MELISSA

Memorandum of understanding
ETC/GF/MMM/97.012

ESA/ESTEC PO :

TECHNICAL NOTE 41.1

Anaerobic biodegradation of human faecal material under highly thermophilic conditions (70 °C)

TECHNICAL NOTE 41.2

Detailed characterisation of the thermophilic biodegradation process

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1. INTRODUCTION

The liquefying compartment in the MELISSA-loop is responsible for the biodegradation of human faecal material and other organic waste generated by the crew. The volatile fatty acids and ammonium produced during the anaerobic fermentation is fed to the second phototrophic anoxygenic compartment with the Rubrum species. The produced carbondioxide is supplied to the photosynthetic Spirulina compartment.

Biodegradation of faecal material by the selected thermophilic strains *Clostridium thermosaccharolyticum, Clostridium thermocellum and Coprothermobacter proteolyticus I8* seemed not to be successful (TN 22.4). Yet, it appeared that autochtonous strains present in human faecal material were able to biodegrade faecal material (TN 22.5). For this reason, an inoculum containing a consortium of strains that are originally present in faecal material was cultivated (TN 26.1). This consortium of autochtonous strains was able to biodegrade faecal material in anaerobic thermophilic conditions (55° C). Experiments performed in a fed batch reactor fed with non diluted faecal material learned that the overall biodegradation efficiency of the organic matter was equal to 40 %. It appeared that proteins were more easily biodegraded (60 %) than other components such as polycarbohydrates (20%). Due to specific consistency of non-diluted faeces, it was not possible to separate the volatile fatty acids and ammonium by centrifuging and/or filtration. For this reason, faeces needed to be diluted from 13 % dry matter [1] to 2 % dry matter.

It appeared that fibrous material such as lignin and cellulose which is present in the faecal material are difficult to biodegrade. Yet, when pure powdered cellulose was fed to the reactor, a biodegradation efficiency of 60 % was obtained. This indicates that the inoculum contained cellulolytic bacteria.

The production of methane must be inhibited in the MELISSA - cycle because it is of no use for the other compartments. A first approach could be the use of a composed inoculum without methanogenic bacteria. Yet, as already mentioned this approach was not successful because of the poor biodegradation capacity of the inoculum. The cultivated inoculum with autochtonous faecal bacteria contained also methanogenic strains. A first approach to inhibit the methanogenic association was the operation of the reactor at a high ammonium concentration. It is well known that methanogenesis is strongly inhibited by free ammonium. Fed-batch experiments were performed whereby urea was fed together with the faecal material. It appeared that at high ammonia concentrations (higher than 5 g/l) and a pH of 8, methanogenesis was completely inhibited. Yet, there were indications that also the production of volatile fatty acids was negatively influenced by the methanogenesis inhibiting conditions. For this reason two other methods were selected by which methanogenesis could be inhibited.

A first method that was tested, was the operation of the reactor at hyperthermophilic conditions (70°C). A second method that was investigated was the operation of the reactor at a pH lower than 6.5. At this pH level methanogenic bacteria are not active anymore.

A new demonstration reactor was build in which the experiments at low pH were performed. The results produced during the experiment will also be used by the University of Clermont Ferrand to perform simulation experiments.

[1] Determined by drying a sample at 105 °C during 24 hours

2. Biodegradation at hyperthermophilic temperatures

2.1 Introduction

Parshina et al. (1997) reported that it was possible to digest manure at high temperature ranging from 60° to 80°. The advantage to digest manure at this temperature is the fact that a complete destruction of pathogenic organisms is obtained. Methanogenesis is completely inhibited at this temperature, but acidogenesis still occurred on the condition that a well adapted inoculum was used. Cultivation of an adapted inoculum can take 0.5 to 1.5 years.

2.2 Results

The possibility to biodegrade faecal material was investigated. Tests were started by cultivating an inoculum by slowly adapting a mixture of anaerobic sludges and faecal material. The activity was screened by closed bottle tests (see set-up in Figure 2-1) . In this tests faecal material was used as test substrate for the cultivated inoculum.

INSERT

Figure 2-1. Set-up of a closed bottle test : 100 ml bottles in the incubator

Up to now, it seemed that it was not possible to get any significant activity on the biodegradation of the faecal material at 70 °C. This is illustrated in Figure 2-2. This confirms the statement that a long adaptation period is necessary to cultivate an adapted inoculum.

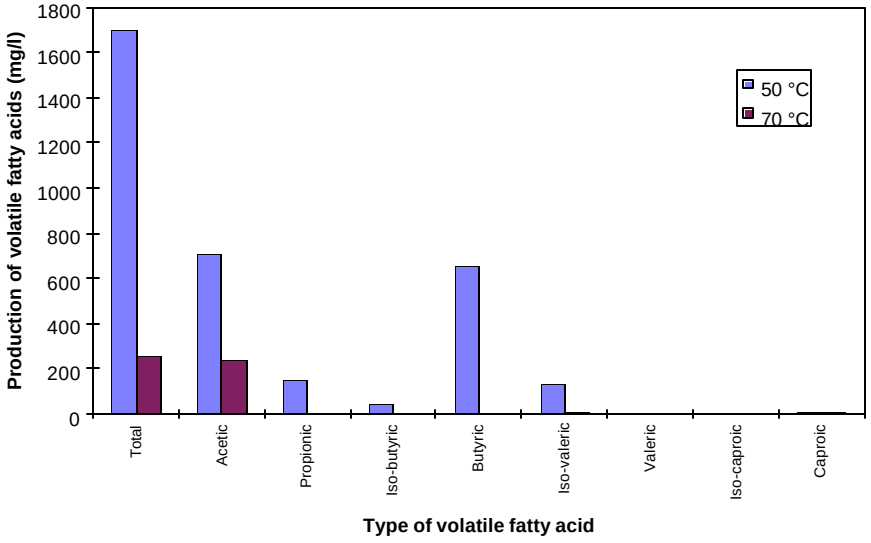

Figure 2-2. Comparison of the volatile fatty acid production during the biodegradation of faecal material with an inoculum cultivated in thermophilic conditions (55°C) and hyperthermophilic conditions (70°C)

3. Biodegradation at low pH

3.1 The demonstration reactor

A demonstration reactor was constructed in which the biodegradation tests were performed. The reactor had a wet volume of 1.5 litre and was kept at a temperature of 55°C by means of a hot water bath. The pH of the reactor had a set-point value between 6.0 and 6.5. The reactor content was continuously stirred by means of a magnetic stirrer. The reactor headspace was automatically flushed by nitrogen-gas every eight hours. During one flushing cycle, the gas volume was equal to three times the headspace volume. In this way, the produced biogas was removed from the reactor. The gas passed a trap containing a solution of 0,1 N KOH to capture the carbondioxide. The rest of the gas was captured in a gas column. Methane was detected by gas chromatography.

The reactor was fed with faecal material collected from different persons aged between 25 and 40 years. The material was ad random supplied to the reactor. The faecal material was received frozen and stored at minus 18 °C. The feed for the reactor was prepared by mixing 100 g faecal material (wet weight) in 1 l of tap water. The mixing was performed with an "IKA" grinder. The amount of the feed solution fed to the reactor is indicated in Figure 3-1. The harvested material was centrifuged and the supernatant and the cake were stored at minus 18 °C. No cake was recycled to the reactor. Table 3–1 gives a summarised overview of the characteristics of the faeces used in the experiment.

Table 3–1. Characteristics of the faecal material (mean values)

Parameter	Unit	Mean value
pH		6.9 (solution 1/10 on wet weight bases)
Dry matter (DM)	g/kg WW	200
Organic matter (OM)	g/kg DM	850
Organic nitrogen	g/kg DM	37.7
Ammonium nitrogen	g/kg DM	3.2
VFA	g/kg DM	45
Acetic acid	% of total VFA	43
Propionic acid		16
Butyric acid		28
Valeric acid		9
Caproic acid		4

The total reported process period is equal to 110 days. During the first 29 days of the test period the hydraulic retention time was equal to 20 days, during the next 48 days equal to 12 days en the last 35 days equal to 17 days. The reactor was diluted at day 30 and day 77. The dilution at day 30 was necessary to decrease the volatile fatty acid concentration in the reactor. The dilution at day 77 was necessary to produce enough effluent that could be used in the separation tests. Separation tests were performed at VITO (Mol, Belgium). More details on the process parameters are given in Figure 3-1 and in Appendix 1.

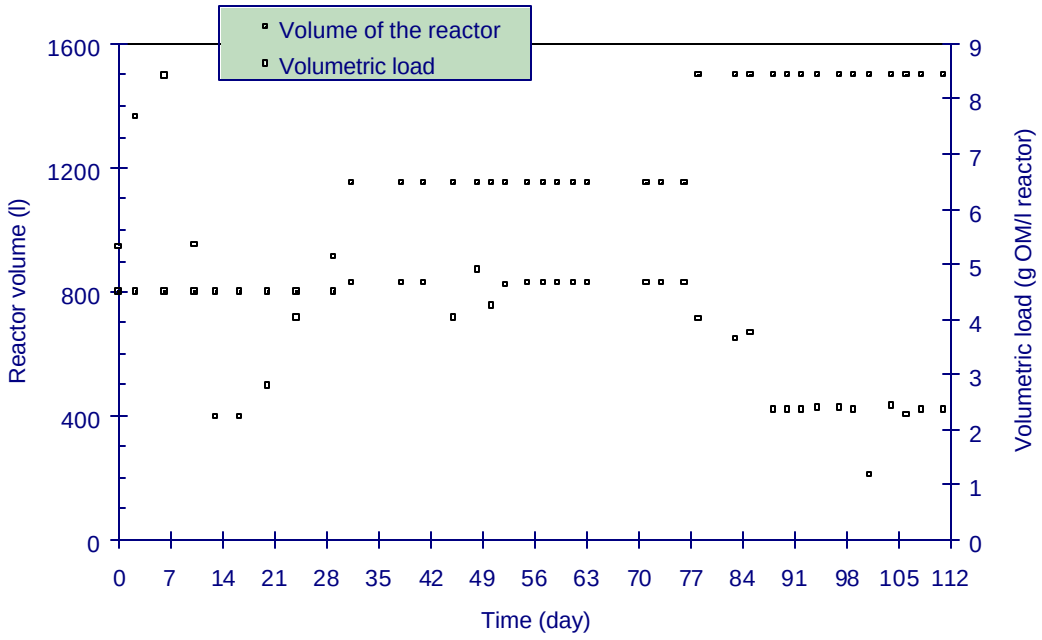

Figure 3-1. Overview of the feeding regime of the reactor

3.2 Results

The evolution of the volatile fatty acid concentration and ammonium concentration is presented in

Figure 3-2. At the beginning of the test period the volatile fatty acid concentration was equal to about 10.000 mg/l. Based on previous experiments (TN 34.2) it was assumed that a concentration higher than 10.000 mg VFA/l hydrolysis is significantly inhibited. At day 30 the reactor content was diluted from 800 ml tot 1150 ml resulting in a decrease in volatile fatty acid concentration to about 7.000 mg/l. During the experiment the concentration of volatile fatty acids evolved to an equilibrium concentration of about 5.000 mg VFA/l. The composition of the volatile fatty acids is presented in Figure 3-3. It appeared that 40 % of the total amount of volatile fatty acids was acetic acid, 10 % propionic acid and 30 % butyric acid.

The evolution of the ammonium concentration is presented in Figure 3-4. The ammonium concentration decreased from 1.400 mg N/l at the beginning of the test run to the equilibrium concentration of about 700 mg N/l at the end of the test run. The ammonium level was lower than the inhibitory level for methanogenesis that is equal to 2 g ammonium/l at a pH equal to 8.0. From Figure 3-4 it can be seen that the measurement of the organic nitrogen concentration was not stable at the beginning of the test. This was mainly due to analytical disturbances. At the end of the test, the organic nitrogen concentration was equal to about 600 mg/l.

The evolution of the process pH is plotted in Figure 3-5. The pH varied between a value of 6.0 and 6.5. At these values, methanogenesis did not occur. There was no methane measured in the produced biogas. Only carbondioxide was present in the biogas. The carbondioxide production was equal to about 6 to 8 mg carbondioxide per 100 mg faecal organic matter.

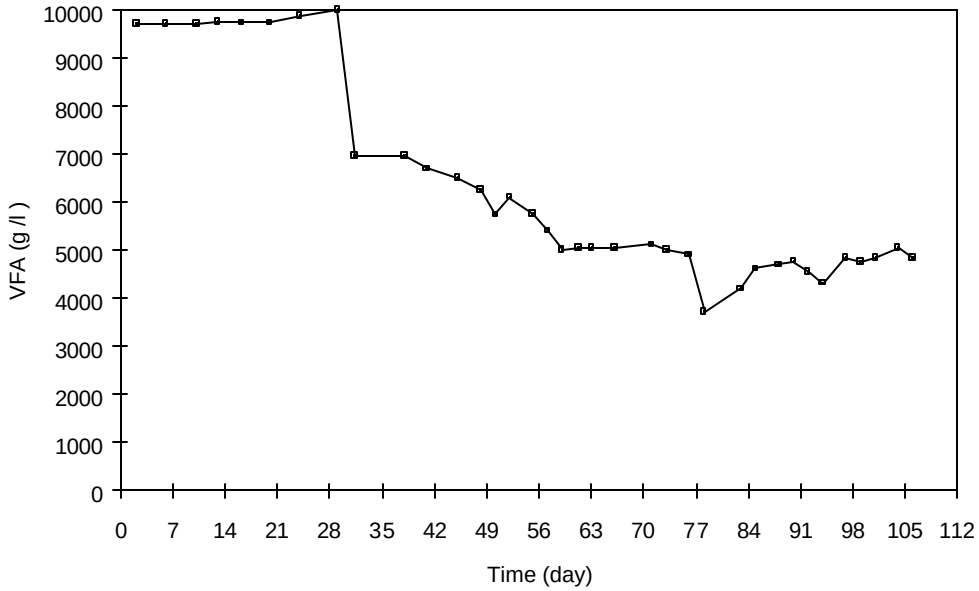

Figure 3-2. Evolution of the volatile fatty acids concentration in the reactor.

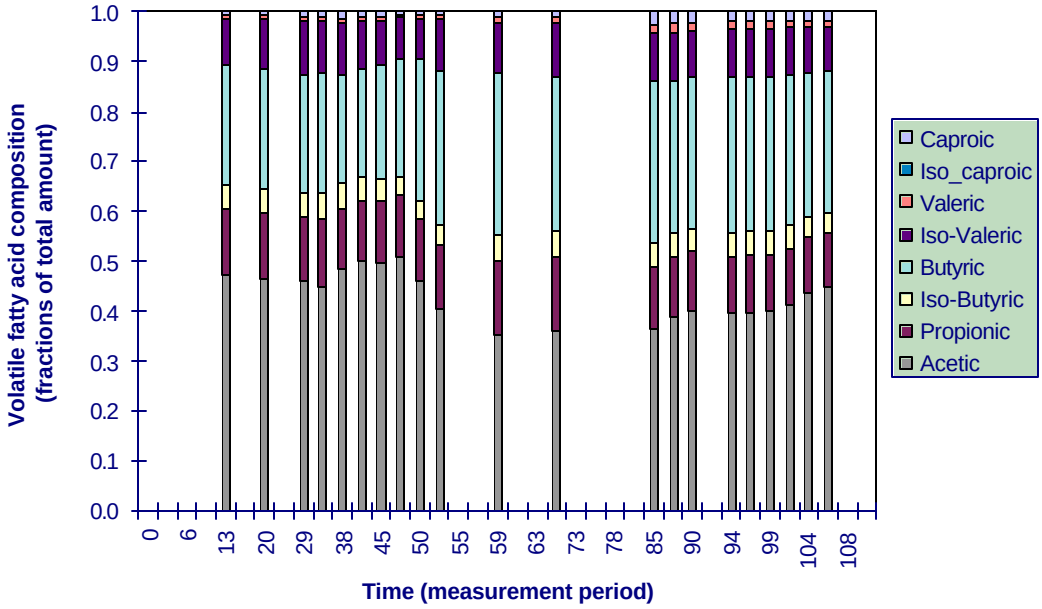

Figure 3-3. Composition of the volatile fatty acids in the reactor

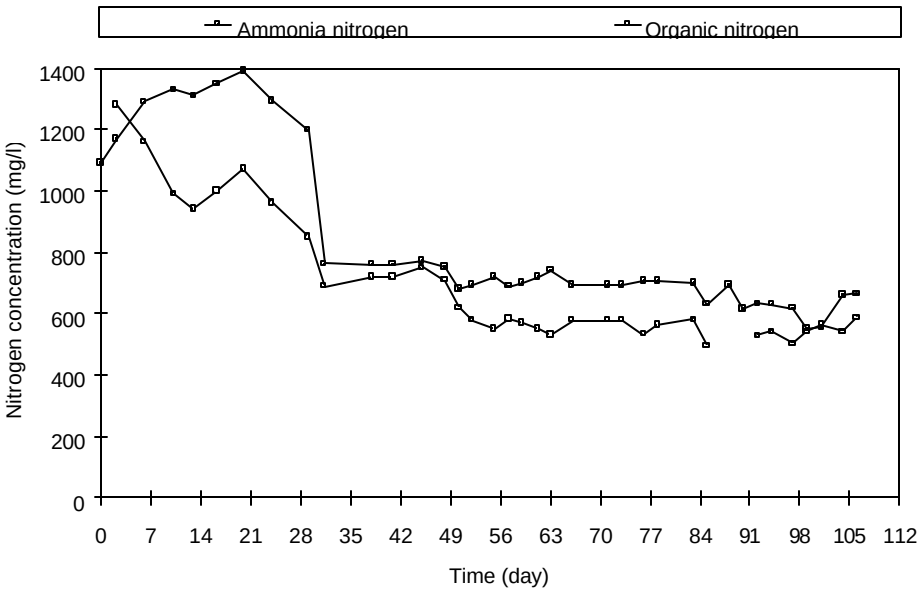

Figure 3-4. Evolution of the ammonium concentration and the organic nitrogen concentration in het reactor

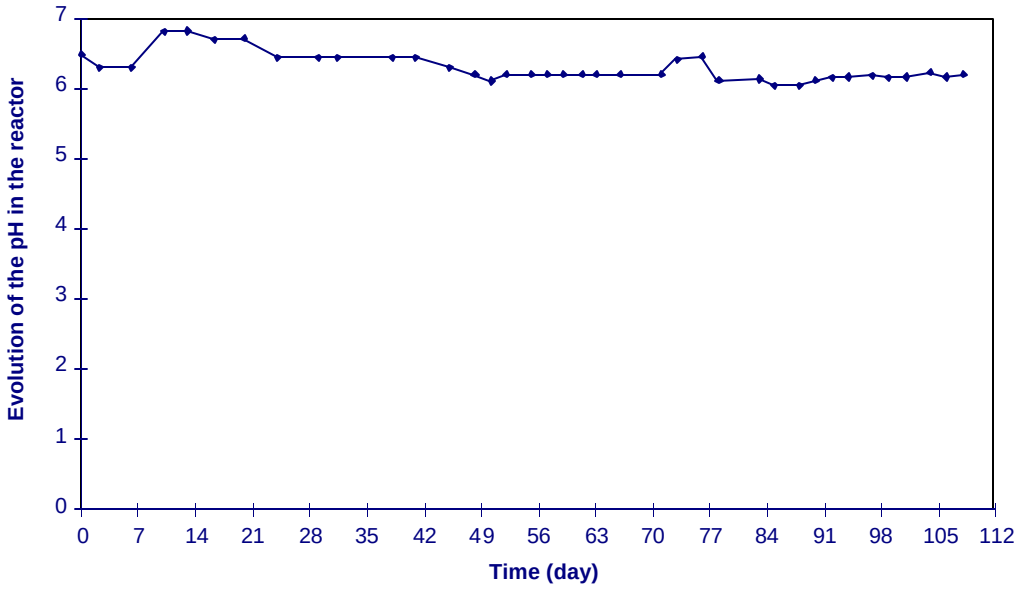

Figure 3-5. Evolution of the process pH

3.3 Calculation of the massbalance

3.3.1 The model of Angelidaki et al. (1993)

The data collected during the test run were used for the calculation of a mass balance. The main parameters that were used to calculate the mass balance were the characteristics of the reactor feed such as organic matter, ammonium and protein content and the production of volatile fatty acids and ammonium in the reactor.

The biodegradation efficiencies of the materials fed to the reactor were calculated taking into account some boundary conditions. It was assumed that during the anaerobic biodegradation polymers were converted into volatile fatty acids. Besides volatile fatty acids, also other products like lactate or alcoholic compounds can be produced. The production of this components was not measured and not taken into consideration to calculate the biodegradation efficiency of the faecal material. In the Melissa-concept organic matter should be converted into volatile fatty acids that are useful for the Rhodospirillum compartment. Also lactate can be used by the Rhodospirillaceae. However, this component was not considered in the mass balance calculation. During the biodegradation of faecal material also new biomass is produced. The transformation of organic matter into biomass can be considered as a non-efficient conversion in terms of the Melissa-concept and may not be considered in the calculation of the biodegradation efficiencies. Because only the produced volatile fatty acids are used to calculate the biodegradation efficiency, it is better to use the term conversion efficiency. This term indicates which part of the organic matter that is directly converted into volatile fatty acids. The conversion efficiency factor is an estimate for the part of the organic matter which may be converted into volatile fatty acids. Notice that carbondioxide was not taken into account in the calculation due to the relative low production compared to the volatile fatty acid production.

Angelidaki et al. (1993) presented a stochiometric model of the anaerobic biodegradation of cattle manure. The model describes the several anaerobic biodegradation steps on a stochiometric basis. Based on this theoretical stochiometric mass-balance it can be calculated that in optimal conditions 1 gram of biodegradable organic matter is converted into 0.153 gram biomass and 0.907 gram of biogas which contains 51.5 vol% carbondioxide and 48.5 vol% methane. The production of biomass is not considered in the Melissa cycle as a useful conversion. Taking this into consideration it can be theoretically calculated that the formation of one gram of biogas containing 48.5 vol% methane, corresponds with an effective conversion of 0.934 gram of organic matter. A mixture of volatile fatty acids is accumulating when the methanogenesis is completely inhibited. The organic matter is converted into volatile fatty acids. One gram of organic matter is converted in 0.08 gram biomass, 0.741 gram volatile fatty acids (37 % acetic acid; 31 % propionic acid; 32 % butyric acid) and 0.1874 gram biogas. This means that a production of 1 gram volatile fatty acids theoretically corresponds with an effective conversion of 1.24 gram organic matter. To calculate the conversion efficiency for the fed-batch reactor based on the experimental data, the theoretical conversion rates were simplified. It was presumed that the production of one gram of volatile fatty acid was presumed to correspond with 1 gram of organic matter converted. So, the calculated conversion efficiencies are indicative and an error on the reported efficiencies must be taken into consideration. The first twenty days were not taken into account and were considered as the start-up phase.

The protein biodegradation efficiency was calculated based on the measurement of the ammonium produced by hydrolysis of the proteins followed by biodegradation of the amino acids. The amount of ammonium produced per amount of organic nitrogen present in the feed gives the protein conversion efficiency. The protein content can be calculated by multiplying the ammonium-nitrogen with a factor 6.25.

Based on the overall conversion efficiency and the protein biodegradation efficiency, the efficiency of non-proteins was calculated.

3.4 Mass balance

The results of the mass-balance calculation are presented in Figure 3-6. The conversion efficiency of the faecal material into volatile fatty acids was equal to about 30 % for the whole period. This means that over the testing period 30 % of the faecal organic matter was converted into volatile fatty acids.

Based on the organic nitrogen hydrolysis efficiency it was possible to calculate the biodegradation efficiency of the proteins present in the faecal material. It appeared that the efficiency of protein biodegradation was equal to 80 % which is a high value. Based on the overall conversion efficiency, the protein biodegradation efficiency and the knowledge that about 27 % of the faecal material are proteins, the conversion efficiency of non-nitrogen containing components could be calculated. It appeared that this efficiency was equal to 10 %. This indicates that under given conditions, the biodegradation of materials such as cellulose and lignin were extremely low and that protein biodegradation was favoured.

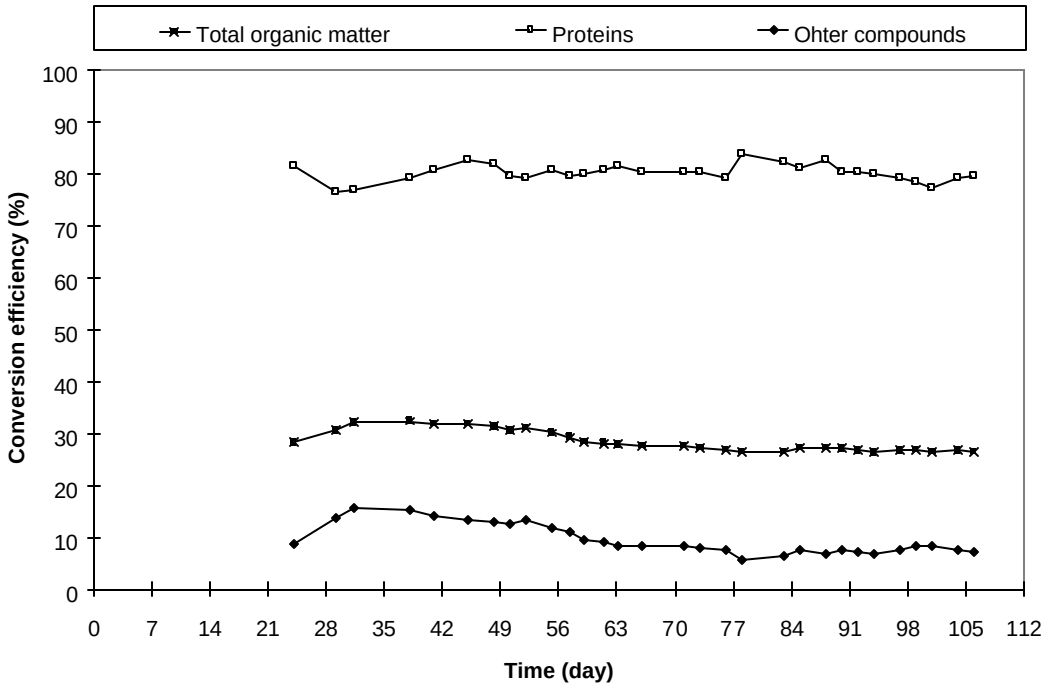

Figure 3-6. Cumulative conversion efficiency of the faecal material into volatile fatty acids

4. Counting of bacterial cells

4.1 Counting techniques

To simulate the anaerobic biodegradation process information, the density of bacteria in the anaerobic reactor can be valuable. Several methods can be used to count bacterial cells (Anderson et al., 1994). Microscopic enumeration and determination can be used, which is a fast and accurate method, but the examiner must have a well grounded experience. The disadvantage of the method is the fact that it is hardly possible to make a difference between viable and non-viable cells. To measure viable cells, counting techniques based on the determination of specific activities of different bacterial groups can be used. The "Most Probable Number" or MPN-technique is such a method that is based on the detection of specific activity of bacteria. Dilution series of the medium containing the bacteria are made and added to a nutrient and substrate solution. After incubation the number of positive reactions is detected in the different vials and based on the MPN-theory. This theory is based on the application of the Poisson distribution for extreme values to the analysis of the number of positive and negative results. The MPN is not the absolute concentration but a statistical estimate of the concentration. The MPN can be determined using the Poisson distribution. The joint probability of obtaining a given result from a series of three dilutions is given by following equation :

$$y = \frac{1}{a} \cdot \left[\left(1 - e^{-n1 \cdot l}\right)^{p1} \left(e^{-n1 \cdot l}\right)^{q1} \right] \left[\left(1 - e^{-n2 \cdot l}\right)^{p2} \left(e^{-n2 \cdot l}\right)^{q2} \right] \left[\left(1 - e^{-n3 \cdot l}\right)^{p3} \left(e^{-n3 \cdot l}\right)^{q3} \right]$$

where

y	=	probability of occurrence of a given result
a	=	constant for a given set of conditions
n1, n2, n3	=	sample size in each dilution
λ	=	bacteria density expressed as number/ml
p1, p2, p3	=	number of positive tubes in each sample dilution
q1, q2, q3	=	number of negative tubes in each sample dilution

4.2 Evaluation of the MPN-method

Dilution series were made from samples from the reactor. 10 ml of dilution was added to 30 ml of mineral medium containing 0,75 g gelatine/l and 0,75 g water soluble starch/l as substrate. Each dilution was set up in triplicate .The pH was set at a value of 7.5. A blanc without bacteria was also incubated. The 100 ml vials containing the test solution was incubated for 5 days at a temperature of 55 °C. After incubation gas samples were taken and analysed for methane. The production of methane was interpreted as a positive result for the presence of methanogens. The presence of acidogenic bacteria was monitored by measurement of a drop in the pH value. Yet, it appeared that it was difficult to evaluate this value in a reliable way as positive or negative. Also volatile fatty acid production could

be monitored, but due to the intensive character of the test, this was not done. Measurement of the ammonium concentration with a test kit appeared to be a good tool to detect positive reactions.

4.3 Results

Based on the test results it can be assumed that the magnitude of viable bacterial cells present in the reactor ranged from 10^8 to 10^9 cells/ml. Taking into account that the mean volume a bacteria is equal to $5.23.10^{-13}$ cm³ and a dry weight of 440 mg/cm³ cells it can be calculated that 0.023 to 0.23 mg bacteria/ml were present. This means that 0,27 % to 2,7 % of the organic matter present in the reactor were bacterial cells. This values are indicative values and the enumeration method needs to be optimised to produce more accurate data.

Concentrations of bacterial cells of in fresh faecal material of human origin were determined by Ikeda et al. (1994). Faecal material contained about $10^{10.8}$ bacteria per gram fresh material. This value corresponds to about 0.072 mg bacteria per mg of faecal organic matter or 7,2 % of the faecal matter are viable bacteria.

5. General conclusions

Biodegradation experiments of faecal material were performed in a thermophilic (55 °C) anaerobic reactor. The process was characterised by a low pH (6.0 - 6.5), no recycling of the organic matter, a residence time increasing from 12 days to 17 days and the absence of methanogenesis (due to the low pH). It appeared that proteins were biodegraded with a high efficiency (80 % biodegradation) but other components were biodegraded for only 10 %. Compared to previous experiments (TN 34.3), in which faecal material was biodegraded in non-methanogenesis inhibiting thermophilic conditions at a process pH of 8.0 and with recycling of the non-biodegraded fraction, the biodegradation efficiency of proteins was high. In the previous experiments (TN 26.1, TN 34.2, TN 34.3) the biodegradation efficiency of proteins ranged between 50 % and 60 %. The overall biodegradation efficiency of the faecal organic material in the previous process was equal to 40 %. This value was higher compared to the value of 30 % obtained in the current experiments. The biodegradation of the fraction of faecal material containing no nitrogen was in the current experiment only 10 % and lower than in the previous experiments where values of 25 % to 30 % were obtained. Yet, it is known from the previous experiments (TN 34.2) that the cultivated strains were able to biodegrade powdered cellulose with an efficiency of 50 %.

The fact that a higher biodegradation efficiency of long chained carbohydrates was obtained in the previous experiments can be due to the higher solid residence time. The solid residence time was equal to the hydraulic residence time in this test (12 to 17 days). This is low compared to the solid residence times of the previous experiments which ranged from 25 to 50 days. A higher solid residence time can be obtained by centrifuging the effluent of the reactor and by feeding back the obtained centrifuge cake. **The results indicate that a process with recycling of the recalcitrant fraction will be necessary**. This fraction can be recycled directly to the thermophilic reactor, or can be fed to another compartment in which for example fungi are active to hydrolyse the recalcitrant fraction mainly consisting of cellulose and lignin. The effluent of the test run was collected and after centrifuging stored at minus 18 °C. The cake can be used in experiments in which the biodegradation of the recalcitrant fraction is studied. The supernatant containing the volatile fatty acids and ammonium can be used as a test substrate for the second compartment.

Anaerobic biodegradation at hyperthermophilic conditions (70 °C) did not result in a significant increase of the biodegradation efficiency. This is mainly due to the long period for cultivating an adapted inoculum of hyperthermophilic organisms.

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Appendix 1. Detailed overview of the feeding regime of the reactor

Day	Volume of the reactor	Volume of the feed	Amount of faecal OM fed	NH_4^+-N in feed	Organic-N in feed	VFA in feed
	ml	ml	g	mg/l	mg/l	mg/l
0	800	100	4.3			
2	800	100	6.1	271	2269	885
6	800	100	6.7	125	1995	885
10	800	100	4.3	123	2155	885
13	800	100	1.8	200	980	885
16	800	100	1.8	90	1100	885
20	800	125	2.2	93	950	885
24	800	180	3.2	70	950	885
29	800	230	4.1	70	950	885
31	1150	300	5.4	70	770	885
38	1150	300	5.4	70	770	885
41	1150	300	5.4	70	770	885
45	1150	300	4.6	60	770	739
48	1150	300	5.6	77	850	641
50	1150	300	4.9	73	689	617
52	1150	300	5.3	72	828	1319
55	1150	300	5.4	69	838	885
57	1150	300	5.4	69	846	885
59	1150	300	5.4	69	846	885
61	1150	300	5.4	69	846	885
63	1150	300	5.4	69	846	885
66	1150	300	5.4	69	846	885
71	1150	300	5.4	69	846	885
73	1150	300	5.4	69	846	885
76	1150	300	6.0	85	1145	747
78	1500	300	5.5	75	1085	998
83	1500	300	5.6	78	1022	800
85	1500	200	3.5	60	795	744
88	1500	200	3.5	60	795	744
90	1500	200	3.5	60	795	744
92	1500	200	3.6	57	903	750
94	1500	200	3.6	57	843	750
97	1500	200	3.5	60	840	750
99	1500	100	1.8	60	840	750
101	1500	200	3.6	63	781	925
104	1500	200	3.4	67	800	1066
106	1500	200	3.5	62	724	1066
108	1500	200	3.5	62	724	
111	1500	200	3.5	62	724	

Appendix 2. Detailed overview of the dry matter concentration, the ammonium concentration, the organic nitrogen concentration and the volatile fatty acids concentration in the reactor.

Day	DM	NH_4^+-N	Organic nitrogen	VFA	AA	PA	IBA	BA	IVA	VA	CA
	g/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l
0	24.5	1090		9700							
2	24.9	1170	1280	9700							
6	24.1	1290	1160	9700							
10	24.7	1330	990	9737	4623	1256	473	2326	921	92	46
13	31.6	1310	940	9732							
16	23.3	1350	1000	9729	4508	1286	477	2342	981	88	47
20	23.3	1390	1070	9863							
24	23.3	1293	960	9997	6259	894	349	1634	721	64	76
29	28.8	1196	850	6955	3132	944	354	1675	725	68	58
31	18.8	763	688	6968	3362	846	359	1539	698	70	93
38	23.3	760	720	6700	3347	825	319	1439	637	64	69
41	23.3	760	720	6500	3226	805	284	1480	591	57	57
45	20.4	771	749	6262	3192	769	239	1481	500	48	33
48	17.6	752	708	5740	2643	712	214	1630	465	45	31
50	18.2	680	620	6108	2476	783	242	1873	648	46	40
52	23.3	692	578	5749	2926	883	331	1565	678	63	54
55	23.3	720	550	5390	2250	721	231	1606	495	49	39
57	23.3	688	582	5007							
59	23.3	700	570	5050							
61	23.3	720	550	5050							
63	23.3	740	530	5050							
66	23.3	692	578	5111	1835	757	282	1560	557	63	58
71	23.3	692	578	5000							
73	23.3	692	578	4902							
76	23.3	707	533	3726							
78	19.5	707	563	4200	1702	601	205	1512	443	77	114
83	19.8	700	580	4619	1682	586	204	1504	441	80	122
85	14.7	630	495	4699	1824	557	228	1434	457	86	113
88	14.3	693		4779	1920	564	212	1452	442	81	105
90	14.0	615		4547	1827	538	206	1386	426	73	92
92	14.0	632	528	4315	1699	499	205	1339	419	70	81
94	13.6	630	540	4838	1926	546	233	1502	469	73	87
97	13.5	618	502	4745	1906	530	226	1466	460	72	83
99	13.6	550	540	4850							
101	14.2	554	566	5040	2210	546	227	1442	462	70	83
104	11.5	660	540	4836	2162	519	213	1360	433	67	81
106	12.0	664	586								
108											
111											