001].

The liquefaction of nitrogen reached higher turnover-ratios. 40% of the feed's nitrogen was liquefied. Of the liquefied nitrogen 20% was ammonia (NH_4^+). The ammonia concentration laid well below the inhibiting value of 2-4g/l.

The stirrer speed optimisation showed, that low stirrer speeds are sufficient. From the *Methanococcus* strain it is known, that it is also very sensible to stirring while *Methanobacterium* and *Pyrococcus furiosus* sustain high mechanical stress [Märkl et. al. 1991, Krahe et al 1996]. *Methanococcus* has a thin cell wall consisting of proteins. The cell wall of M*ethanobacterium* and *Pyrococcus furiosus* consist of pseudomurein. This might be a hint, that the cell wall of the consortium also consists of protein.

In batch fermentations a greater liquefaction was achieved. The best pH-value for liquefaction is 7, though Sodium-hydroxide had to be added. Without the addition of base the pH will drop to 5.3 and the consortium dies after 20 hours.

The best carbon liquefaction was attained in dialysis fermentation with addition of 2g/l yeast extract.

9. Conclusions + Outlook

The hyperthermophilic pretreatment showed a moderate liquefaction performance in continuous fermentation (27%) and a good liquefaction performance in batch fermentation (43%). The introduction of dialysis fermentation increased the liquefaction performance to 66%.

Further tasks should be the decrease of the total residence time of the whole liquefaction unit. To prove whether this is possible, effluent of the reactor should be send to partner 1. The pre-treated substrate should be digested much faster.

Additionally the non biodegradable residues of partner 1 are to be liquefied in the hyperthermophilic pretreatment.

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