EPAS n.v.

Eco Process Assistance

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MELISSA
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TN 26.3

Optimisation of the Melissa substrate biodegradation II:

Process control and optimisation

March 1996

ir. Dries Demey
Ing. Peter Van Meenen

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TECHNICAL NOTE 26.3
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1. INTRODUCTION

Based on the results retrieved during the first process run, reported in the technical notes TN 26.1 & TN 26.2, a preliminary mass balance was calculated. The biodegradation efficiency of proteins and total carbohydrates + lipids was equal to respectively 59 % and 40 %. Assuming that one third of the organic matter consists out of bacterial biomass, the biodegradation efficiency of non-bacterial proteins and non-bacterial carbohydrates + lipids was equal to 89 % and 61 %, respectively.

The separation test described in TN 26.1 & TN 26.2 demonstrated the positive effect of the dilution of the reactor content on the separation efficiency of ammonia and volatile fatty acids. The separation efficiency of the centrifuging step was 62 % for ammonia and 74 % for volatile fatty acids. During the ultrafiltration step, 80 % of the ammonium and organic nitrogen passed the 8 µm membrane and 100 % of the volatile fatty acids. It wasn't possible to separate the ammonium-nitrogen and the volatile fatty acids without remaining fractions of organic nitrogen.

The fed-batch and separation experiments were continued and results are reported in this technical note. Furthermore the inhibitive effect of free ammonia on the methanogenesis was studied by closed bottle tests.
Some preliminary tests on the die-off of pathogenic organisms were also performed.

2. EXPERIMENTS

2.1. Methanogenesis inhibition

Closed bottle tests were performed to determine the concentration range at which ammonia-inhibition of the methanogenesis occurs. A thermophilic methanogenic inoculum obtained from reactor RI2 was fed with starch (1 g/l) and ammonium (as NH_4Cl) in increasing concentrations up to 14 grams per liter. The pH was set at 7.5. Gas production and gas composition were measured.

The corresponding free ammonia concentration was calculated, using following equation:

$$[NH_3] = \frac{\left[NH_4^+ + NH_3\right]}{\left(1 + \frac{\left[H^+\right]}{K_a}\right)}$$

At 50°C K_a equals 38.1×10^{-10}

2.2 Fed-batch experiments

The experiments were performed in stirred fed-batch reactors as described in TN 26.1 & 26.2.
Several steps can be distinguished in the fed-batch experiments, as summarised in Figure 2.1. Reactors were fed every two days.

** Process run 1 (Reactor RI1)*

During the first process run, reported in TN 26.1 & 26.2, a microbial consortium was cultivated by natural selection. After an acclimatisation period of two weeks, the reactor was fed with undiluted human faeces. Biogas production and fermentation products were monitored in order to calculate a preliminary mass balance. The analytical dry matter content (DM_A) of the reactor was equal to 12.9 %.

** Process run 2 (Reactor RI2)*

The reactor from process run 1 was diluted to 5.2 % analytical dry matter and fed with human faeces which were diluted with water (ratio water/human faeces = 1.75). The dilution was necessary to obtain an optimal separation of volatile fatty acids and ammonium using a centrifuge during process run 3.

** Process Run 3 (Reactor RI3 + Reactor RII3)*

The reactor content of the first reactor was divided over two reactors. Reactor RII3 was inoculated with an inoculum obtained from a thermophilic anaerobic reactor digesting garden, fruit and vegetable waste, in order to stimulate the cellulolytic activity. Reactor RI3 had the same reactor content as reactor RI2. Before feeding, 10 to 20 % of the reactor content of both reactors was centrifuged to separate the soluble end products. The settled particulate material was fed again to the reactor together with the human faeces, thus the residence time of the particulate material will be infinite.

** Process Run 4*

A third mesophillic reactor (*Reactor RIII4*) was set-up and is still in operation. Preliminary results are reported.
At the end of Process Run 3, the reactor content of reactor RI3 was divided in two reactors (*Reactor RI4* and *Reactor RIV4*). Both reactors were fed with human faeces and the ammonium concentration of reactor IV was increased by adding urea. The reactors are still in operation and results will be reported in the next technical note.

The process conditions during the experiments are summarised in Table 2.1.

Table 2.1. Process conditions during the fed-batch experiments

		RI1 (TN 26.1 & 26.2)	RI2	RI3	RII3	RIII4
Volumetric load [1]	g OM/day.liter HF	5.7	5.8	5.1	4	4
Recycle [2]	% of OM	-	-	38	35	35
HF / water - ratio [3]		-	1.75	2.70	4.7	4.7
Mean Residence Time[5]	days	25	12	∞	∞	∞
Temperature	°C	55	55	55	55	32
pH		7	6.7	6.8	7.3	7
Dry matter (analytical)[4]	%	12.9 ± 0.3	5.2 ± 0.4	5.2 ± 0.4	2 ± 0.3	2 ± 0.4
Steady-state period	days	53	23	20	20	-

1. Amount of organic matter fed to the reactor; 2. Amount of organic matter originating from settled material after centrifuging; 3. Dilution; 4. Mean residence time of the organic matter; 5. Dry matter content without taking volatile organic components into account. * Residence time for the recycled particulate material.

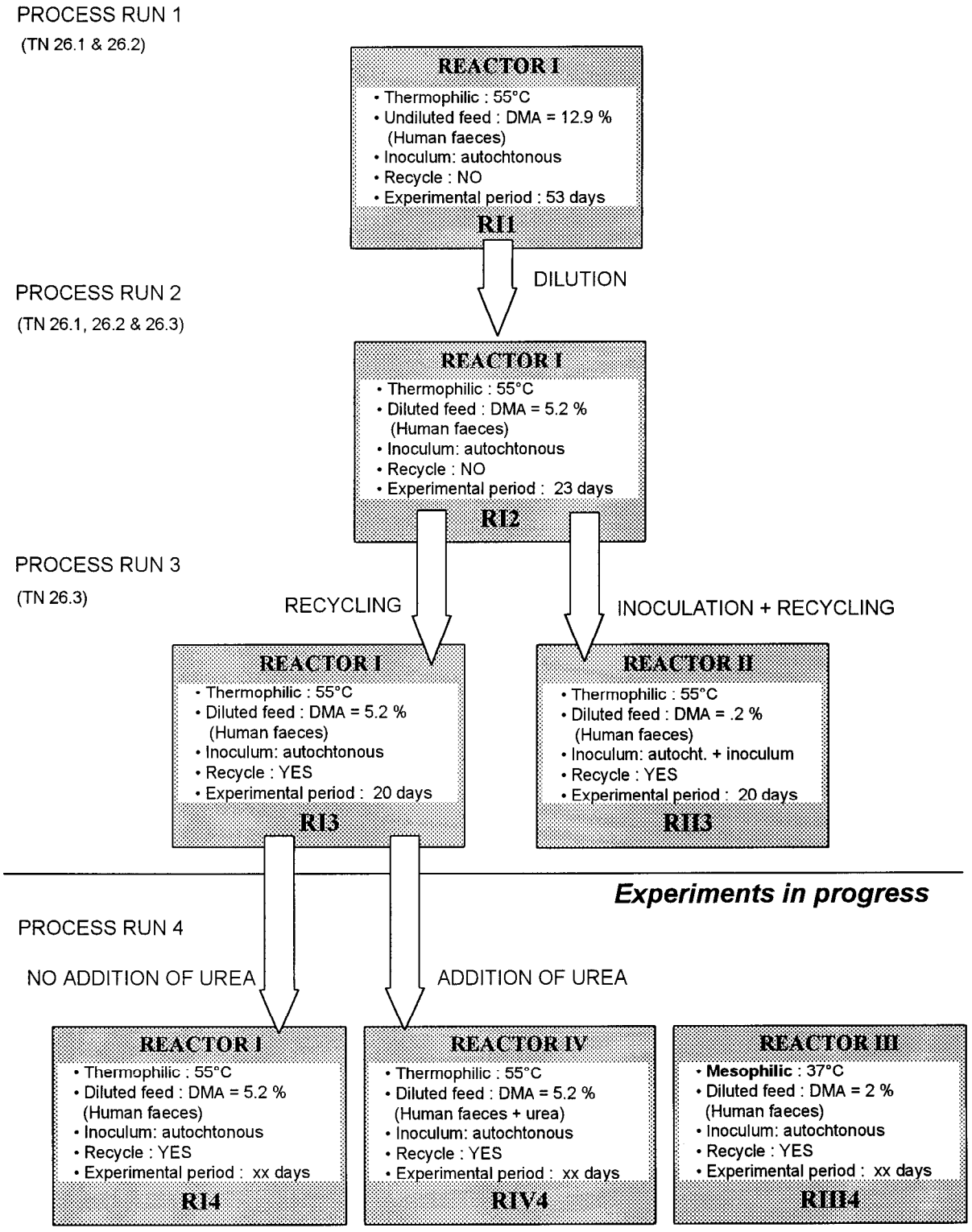

Figure 2.1. Schematic overview of the experiments

2.3. Separation tests

The reactor content of reactors RII3 and RIII4 was separated with a high speed centrifuge operating at an angular velocity of 12.10^3 rounds per minute. The efficiency of the removal of soluble organic matter was determined and compared with previous results.

2.4. Pathogenic organisms

2.4.1. Introduction

Pathogenic organisms may not be present in the effluent generated by the liquefying compartment. Potentially pathogenic organisms may be present in human faeces and can be bacteria, yeasts and viruses. A valuable concept generally used to screen for pathogenic organisms is the determination of *indicator organisms* (Bendixen, 1994; Catunda et al., 1994, Metcalf and Eddy, 1991). Indicator organisms can be isolated and determined in a fast and reliable way. If no indicator organisms are detected in a sample, there is no significant risk that the sample contains pathogenic organisms.

The reactor content was screened for the presence of three indicator organisms, namely *faecal coliforms*, *total coliforms* and *faecal streptococci* or *enterococci*. The analyses were carried out immediately after feeding the faecal material to the reactor and after a period of two days.

2.4.2. Determination procedure

* *Total and faecal coliforms*

A sample of the reactor content was diluted in physiological solution and a serial dilution was made. One ml of the dilution was brought on a petri-dish and mixed with 20 ml of Mc Conkey agar. After solidification of the agar, the plates were incubated. The incubation parameters are indicated in Table 2.2.

Table 2.2. Incubation parameters for the determination of total and faecal coliforms

Indicator organism	Incubation period (days)	Incubation Temperature (°C)
Total coliforms	3	37
Faecal coliforms	1	43

* *Faecal streptococci*

The same procedure was used as for the determination of coliforms. Slanetz and Bartley medium was used and the petri-dishes were incubated at 37°C for a period of 3 days.

3. RESULTS

3.1. Methane inhibition

Figure 3.1 shows the results of the closed bottle tests. The biogas production was monitored by a pressure measurement. The amount of biogas produced decreased with an increasing concentration of ammonium.
When the ammonium concentration was lower than 2 grams per liter, about 40% of the biogas produced was methane. This concentration corresponded with 204 mg free ammonia per liter. Between 2 and 6 grams ammonium per liter, or 204 and 612 mg NH_3/l, about 20% of the biogas was still methane. Between 6 and 8 grams ammonium per liter (612 to 816 mg NH_3 per liter) the methane production dropped to zero. When the ammonium concentration was higher than 8 grams per liter (816 mg NH_3/l), methanogenesis was completely inhibited.
The curve of the H_2 concentration in the headspace of the bottles is the opposite of the methane concentration in the headspace. When the methane production decreases, the H_2-production increases. At a concentration above 8 grams ammonium per liter, the H_2-production decreased to reach a minimal value at a concentration of 14 grams ammonium per liter. This indicates that also acetogenesis was inhibited when the ammonium level was higher than 8 grams per liter.

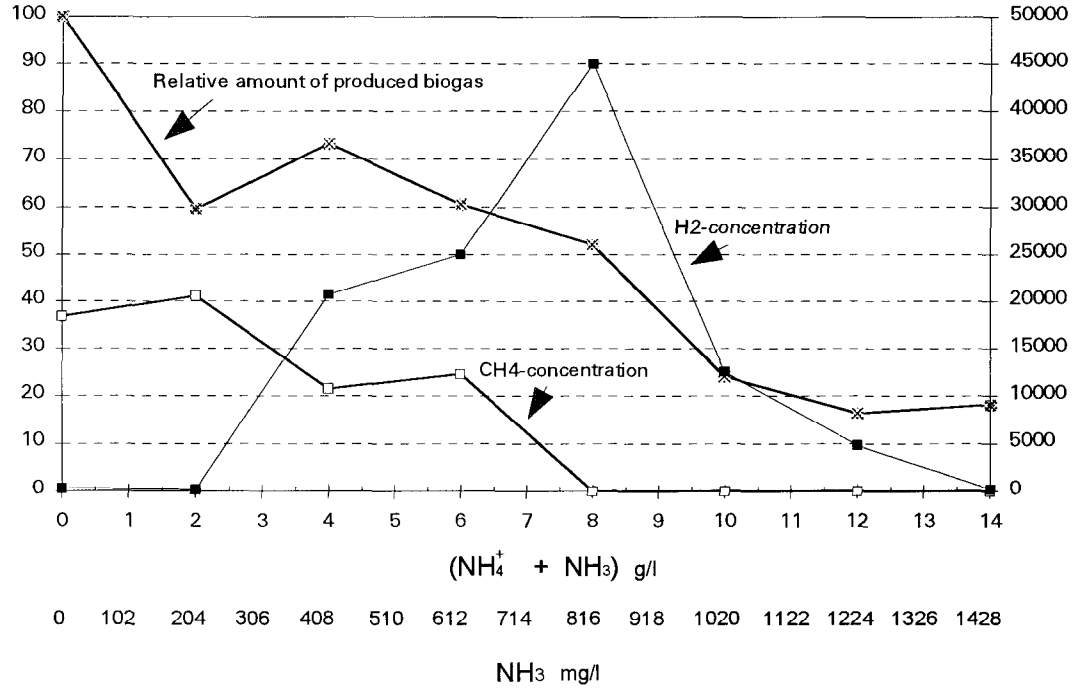

Figure 3.1. Relative amount of produced biogas and the methane and hydrogen content of the biogas in function of the total ammonium and free ammonia concentration

The effect of the ammonia inhibition is summarised in Table 3.1.

Table 3.1. Influence of ammonia on methanogenesis at pH = 7.5 and temp. = 55°C

Ammonium	Ammonia	Effect
< 2 g/l	< 204 mg/l	no inhibition
2 - 6 g/l	204 - 612 mg/l	± 50 % inhibition
6 - 8 g/l	612 - 816 mg/l	50 - 100 % inhibition
> 8 g/l	> 816 mg/l	100 % inhibition

3.2. Fed-batch experiments

As reported in TN 26.1 and TN 26.2 reactor RI1 was fed with undiluted faeces. It wasn't possible to obtain an efficient separation of the volatile fatty acids by centrifuging the reactor content. Therefore the reactor content was diluted to 5.2 gram analytical dry matter per liter (Experiment RI2). The biogas production during experiment RI2 was equal to 73 ml / gram organic matter fed. About 16 % of the produced biogas was methane. The ammonium content ranged from 2.4 to 3.6 gram per liter. After 20 days of stable processing, the reactor content was centrifuged (experiment RI3) to separate successfully the volatile fatty acids and ammonium (see Point 3.3.). The mean ammonium concentration in the reactor was equal to 2.2 gram per liter. About 50 ml of biogas per gram organic matter was produced. 28 % of the biogas was methane. Reactor RII3 which was inoculated with thermophilic bacteria obtained from a organic waste digester, produced 180 ml gas per gram organic material fed. About 66 % of the biogas was methane. The ammonium content of the reactor was low, namely 0.8 grams per liter. The production of biogas in the mesophilic reactor was equal to 35 ml per gram organic matter fed.

About half of the organic matter present in the reactors RI1 and RI2 was soluble. Reactors RI3 and RII3 were containing a higher amount of particulate organic matter. This can be explained by the fact that the soluble material was separated by centrifuging and the non-hydrolysed material was recycled to the reactor. Only 35 % of the total organic matter present in reactor RII3 was soluble organic matter. This was due to the conversion of soluble organic matter into biogas. In spite of the inoculation with a consortium of bacteria with a high cellulolytic activity, the amount of particulate organic matter present in this reactor stayed high. About 50 % of the organic matter present in the mesophilic reactor was soluble.

The pH of the different reactors was very stable and no pH correction was needed. This can be explained by the fact that the reactor contents were strongly buffered.

In all cases volatile fatty acids were formed. The composition of the volatile fatty acids of the four reactors and the human faeces is compared in Figure 3.2. The amount of each volatile fatty acid is expressed as a part of the total concentration (%). About half of the total amount of volatile fatty acids present in faeces is acetic acid. Acetic acid is also the most important fatty acid in reactors RI1, RI2 and RI3. RII3 has a low amount of acetic acid, which can be explained by the conversion into methane. The second most important fatty acid in reactor RI1 is butyric acid, followed by iso-valeric and propionic acid. In the reactors RI2 and RI3, propionic acid, butyric acid and iso-valeric acid are the most important acids. The concentrations of valeric acid and capronic acid were low. The production pattern of volatile fatty acids in the mesophilic reactor (RIII4) was comparable with the thermophilic reactor RI3. Temperature did not have any effect on the composition of the volatile fatty acids produced.
The relative high amounts of propionic, butyric and valeric acid indicates that acetogenesis was not favoured.

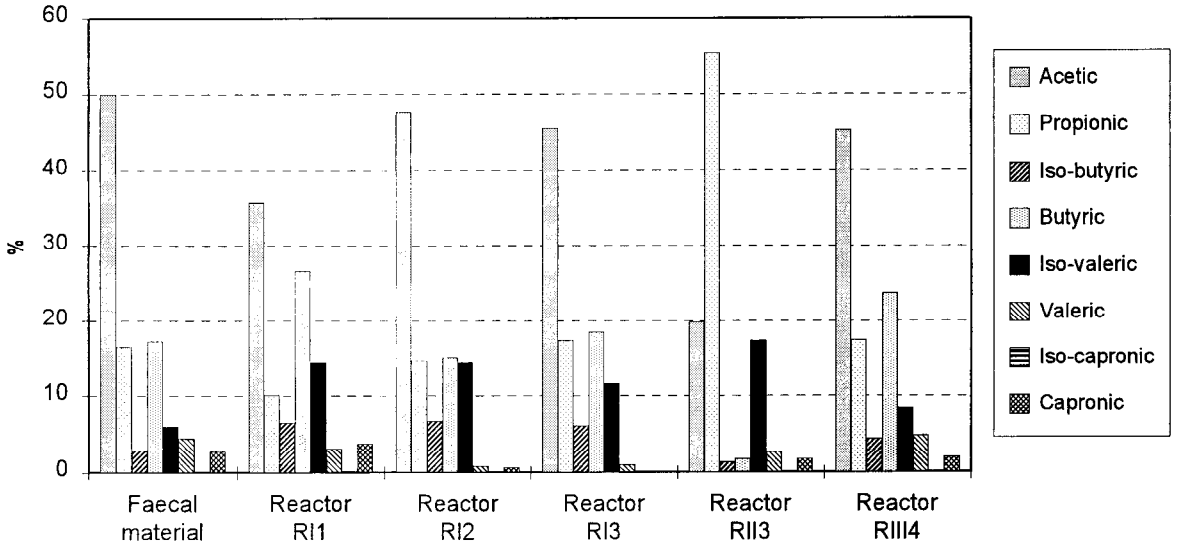

Figure 3.2. Fatty acid composition of the four different reactor contents and human faeces.

Table 3.2. Process conditions and results obtained during the different process runs.

		RI1 (TN 26.1 & 26.2)	RI2	RI3	RII3	RIII4
Process parameters						
Volumetric load [1]	g OM/day. liter HF	5.7	5.8	5.1	4	4
Recycle [2]	% of OM	-	-	38	35	35
HF / water - ratio [3]		-	1.75	2.70	4.7	4.7
Mean Residence Time	days	45	86	∞	∞	∞
Temperature	°C	55	55	55	55	32
pH		7	6.7	6.8	7.3	7
Dry matter (analytical)	%	12.9 ± 0.3	5.2 ± 0.4	5.2 ± 0.4	2 ± 0.3	2 ± 0.4
Biogas						
Production[5]	ml/g OM	30	73 ± 13	51 ± 15	180 ± 18	35
Composition: CH_4	%	0	16 ± 3	28 ± 5	66 ± 17	10 - 20
CO_2	%	100	84 ± 3	72 ± 5	34 ± 17	20 - 80
Reactor Content						
NH_4^+-Nitrogen	mg/l	6840 ± 600	2460 - 3660	2240 ± 110	815 ± 170	633 ± 107
Organic Nitrogen	mg/l	3970 ± 520	1880 - 2780	1340 ± 420	800 ± 220	761 ± 198
Total nitrogen	mg/l	10450 ± 790	5400	3580 ± 430	1615 ± 380	1394 ± 225
Volatile Fatty Acids	g/l	41.2 ± 6.2	15 - 20	13.1 ± 1.6	2.3 ± 0.60	5.1 ± 1.2
% soluble OM[6]	%	50	54	45	35	52
NH_4^+-N / Total-N	%	65	45 - 67	62	50	46

[1] Amount of organic matter fed to the reactor
[2] Amount of organic matter originating from settled material after centrifuging
[3] Dilution
[4] Dry matter content without taking volatile organic components into account
[5] Amount of biogas produced per amount of organic matter fed
[6] Percentage of organic matter which is soluble

3.3. Separation tests

Volatile fatty acids and the ammonia formed in the anaerobic compartment must be separated from the reactor content before these end products can flow to the second photoheterotrophic compartment. Reactors RII2 and RIII3 had a analytical dry matter content equal to 2 %. The separation of ammonia and volatile fatty acids was more efficient compared to the reactors with a higher dry matter content. The separation efficiency of ammonium was equal to 73 % for reactor RII3 and 88 % for reactor RIII4. Only 20 % of the organic nitrogen was separated from the reactor content. Volatile fatty acids were separated for 79 % from the reactor content of reactor RII3 and 88 % for reactor RIII4.

Table 3.3. Partition of the centrifuged reactor content and removal efficiency of soluble compounds by centrifuging

	RI1 (TN 26.1 & 26.2) **12.9 % DM_A**	**RI3** (TN 26.1 & 26.2) **5.5 % DM_A**	**RII3** **2 % DM_A**	**RIII4** **2 % DM_A**
Partition of centrifuged reactor-content (%)				
Volume supernatant	54	68	84	87
Volume precipitate	46	32	16	13
Removal efficiency (% of initial amount in reactor content)				
Dry matter	9	21	27	25
NH4+-N	46	62	73	88
Organic nitrogen	45	24	24	17
Volatile fatty acids	48	74	79	89
Acetic acid	70	76	81	88
Propionic acid	50	74	79	60
Iso-butyric acid	39	74	81	56
Butyric acid	40	73	77	86
Iso-valeric acid	35	73	72	60
Valeric acid	34	71	84	73

3.4. Pathogenic organisms

Table 3.4 shows the results of the experiment. Immediately after feeding total and faecal coliforms and faecal streptococci were determined in the reactor content. After a period of two days (without feeding) no indicator organisms were present anymore. This indicates that the reactor conditions were unfavourable for the survival of the indicator organisms. In future experiments more screenings will be performed.

Table 3.4. Die-off of pathogenic organisms in a thermophilic (55°C) anaerobic reactor.

Indicator organism	**Sample immediately after feeding**	**2 days after feeding**
Total coliforms	10^2 - 10^3	0
Faecal coliforms	10^2 - 10^3	0
Faecal streptococci	10 - 10^2	0

4 CONCLUSIONS

Inhibitive concentrations of free ammonia towards methanogenesis were determined by closed-bottle experiments. Methanogenesis was completely inhibited at a concentration of 800 mg/l free ammonia. Based on the results received by the closed-bottle experiments, the ammonia concentration in the fed batch reactors will be stepwise increased by adding urea to inhibit methanogenesis.

Several experiments with fed-batch reactors were performed. In a first step the reactor was fed with undiluted faeces (TN 26.1 & 26.2). The reactor had an analytical dry matter content of about 13 %. In steady-state conditions about 50 % of the organic matter was present in soluble form and about 65% of the total nitrogen was ammonium nitrogen. The soluble organic matter content of human faeces was equal to 30 % of the total organic matter and only 10 % of the total nitrogen was ammonia nitrogen. This means that in the reactor hydrolysis and acidification of the organic matter occurred. Proteins and carbohydrates and lipids were biodegraded for respectively 59 % and 89 %. Although it wasn't possible to separate the produced volatile fatty acids and ammonia from the reactor content by centrifuging or filtration. In order to obtain an efficient separation of the biodegradation products, the reactor content was diluted up to 5.2 % analytical dry matter content and fed with diluted human faeces (Reactor RI2). This had a positive effect on the separation efficiency of the end-products by means of a centrifuge. About 74 % of the volatile fatty acids and 62 % of the ammonium could be separated by centrifuging the reactor content (Reactor RI3). When the dry matter content of the reactor was equal to 2 %, the separation efficiency increased to about 85 % for volatile fatty acids and 80 % for ammonium. The settled material left after centrifuging was recycled to the reactor for further biodegradation. Besides volatile fatty acids and ammonium, also 24 % of the organic matter was still present in the supernatant. At this moment it wasn't possible yet to separate this fraction from the supernatant by filtration techniques. The soluble organic matter present in a reactor with recycling of the settled material obtained after centrifuging (reactor RI3) was equal to 45 % which was lower than in the reactor without recycle (54 % in reactor RI2). This indicates that the recycled material is slower biodegraded then the new fed human faeces. The ammonium nitrogen/total nitrogen-ratio in the thermophilic reactor with recycle was comparable with the undiluted rector and reactor without recycle (62 to 65 %). This indicates that proteins are better biodegraded than non-protein organic matter. Proteins are not accumulated in the reactor with recycle. In order to stimulate the biodegradation of carbohydrates and lipids, a reactor inoculated with an inoculum obtained from a thermophilic digester of fruit, vegetable and garden waste was taken in operation (reactor RII3). In this case a lower soluble organic matter content (35 %) was measured compared to the reactor without this inoculum (52 %). This was due to the higher conversion of volatile fatty acids to biogas (66 % methane). The ammonium/total nitrogen-ratio was lower equal to 50 % and lower than the other reactors. This indicates that proteolysis was not that efficient as in the other reactors Besides the thermophilic reactors a mesophilic reactor was started. In the beginning the reactor was not operating stable. After a period of three weeks a more steady state operation was reached. The reactor is still in operation. The percentage of soluble organic matter present in the mesophilic reactor was even higher than measured in the thermophilic reactor RII3, but the ammonium/total nitrogen ration was lower. This indicates that biodegradation of carbohydrates was more efficient at mesophilic conditions, but that proteins are better biodegraded in thermophilic conditions. Further experiments are needed to confirm this hypothesis.
Reactors fed with human faeces and an increasing amount of urea in order to inhibit methanogenesis were started up.

5 FUTURE ACTIVITIES

During the period April '95 - April '96 indicative information was obtained concerning the biodegradation efficiency of proteins, carbohydrates and lipids in thermophilic reactors fed with diluted and undiluted faeces and with or without recycling the material settled after centrifuging the reactor content. It was proven that ammonium and volatile fatty acids were formed during the biodegradation of the human faeces.
The centrifuging experiments showed that it was possible to separate the formed biodegradation products. Ultrafiltration of the supernatant obtained from centrifuging was not successful up to this moment.
Preliminary experiments on the removal of pathogenic organisms showed that in thermophilic conditions, pathogenic indicator organisms were removed.

Based on the orientating tests obtained during the period April '95 - April '96, further research will be focused on following topics:

- The improvement of the biodegradation of the recalcitrant part of the human faeces present in the recycle stream.
- Effect of the addition of cellulose on the reactor feed
- Evaluation of the pathogenity of the effluent from the anaerobic compartment
- Separation of the end products formed during biodegradation

Improvement of the biodegradation

Based on the indicative results obtained from previous fed-batch experiments, the complete anaerobic biodegradation process will be designed using modelling techniques in order to predict and control the operation of the fed-batch reactors. Important control parameters are the feeding rate, dilution ratio of the feed, the recycle ratio and temperature.
Additionally treatments to improve the biodegradability of the recycled material will be taken in consideration.
Finally a reactor will be designed to produce substrate which can be used for further characterisation and as a feeding substrate for the next compartments.

Addition of cellulose

The biodegradation of the co-substrate cellulose together with human faeces will be first investigated on small scale experiments (closed-bottle tests). Based on the results fed-batch experiments will be performed.

Evaluation of the pathogenity

The elimination of pathogenic organisms based on the screening of pathogenic indicator organisms will be studied in detail.

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