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Biodegradation of cellulose in batch tests

Biodegradation of cellulose in methanogenis inhibiting and non-inhibiting conditions (fed-batch tests)

Preliminary pre-acidifcation test

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1. INTRODUCTION

TN 34.1 gives an overview of the main results and conclusions obtained in previous experiments. It was concluded that further research needs to be focused on the following main topics:

- Biodegradation efficiency of cellulose and faecal material combined with cellulose in methanogenesis inhibiting and non-inhibiting conditions
- Improvement of the biodegradation efficiency of the faecal material and cellulose by adding additional organisms or by pretreatment of the feed

This technical note reports the results of closed-bottle tests for the determination of the optimal conditions of cellulose biodegradation and the results of the fed-batch reactor tests

2. BATCH REACTOR TESTS AND CELLULOSE BIODEGRADATION

2.1 Introduction

Two batch reactor tests were set up to determine the optimal conditions for cellulose biodegradation. Table 2.1 gives an overview of the different conditions at which the closed-bottle tests were performed. It was difficult to maintain a constant pH in the test medium during the batch reactor tests. An additional experiment was performed to test if the buffer capacity of the test medium could be improved.

Table 2.1. Overview of the experimental conditions for the batch reactor tests

BATCH REACTOR EXPERIMENT 1 The cellulolytic activity of the mesophilic and thermophilic inocula
Substrate: cellulose **Inoculum:** 1. Inoculum containing a consortium of autochtonous bacteria present in faecal material 2. Inoculum containing a consortium of autochtonous bacteria present in faecal material with additional Clostridia strains (*Clostridium thermocellum* and *Clostridium thermosaccharolyticum*) **Temperature:** 1. Mesophilic conditions (37 °C) 2. Thermophilic conditions (55 °C) **pH:** between 7 - 7.5
BATCH REACTOR EXPERIMENT 2 Effect of the pH on the biodegradation efficiency of cellulose in mineral medium
Substrate: cellulose **Inoculum:** inoculum containing a consortium of autochtonous bacteria present in faecal material **Temperature:** thermophilic conditions (55 °C) **pH:** different pH levels : 6 - 7 - 8
Additional test "BUFFER CAPACITY"
Improvement of the buffer capacity of the mineral medium

2.2 The cellulolytic activity of the mesophilic and thermophilic inocula

2.2.1 Introduction

A major part of the slowly biodegradable components present in the human faeces are long chained carbohydrates such as cellulose and lignin compounds. Additional cellulose can be added under the form of toilet paper. The proteolytic activity of the cultivated inoculum was already established in previous experiments. The aim of this experiment was to test the cellulolytic activity of the inocula.

2.2.2 Experimental set-up

The tests were performed in 100 ml closed bottles. The bottles were filled with reactor content of a thermophilic and mesophilic fed batch reactor which was diluted with water (ratio inoculum/tap water: 1/1)

The initial volatile fatty acid concentration was equal to 620 mg per liter for the thermophilic reactor and equal to 515 mg per liter for the mesophilic reactor. The initial pH was equal to 8. Cellulose was added to the medium in a concentration of 1 g per liter. No cellulose was added in the reference. The mesophilic set-ups were incubated at a temperature of 37 °C and the thermophilic set-ups at a temperature of 55 °C. Table 2.2 gives an overview of the set up of the experiment.

Table 2.2. Schematic overview of the different set-ups of the cellulolytic activity test

	MR	MC	MCC	TR	TC	TCC
Mesophilic inoculum	x	x	x	-	-	-
Thermophilic inoculum	-	-	-	x	x	x
Addition of cellulose (1 g/l)	-	x	x	-	x	x
pH correction to pH 7 at day 10	x	x	x	x	x	x
Inoculation with the strains *Clostridium thermocellum* and *Clostridium thermosaccharolyticum* at day 10	x	-	x	x	-	x

MR: mesophilic reference ; MC: mesophilic + cellulose ; MCC: mesophilic + cellulose + Clostridia strains
TR: thermophilic reference ; TC: thermophilic + cellulose ; TCC: thermophilic + cellulose + Clostridia strains

2.2.3 Results

Table 2.3 gives an overview of the amount of volatile fatty acids produced in the different set-ups. Table 2.4 shows the evolution of the pH during the test.

During the test, the pH increased in most of the set-ups above the initial value of 8. Therefore, at day 10, the pH was corrected in the bottles to a value of 7. It seemed that in the set-ups without Clostridia inoculum, the pH increased again above a value of 8. It was not possible to keep the pH at a constant level. The pH evolves to the pH of a $CaCO_3$ saturated solution. The high pH can be maintained due to the presence of carbonates formed in the fed batch reactors from which the inocula for the test were taken. It is recommended to use mineral medium with a smaller amount of inoculum instead of diluted reactor content in future batch reactor experiments.

After 5 days of incubation practically no volatile fatty acid production occurred in the mesophilic set-ups. In the thermophilic set-ups the volatile fatty acid production varied from 60 to 300 mg/l. There was also a volatile fatty acid production of about 300 mg/l in the reference TR (without cellulose). Because of the low volatile fatty acid production, the set-ups MR, MCC, TR and TCC were inoculated with the cellulolytic strains *Clostridium thermocellum* and *Clostridium thermosaccharolyticum*. Five days after the inoculation, the volatile fatty acids concentration in all applications raised significantly. The production of volatile fatty acids under thermophilic conditions was higher than under mesophilic conditions. The increase of the volatile fatty acids in the reference application "TR" was practically as high as in the set-up where extra cellulose was added (TCC). This means that also the organic material present in the inoculum was further biodegraded by the additional strains *Clostridium thermocellum* and *Clostridium thermosaccharolyticum*.

To verify the results, a fed batch reactor experiment was set-up to compare the biodegradation of cellulose by the cultivated inoculum and by the cultivated inoculum enriched with the cellulolytic strains *Clostridium thermocellum* and *Clostridium thermosaccharolyticum*.

Table 2.3. Volatile fatty acids production during the cellulolytic activity test.

Time (day)	MR	MC	MCC	TR	TC	TCC
Without pH correction and inoculation with *Clostridium thermocellum* and *Clostridium thermosaccharolyticum* at day 10						
0	0	0	0	0	0	0
5	11	10	0	297	60	316
10	-	-	-	-	-	-
15	0	25	14	320	316	NR
After pH correction to 7.0 at day 10						
15	189	343	99	545	262	322
After pH correction and inoculation with *Clostridium thermocellum* and *Clostridium thermosaccharolyticum* at day 10						
15	444	-	940	1248	-	1352

MR: mesophilic reference ; MC: mesophilic + cellulose ; MCC: mesophilic + cellulose + Clostridia strains
TR: thermophilic reference ; TC: thermophilic + cellulose ; TCC: thermophilic + cellulose + Clostridia strains
NR: no representative value

Table 2.4. pH evolution during the cellulolytic activity test.

Time (day)	MR	MC	MCC	TR	TC	TCC
Without pH correction and inoculation with *Clostridium thermocellum* and *Clostridium thermosaccharolyticum* at day 10						
0	8	7.4	8.0	8.0	7.4	8.0
5	8.6	8.8	8.5	8.4	8.8	8.5
10	8.7	8.9	8.5	8.0	8.9	8.3
15	8.8	8.8	8.4	8.0	8.9	8.2
After pH correction to 7.0 at day 10						
15	8.4	7.7	8.4	8.2	8.0	7.9
After pH correction and inoculation with *Clostridium thermocellum* and *Clostridium thermosaccharolyticum* at day 10						
15	7.9	-	8.5	7.3	-	6.9

MR: mesophilic reference ; MC: mesophilic + cellulose ; MCC: mesophilic + cellulose + Clostridia strains
TR: thermophilic reference ; TC: thermophilic + cellulose ; TCC: thermophilic + cellulose + Clostridia strains

2.3 EFFECT OF THE PH ON THE BIODEGRADATION EFFICIENCY OF CELLULOSE IN MINERAL MEDIUM

2.3.1 Introduction

A batch reactor experiment was performed in order to test the influence of the pH on the biodegradation efficiency of cellulose.

2.3.2 Experimental set-up

Bottles of 100 ml were filled with 30 ml of mineral medium (Addendum 1). The pH in the different set-ups was corrected to a value of 6, 7 and 8. After autoclaving during 20 minutes at a temperature of 121 °C, 0.6 ml Na_2S and 10 ml cellulose solution were injected corresponding with a cellulose concentration of 1 g/l. Next, 5 ml of thermophilic anaerobic inoculum was injected. The reference applications were injected with 5 ml of water. The bottles were incubated on a shaker during 5 days at a temperature of 55 °C. After incubation the volatile fatty acids concentration, the pH and the gas production were measured.

2.3.3 Results

The results of the experiment are presented in Table 2.5. It was not possible to maintain the pH at a constant level. At the start of the experiment the pH already decreased in all the different set-ups. The reference where the initial pH was equal to 8 showed a decrease down to a value of 6.7.

Based on the results it could be calculated that the biodegradation efficiency of cellulose at a pH equal to 6.0 is only 7 % to 8 %. The biodegradation efficiency of cellulose in a medium with an initial pH of 6.5 to 6.7 is ranging from 31 % to 49 %. This result indicates that the biodegradation is inhibited at a pH lower than 6. Although, it was not possible in the experiment to investigate the optimal pH-range because of the instability of the pH of the test medium. To improve the buffer capacity of the medium some additional tests were performed with some media with varying alcalinity.

Table 2.5. Results of the biodegradation experiments of cellulose at different pH values in mineral medium

	pH initial	pH final	Gas product. mg	VFA mg/l	AA mg/l	PA mg/l	IBA mg/l	BA mg/l	IVA mg/l	VA mg/l	ICA mg/l
Ref. time 0	6	5.95	0	300	64	25	10	169	12	8	7
Ref. time 5	6	5.88	3	402	159	30	11	173	13	7	6
TI time 0	6	5.96	3	240	62	22	4	143	5	4	0
TI1 time 5	6	5.90	14	579	227	108	36	115	93	0	0
TI2 time 5	6	5.90	17	472	194	93	27	79	80	0	0
Ref. time 0	7	6.54	0	265	81	6	0	176	2	0	0
Ref. time 5	7	6.53	1	300	122	10	2	162	4	0	0
TI time 0	7	9.53	1	253	100	13	1	137	2	0	0
TI1 time 5	7	6.41	19	789	425	66	32	194	71	3	0
TI2 time 5	7	6.40	20	610	316	50	22	167	54	2	0
Ref. time 0	8	6.66	0	237	52	14	2	163	3	3	0
Ref. time 5	8	6.64	1	276	104	6	1	163	2	0	0
TI time 0	8	6.65	2	221	54	14	3	146	4	0	0
TI1 time 5	8	6.50	13	643	321	56	29	170	66	2	0
TI2 time 5	8	6.52	21	712	331	118	38	147	77	2	0

Ref. time 0 : reference set-up at the beginning of the incubation period
Ref. time 5 : reference set-up at the end of the incubation period
TI time 0 : set-up inoculated with thermophilic inoculum at the beginning of the incubation period.
TI1 time 5 : set-up inoculated with thermophilic inoculum at the end of the incubation period (two repetitions TI1 & TI2)

2.4 IMPROVEMENT OF THE BUFFER CAPACITY OF THE MINERAL MEDIUM

2.4.1 Introduction

Because it was not possible to maintain a constant pH during the batch reactor tests, the buffer capacity of the mineral media was tested.

2.4.2 Experimental set-up

The buffer capacity of the mineral medium was adjusted by using $NaHCO_3$ in combination with K_2HPO_4 or CaCO3. The buffer capacity was varying from 50 to 400 meq/l. The volatile fatty acid production in the buffered media was simulated using acetic acid (0.3 N).

2.4.3 Results

A normal alcalinity range to ensure a stable pH in anaerobic digesters ranges from to 25 to 100 meq/l. Components of the buffer system of anaerobic digesters are HCO_3^-, CO_2, NH_4^+, VFA. Because of the fact that a high ammonia concentration inhibits methanogenesis, no ammonia was added to the buffer system of the mineral medium. Based on the results represented in Table 2.6, it can be concluded that a solution with an alcalinity of 100 meq/l $NaHCO_3$ can not prevent a significant pH drop during volatile fatty acid production. Only solutions up to 200 meq/l $NaHCO_3$ and 200 meq/l K_2HPO_4 can compensate to a certain extent the volatile fatty acid production.

It can be concluded that for pH depending biodegradation experiments fed batch reactors with pH control should be used.

Table 2.6. Influence of the volatile fatty acid production on the pH - evolution in media with different alcalinity

CH_3COOH	pH			
mg/l	50 meq/l $NaHCO_3$	100 meq/l $NaHCO_3$	150 meq/l $NaHCO_3$	200 meq/l $NaHCO_3$
0	8.00	8.00	8.00	8.00
180	7.50	7.68	7.67	7.81
360	7.28	7.42	7.45	7.67
540	7.08	7.23	7.36	7.55
720	6.91	7.10	7.27	7.45
900	6.76	7.03	7.18	7.36
1080	6.66	6.95	7.14	7.26
1260	6.55	6.89	7.06	7.21
1440	6.45	6.85	6.98	7.14
1620	6.33	6.75	6.92	7.06
1800	6.21	6.64	6.88	7.01
	200 meq/l $NaHCO_3$ 50 meq/l K_2HPO_4	200 meq/l $NaHCO_3$ 100 meq/l K_2HPO_4	200meq/l $NaHCO_3$ 150 meq/l K_2HPO_4	200meq/l $NaHCO_3$ 200 meq/l K_2HPO_4
0	8.00	8.00	8.00	8.00
180	7.85	7.84	7.85	7.85
360	7.70	7.70	7.71	7.74
540	7.58	7.60	7.62	7.65
720	7.48	7.50	7.53	7.57
900	7.41	7.44	7.46	7.50
1080	7.35	7.37	7.39	7.44
1260	7.29	7.31	7.30	7.37
1440	7.24	7.26	7.26	7.33
1620	7.19	7.21	7.22	7.29
1800	7.15	7.18	7.19	7.25
	50 meq/l NaHCO3 50 meq/l CaCO3	50 meq/l NaHCO3 100 meq/l CaCO3	100 meq/l NaHCO3 50 meq/l CaCO3	
0	8.51	8.40	8.75	
180	7.69	7.62	8.51	
360	7.26	7.27	8.12	
540	7.02	7.07	7.80	
720	6.86	6.92	7.59	
900	6.72	6.8	7.45	
1080	6.61	6.7	7.27	
1260	6.53	6.59	7.14	
1440	6.40	6.51	7.04	
1620	6.35	6.44	7.01	
1800	6.28	6.37	6.96	

3. FED BATCH EXPERIMENTS

3.1 Overview

Figure 3.1 gives a schematic overview of the fed-batch experiments that were done. Three major parts can be distinguished in the experiments.

FB Experiment I

The biodegradation efficiency of cellulose by thermophilic bacteria was tested in conditions at which methanogenesis was not inhibited.

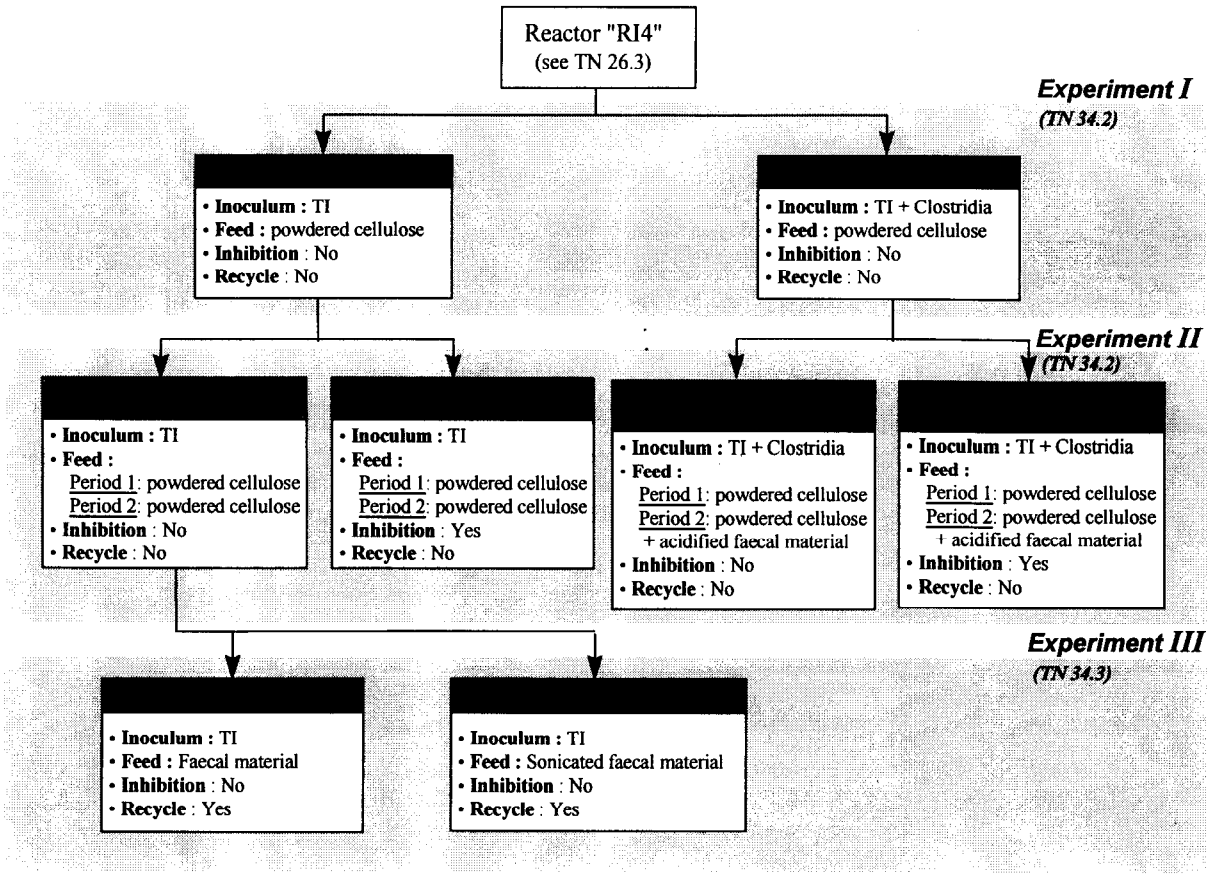
The reactor content of a reactor containing a thermophilic inoculum with a consortium of autochtonous strains present in human faecal material (Reactor "RI4" TN 26.3) was devided into two reactors. Reactor "TI" contained the pure thermophilic inoculum and in reactor "TI+CI" additional cellulolytic Clostridia strains (*Clostridium thermocellum* and *Clostridium thermosaccharolyticum*) were added. The reactors were operated at thermophilic conditions and fed with powdered cellulose.

FB Experiment II

During the first period of experiment II, the biodegradation of cellulose by thermophillic bacteria in methanognesis inhibiting and non-inhibting conditions was tested. The ammonium content of the reactors was increased by addition of urea to inhibit the methanogenesis. The biodegradation of a mixture of cellulose and acidified faecal material will be tested during the second period of the test. Figure 3.1. gives a schematic overview of the reactor configuration during "Experiment II".

FB Experiment III

The effect of the sonication of the faecal material on the biodegrability will be tested in an next experiment. A part of the reactor content will also be centrifuged and the centrifuged cake will be fed again together with new faecal material to the reactor. The results of the test will be presented in TN 34.3



TI : thermophilic inoculum

Figure 3-1 Overview of the fed batch reactor tests

3.2 FB Experiment I

3.2.1 Introduction

The results of the batch reactor experiments indicated that the addition of the cellulolytic strains *Clostridium thermocellum* and *Clostridium thermosaccharolyticum* to the cultivated thermophilic inoculum may have a positive effect on the biodegradation of cellulose. Therefore a fed batch experiment was performed in which the biodegradation efficiencies of powdered cellulose by autochtonous strains present in human faeces and by autochtonous strains mixed with the strains *Clostridium thermocellum* and *Clostridium thermosaccharolyticum* were compared.

3.2.2 Experimental set-up

The experiments were performed using magnetically stirred reactors of one liter wet volume. Two reactors were taken in operation. The reactor "TI" with the cultivated anaerobic inoculum (see TN 26.1 & 26.2) and another reactor "TI+CI" with the same thermophilic inoculum to which the strains *Clostridium thermocellum* and *Clostridium thermosaccharolyticum* were added. At the start of the experiment, the reactors were fed with 0,6 grams faecal material (expressed as organic matter). Two weeks after the start of the experiment, the reactors were fed for a period of ten days with powdered cellobiose as a primer for the biodegradation of cellulose. After this period the reactors were fed with powdered cellulose. The detailed feeding regime of the reactors is illustrated in Figures 3.2 and 3.4 and Addendum 2. Samples were taken for the analysis of volatile fatty acids and pH. The biogas

production was measured and sampled for the determination of the concentration of CH_4 and CO_2. The mass balance was calculated based on the concept described in TN 34.1.

3.2.3 Results

Polymers are hydrolysed and converted into volatile fatty acids by fermentative and hydrolysing bacteria. The acetic acid formed during the acetogenesis is converted into methane and carbon dioxide by the methanogenic bacteria. Based on the amount of volatile fatty acids and biogas produced, the biodegradation efficiency of the substrate was calculated for the two reactors. The biodegradation efficiency during each feeding period is illustrated in Figure 3.6. The calculated biodegradation efficiency reflects the amount of substrate biodegraded of the cumulative amount of substrate fed to the reactor.

For the reactor "TI+Cl" with the thermophilic strains and inoculated with the strains *Clostridium thermocellum* and *Clostridium thermosaccharolyticum*, the final biodegradation efficiency of the substrate was equal to 57 %. The maximum biodegradation efficiency was reached between day 14 and 18 and equalled 68 %. Between day 18 and 21 the biodegradation efficiency decreased to 53 %. Between day 21 and 32 the biodegradation efficiency further decreased to 37 to 48 %. This was due to the fact that the volatile fatty acids, which were accumulated in the reactor upto a concentration of 3800 mg/l, were converted into biogas (Figures 3.2 & 3.3). At day 18 about 21 % of the total amount of volatile fatty acids was valeric acid, 64 % propionic acid and 11 % acetic acid. In the following period the total amount of valeric acid was converted. The decrease of volatile fatty acids was probably due to the conversion of propionic acid and valeric acid into acetic acid by the Clostridium strains. Acetic acid was further converted into biogas by methanogenic bacteria. The first period of 21 days can be considered as a lag-phase.

For the reactor "TI" with the thermophilic inoculum without additional Clostridium strains, the final biodegradation efficiency was 61 % (Figure 3.8). The maximum biodegradation efficiency was equal to 69 %. and was reached between day 35 and 39. The concentration of the volatile fatty acids stayed high during the major part of the test period and fluctuated in the range of 1000 to 1500 mg volatile fatty acids per liter. The volatile fatty acids consisted mainly of propionic acid (Figure 3.5). At day 46 the VFA-concentration decreased to about 400 mg/l.

In both reactors only a minimal fluctuation of the pH around a value of 8 was measured during the experiments. This was due to the conversion of volatile fatty acids into biogas and also to the high buffer capacity of the reactor content.

3.2.4 Discussion

Based on the biodegradation results of the fed batch experiments and the mass-balances (Figures 3.6 & 3.8), it can be concluded that the inoculation with additional Clostridia strains did not result in a higher biodegradation efficiency of the cellulose. The reactor "TI" without additional inoculated Clostridia strains even had a higher biodegradation efficiency than the reactor with the selected cellulolytic strains.

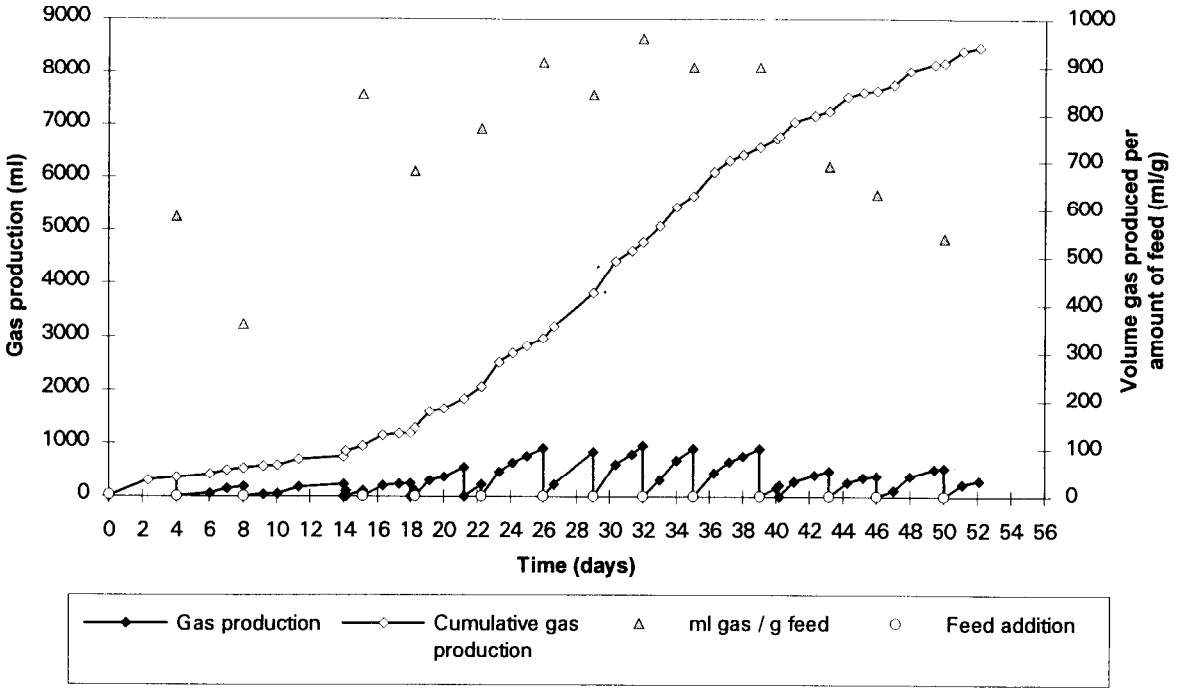


Figure 3-2. Feeding regime, cumulative biogas production and biogas production per amount of feed of the reactor with thermophilic inoculum and additional Clostridium strains (reactor "TI+Cl")

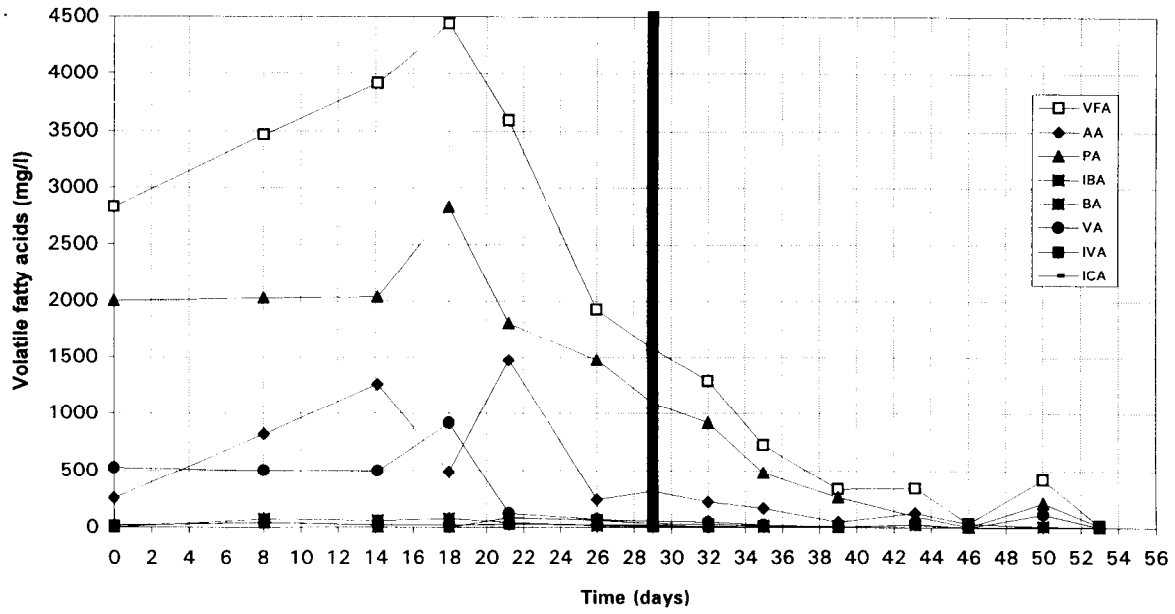


Figure 3-3. Evolution of the volatile fatty acid concentration in the set-up with thermophilic inoculum and additional Clostridium strains (reactor "TI+Cl")

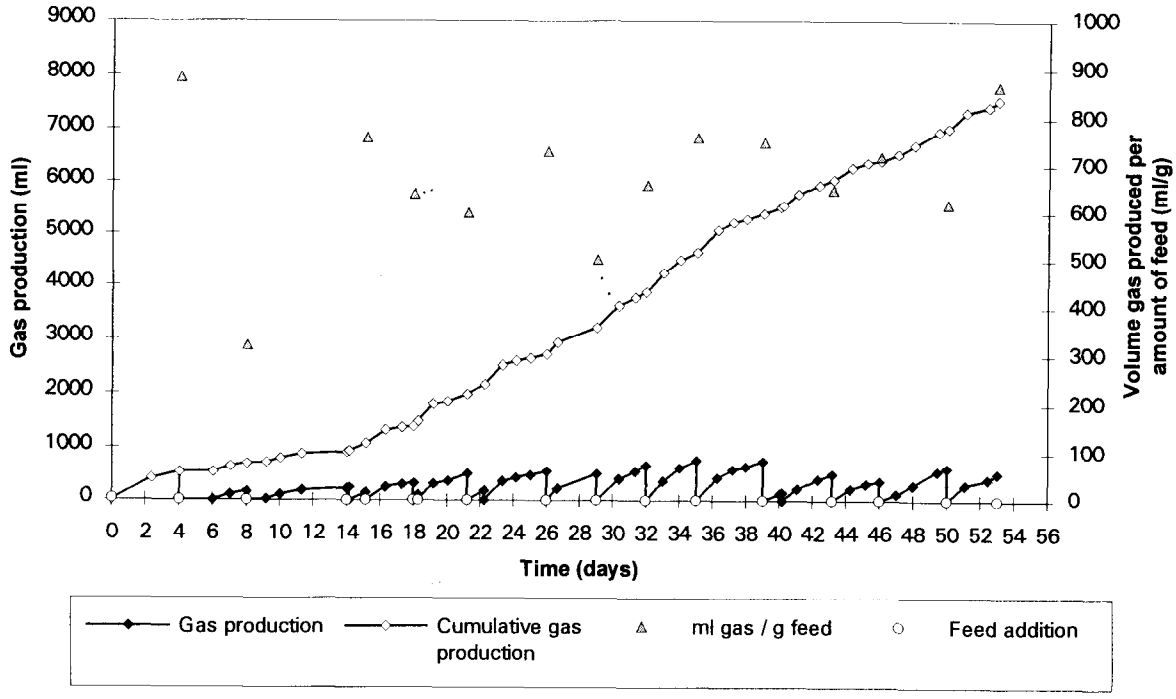


Figure 3-4. Feeding regime, cumulative biogasproduction and biogas production per amount of feed of the set-up with thermophilic inoculum (reactor "TI")

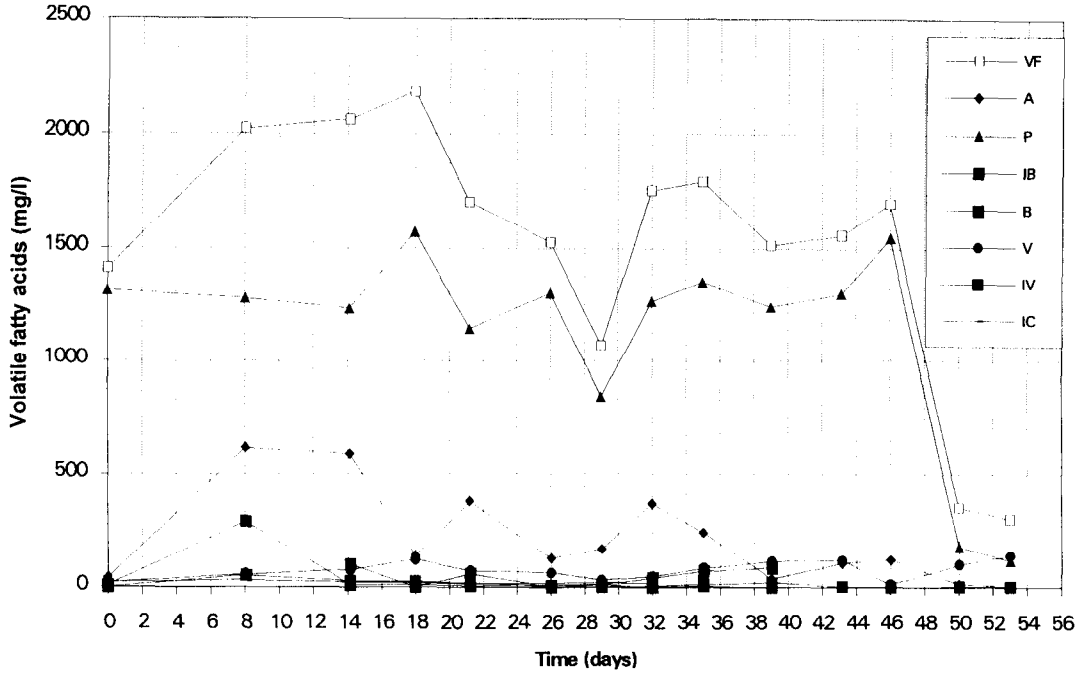


Figure 3-5. Evolution of the volatile fatty acid concentration in the set-up with thermophilic inoculum (reactor "TI")

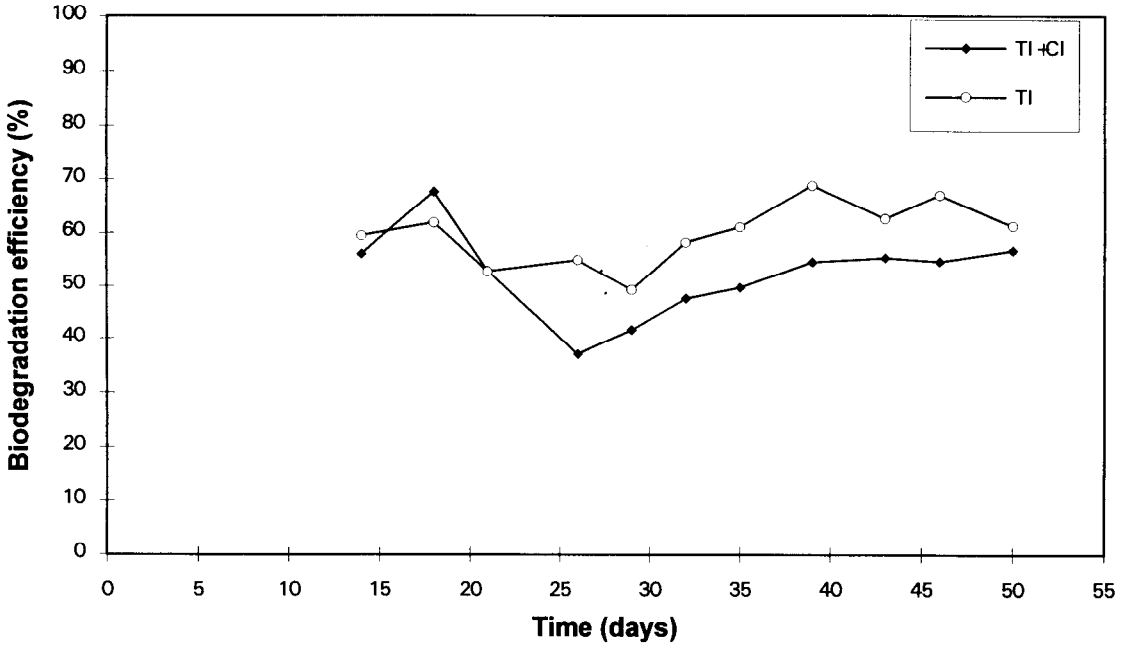


Figure 3-6. Biodegradation efficiency of the cumulatively fed material during the test period in the set-ups with thermophilic inoculum "TI" and thermophilic inoculum with additional Clostridia strains "TI + Cl"

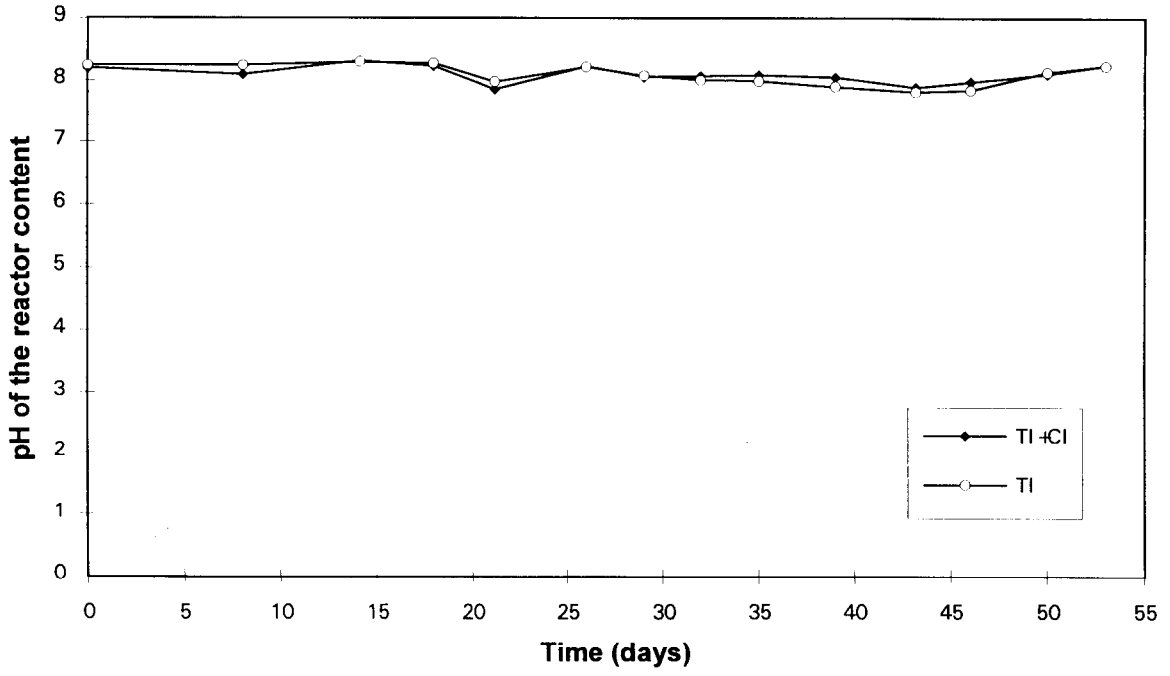
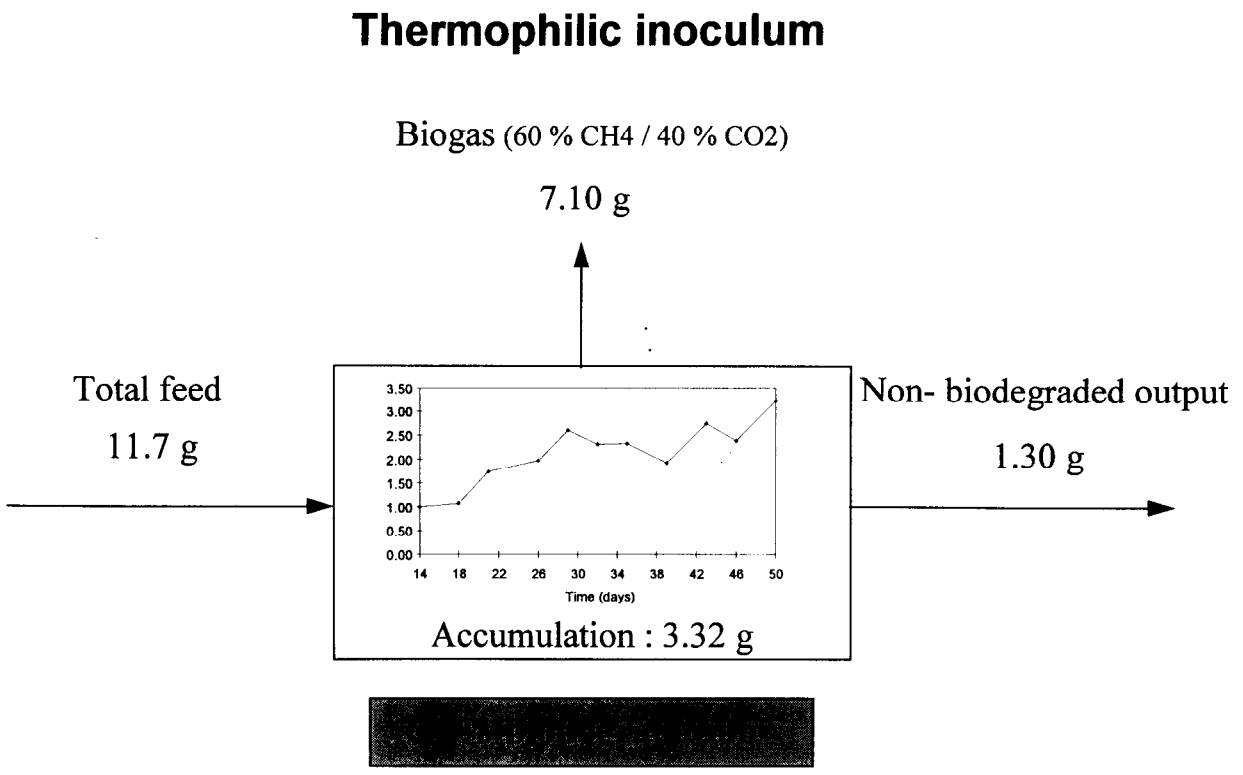


Figure 3-7. Evolution of the pH in the reactors with thermophilic inoculum "TI" and thermophilic inoculum with additional Clostridia strains "TI + Cl"



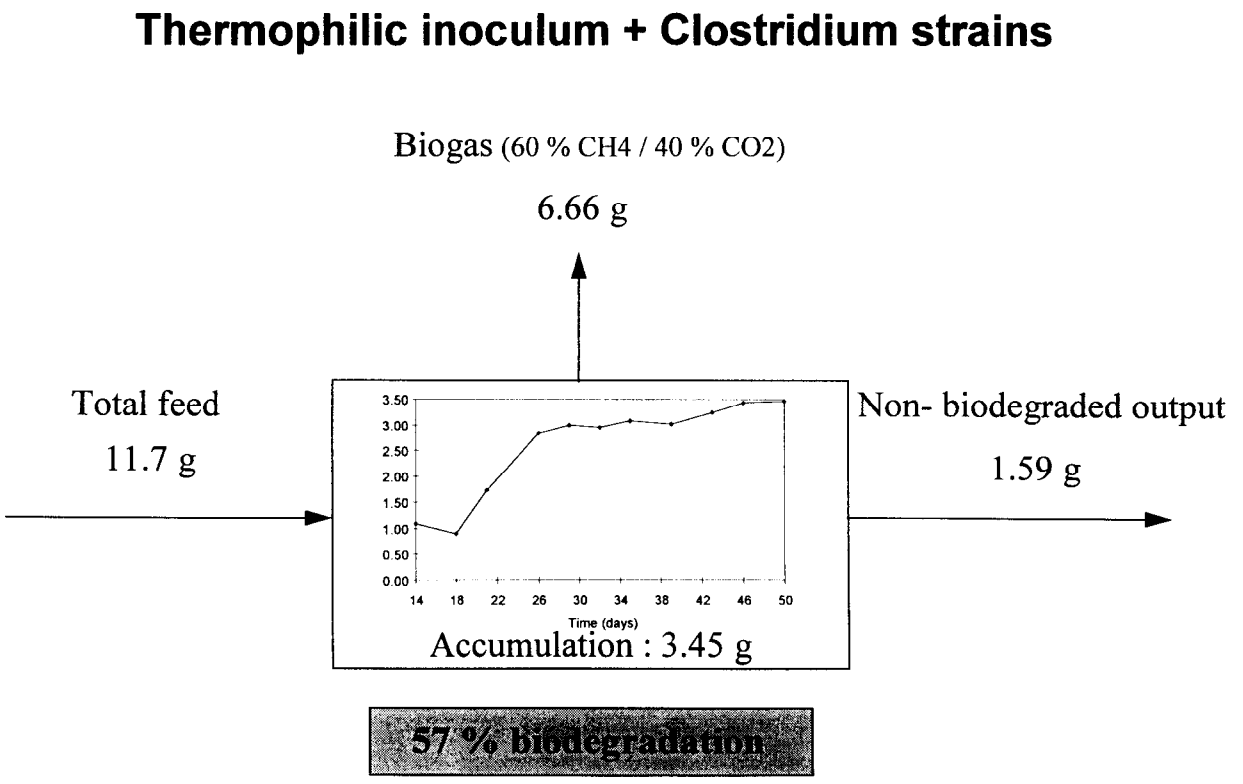


Figure 3-8. Global mass-balance of the fed batch experiment

3.3 FB Experiment II

3.3.1 Introduction

Powdered cellulose was for about 60 % converted into volatile fatty and/or biogas during the fed-batch experiment "Experiment I". During "Experiment I", but the methanogenesis was not inhibited. In the MELISSA-concept the production of volatile fatty acids out of organic matter needs to be maximised. Therefore must the methanegenesis be inhibited. The concept of methane inhibition is described in TN 26.1 & TN 26.2. During this experiment the biodegradation of cellulose in methanogenis inhibiting conditions. The methane production was inhibited by increasing the ammonium concentration in the reactors by addition of urea.

3.3.2 Experimental set-up

Fed-batch reactors

Four thermophilic reactors were taken in operation. The conversion of powdered cellulose into volatile fatty acids was investigated during the first period of the fed-batch experiment. The ammonium concentration in the reactors was stepwise increased to inhibit the methanogenesis. The ammonium was added to the reactors TRci and TRci/TRcfi in the form of urea. Ammonium is produced by the hydrolysis of urea. During the second period of the test a non-inhibited and a inhibited reactor was fed with faecal material and with centrifuged pre-acidified faecal material. Table 3.1 & Figure 3.1 gives a overview of the set-up of the fed-batch experiments and Table 3.2 gives the detailed feeding regime.

Table 3.1 Overview of the set-up of the fed-batch experiments

TRc	Reactor fed with cellulose
TRc	Reactor fed with cellulose
Trci	Reactor fed with cellulose + urea addition
Trci	Reactor fed with cellulose + urea addition
TRc	Reactor fed with cellulose
TRcf	Reactor fed with cellulose + faecal material
Trci	Reactor fed with cellulose + urea addition
Trcfi	Reactor fed with cellulose + faecal material + urea addition

Table 3.2 Feeding regime of the fed-batch reactors (expressed in grams of cellulose -c- , faecal organic material -FM- , centrifuged acidified faecal organic material CAFM)

Time	Reactor			
(day)	TRc/Trcf	TRc	TRci/Trcif	Trci
0	1 c	1 c	1 c	1 c
7	1 c	1 c	1 c	1 c
10	1 c	1 c	1 c	1 c
15	1 c	1 c	1 c	1 c
21	1 c	1 c	1 c	1 c
25	1 c	1 c	1 c	1 c
28	1 c	1 c	1 c	1 c

35	1 c	1 c	1 c	1 c
42	1 c + 3.48 FM	1 c	1 c + 3.48 FM	1 c
49	1 c+1.55 CAFM	1 c	1 c+1.55 CAFM	1 c
56	1 c + 1.9 CAFM	1 c	1 c + 1.9 CAFM	1 c

Pre-acidification of the faecal material

In TN 34.1 the concept of pre-acidifcation of the organic fraction of municipal solid waste was described to improve biodegradation (Kübler and Schertler, 1994). By using this system a remarkable biodegradation of cellulose was noticed. This concept of pre-acidification was also used to produce partly biodegraded material to feed the reactors "TRc/TRcf" & "Trci/Trcif" during the second period of the fed batch experiment. About 20 grams of fresh collected faecal material with a mean dry matter content of 18 % was diluted in 100 ml water and incubated during 4 days in an anaerobic thermophilic acidification reactor. The intial pH was equal to 7.24. After the acidification process the solid remaining material was seperated from the liquid phase containing volatile fatty acids by centrifuging. The cake remaining after centrifuging was fed to the reactors 'TRc/TRcf" and "TRci/TRcfi".

3.3.3 Results of fed-batch reactors

Reactor TRc/TRcf

Reactor TRc/TRcf was fed with powdered cellulose during the first part of the biodegradation experiment and with powdered cellulose and faecal material during the second part of the experiment. There was no additional urea added to the reactor to inhibit the methanogenesis.

The evolution of the ammonium concentration is represented in Figure 3.9. During the first period the ammonium concentration decreased from 500 mg/l to 300 mg/l because of the fact that no additional ammonium was fed to the reactor. The ammonium concentration was increasing again during the second period due to the feeding with faecal material. At this low ammonia concentration no inhibition of the methanogenesis was noticed. The methane concentration in the produced biogas was varying from 60 vol% to 70 vol% (Figure 3.11). Feeding with the faecal material and the centrifuged cake of the pre-acidified faecal material didn't have an influence on the biogas composition. The volatile fatty acids concentration of the reactor was low (Figure 3.9). During the first period the volatile fatty acid concentration was equal to about 75 mg/l and only increased once to 200 mg/l at day 16. The feeding of the reactor with faecal material resulted also in a increase of volatile fatty acids concentration up to 200 mg/l. After the first feeding particularly propionic acid was produced (Figure 3.10). During the next days the propionic acid was converted into acetic acid. The pH was varying from 7.6 and 8 during the test period (Figure 3.26).

It can be concluded that the hydrogenotrophic and acetoclastic methanogenis was not inhibited and that the volatile fatty acids produced during the biodegradation of the cellulose were completely converted into biogas. The cumulative conversion efficiency of the cellulose into biogas was calculated based on the cumulative amount of biogas produced and the cumulative amount of organic matter fed to the reactor. In Figure 3.12 is the total amount of feed added to the reactor is plotted as line "Feed". The line "Total conversion" is indicating the amount (expressed in grams per reactor) of biogas and volatile fatty acids produced. The line "Gas conversion" is indicating the amount of biogas produced (expressed in grams). The space between the lines "Total conversion" and "Gas conversion" is a measure for the amount of volatile fatty acids produced. In this case the two lines are overlapping because of the fact that no volatile fatty acid accumulation was noticed in reactor "TRc/TRcf". The conversion efficiency of the cellulose was increasing from 54 % to 68 % during the first period. By

supplying faecal material to the reactor feed the cumulative conversion efficiency decreased again to 60 %.

Reactor TRc

The feeding regime of reactor TRc was during the first period identical to the feeding regime of reactor TRc/TRcf. The reactor TRc was continuously fed with cellulose and no faecal material was added during the second period. There was no additional urea added to inhibit the methanogenesis. The pH of the reactor was varying between 7.8 and 8 (Figure 3.26).

The volatile fatty acids concentration in the reactor was increasing during the test period from 100 mg/l to 800 mg/l. (Figure 3.19). Between day 56 and day 61 the concentration of volatile fatty acids stayed stable. During this last period there was also a decrease in gas production measured (Figure 3.5). The produced biogas contained about 60 vol% methane (Figure 3.15). The conversion efficiency was calculated and graphically presented in Figure 3.16 From the begin of the experiment to day 56 the conversion efficiency was varying from 52 to 62 %. At day 61 the cumulative conversion efficiency decreased to 54 %.

Reactor TRci/TRcfi

Reactor TRci/TRcif was fed with powdered cellulose during the first part of the biodegradation experiment and with powdered cellulose and with faecal material during the second part of the experiment. During the experiment the ammonium concentration was increased by adding urea to the reactor.

Between day 0 and day 15 was the ammonium concentration equal to about 1000 mg/l (Figure 3.17). The concentration of methane in the biogas during this period was equal to 70 vol% and it can be stated that methanogenesis was not inhibited (Figure 3.19). Between day 15 and day 49 the ammonium concentration was gradually increased from 1000 mg/l to 10000 mg/l. The volatile fatty acids concentration increased from 740 mg/l to 2150 mg/l from the moment that the ammonium concentration was equal to 3000 mg/l. The amount of methane in the produced biogas decreased from 70 vol% tot 40 vol%. At an ammonium concentration of 4300 mg/l the volatile fatty acid production still increased to 2900 mg/l. About 80 % of the amount of volatile fatty acids produced was acetic acid (Figure 3.18). The methane concentration in the biogas decreased to 20 vol%. When the ammonium concentration was equal to 9700 mg/l there was no production of volatile fatty acids noticed anymore and only a small amount of biogas was produced (Figure 3.17 and Figure 3.19). The ammonium concentration in the reactor was increased by addition of urea. Figure 3.25 shows that the ratio of the ammonium nitrogen over the total nitrogen was varying from 50 % to 60 % during the first period of the test. This ratio increased to maximum 87 % once faecal material was added to the reactor. The increase in ammonium concentration was not resulting from the addition of faecal material because not that much ammonia was present in the feed. It can be concluded that the increase was due to stimilution of the hydrolysis of urea by addition of faecal material to the reactor. The fact that the pH of the reactor raised significantly (Figure 3.26) confirms this hypothesis, because hydrolysis of urea increases the pH from 7 to 8.7. The increase of the volatile fatty acid concentration resulted also in a pH drop from 7.9 to 7.0 beetween day 15 and day 42. The buffer capacity of the reactor was not high enough to ensure a stable pH during volaile fatty acis production and urea hydrolysis.

The calculated conversion efficiency increased during the first period from 39 % to a maximum of 78 %. The high maximum was reached because of the high increase of the volatile fatty acids concentration. The cumulative conversion efficiency decreased to a final value 24 % after the peak noticed on day 28. This was due the fact that the production of biogas and volatile fatty acids were inhibited. The faecal material added to the reactor during the second period was not biodegraded. It

can be concluded that as well the acidogenesis as the methanogenesis was completely inhibited during the experiment. The hydrolysis of urea to ammonium was not inhibited during the second period.

Reactor TRci

Reactor TRci was during period 1 and 2 fed with cellulose. The ammonia concentration in the reactor was during the experiment increased by the addition of urea.

Based on the data presented in Figure 3.21 it can be concluded that for a ammonium concentration lower than 3000 mg/l no significant accumulation of volatile fatty acids was noticed. The methane concentration in the biogas decreased from 70 vol% to 55 vol% between day 0 and day 21. When the ammonium concentration is increased to 3000 mg/l, the volatile fatty acid concentration increased from 500 mg/l to 1550 mg/l. The methane concentration decreased to 38 vol% of the biogas. A further increase of the ammonium concentration resulted in a practically complete inhibition of the volatile fatty acid and biogas production (Figure 3.21 and 3.23). In the biogas produced after day 35 there was no methane present.

The cumulative biodegradation efficiency increased from the start of the test to day 28 from 33 % to 40 %. The conversion efficiency decreased after this period to 32 %. The methanogenesis and acetogenesis was completely inhibited after day 28. There was a pH drop measured from 7.2 to 5.0 at day 35. The reason of this sudden pH decrease could not be superseded. A backflow of the acid water from the gas measurement system to the reactor could be a possible reason. The pH was corrected again by addition of sodiumhydroxide.

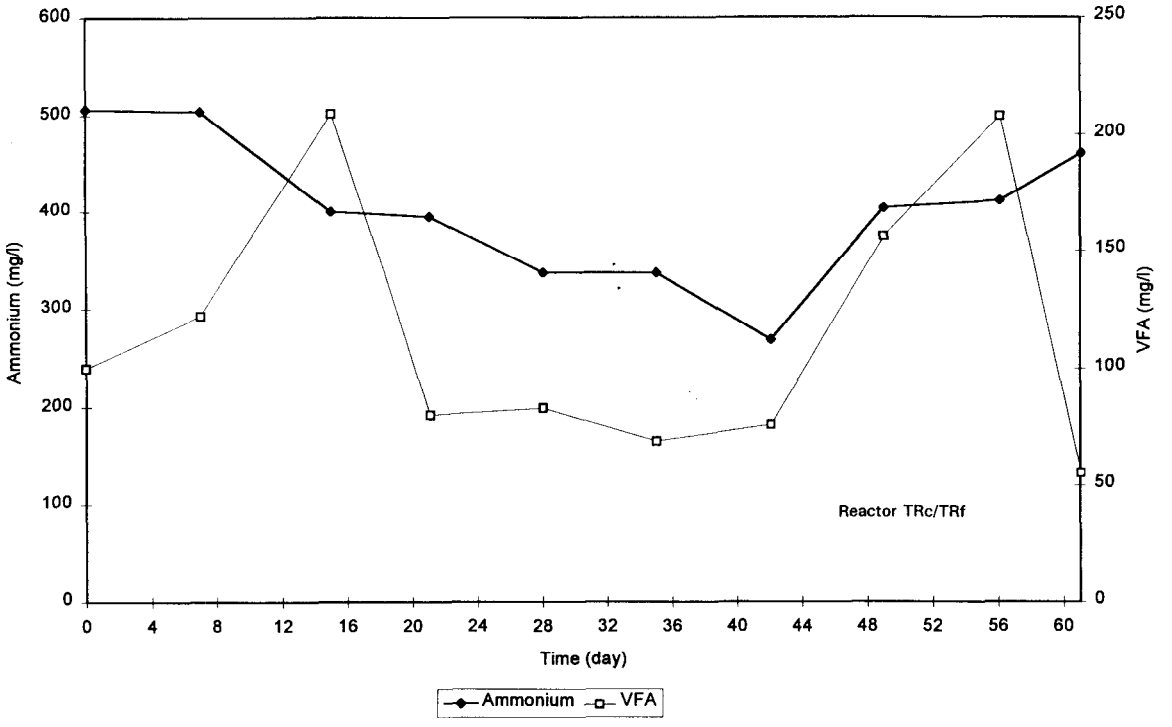


Figure 3-9. Evolution of the ammonium concentration and volatile fatty acid concentration in the reactor TRc/TRcf

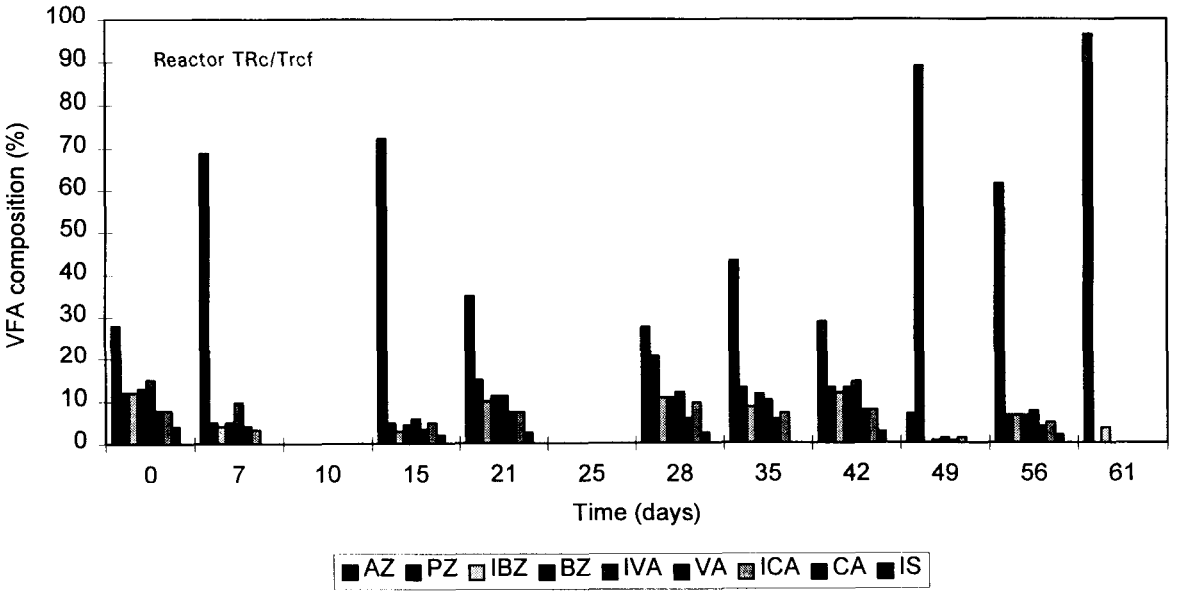


Figure 3-10. Composition of the volatile fatty acids produced in reactor TRc/TRcf

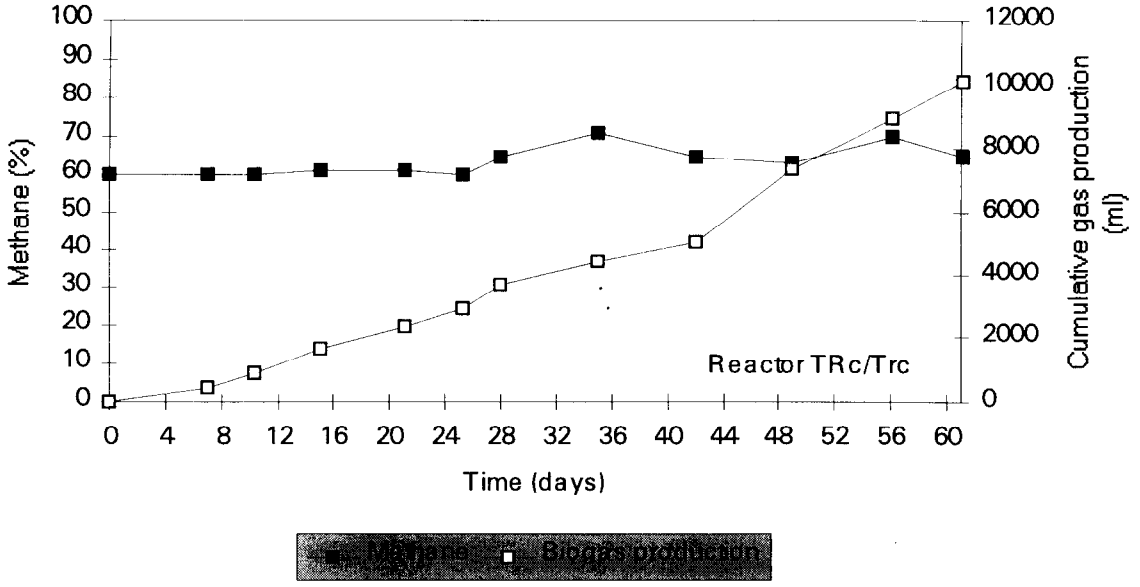


Figure 3-11. Evolution of the ammonium concentration and volatile fatty acid concentration in the reactor TRc/TRcf

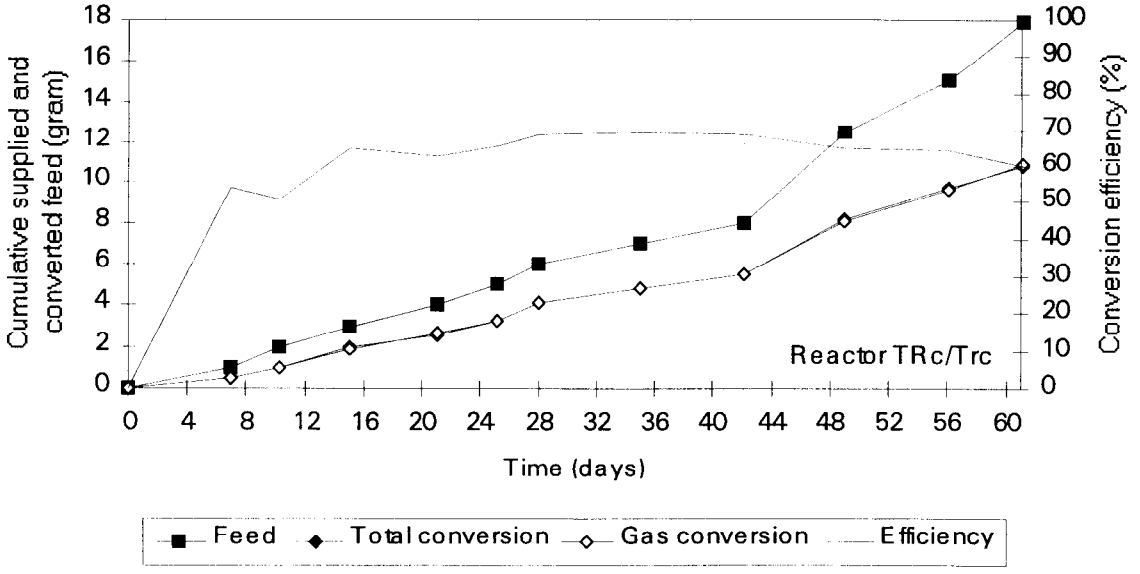


Figure 3-12. Cumulative amount of substrate fed to the reactor TRc/TRcf, cumulative amount of converted substrate and the calculated conversion efficiency

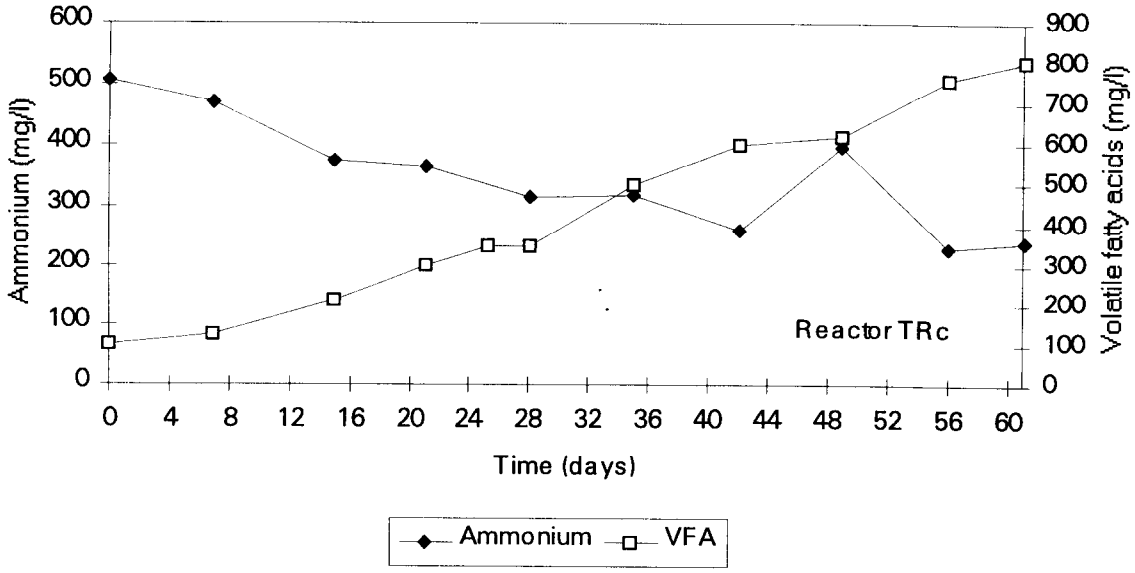


Figure 3-13. Evolution of the ammonium concentration and volatile fatty acid concentration in the reactor TRc

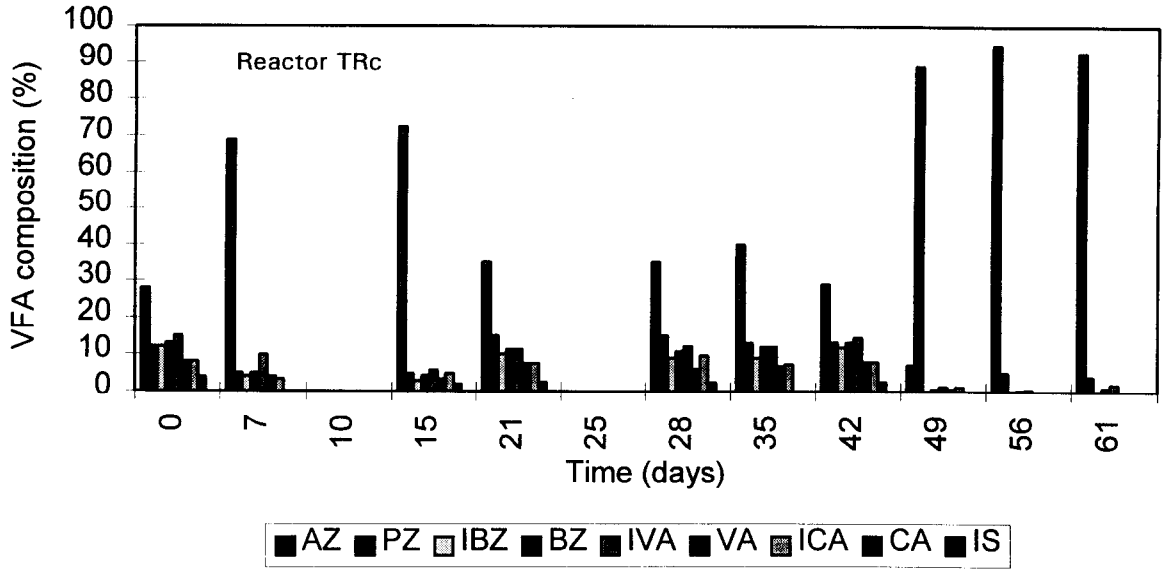


Figure 3-14. Composition of the volatile fatty acids produced in reactor TRc

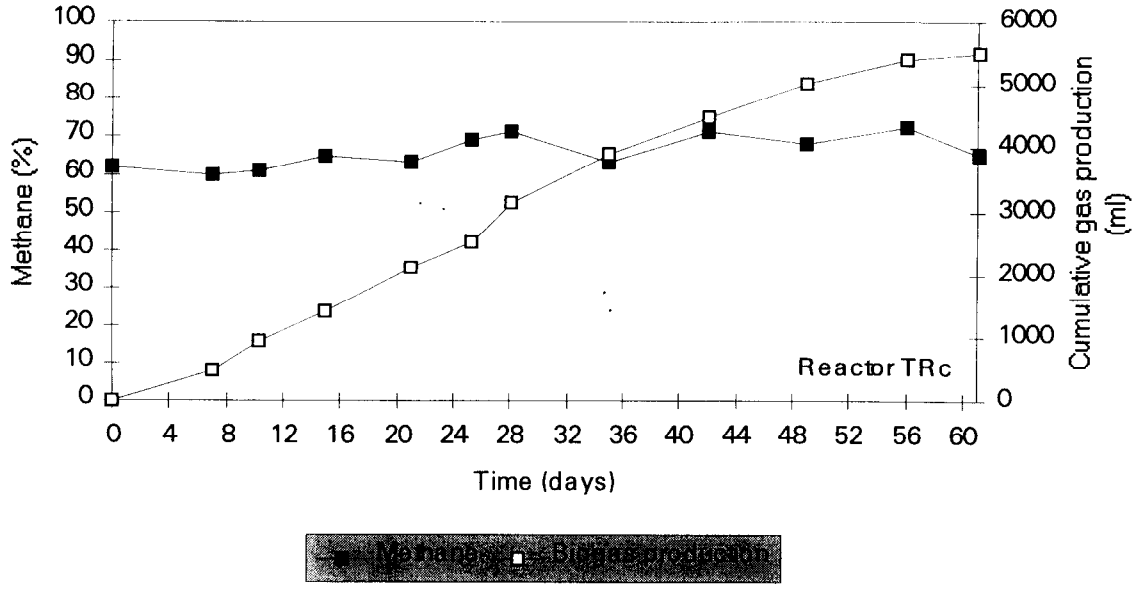


Figure 3-15. Evolution of the ammonium concentration and volatile fatty acid concentration in the reactor TRc

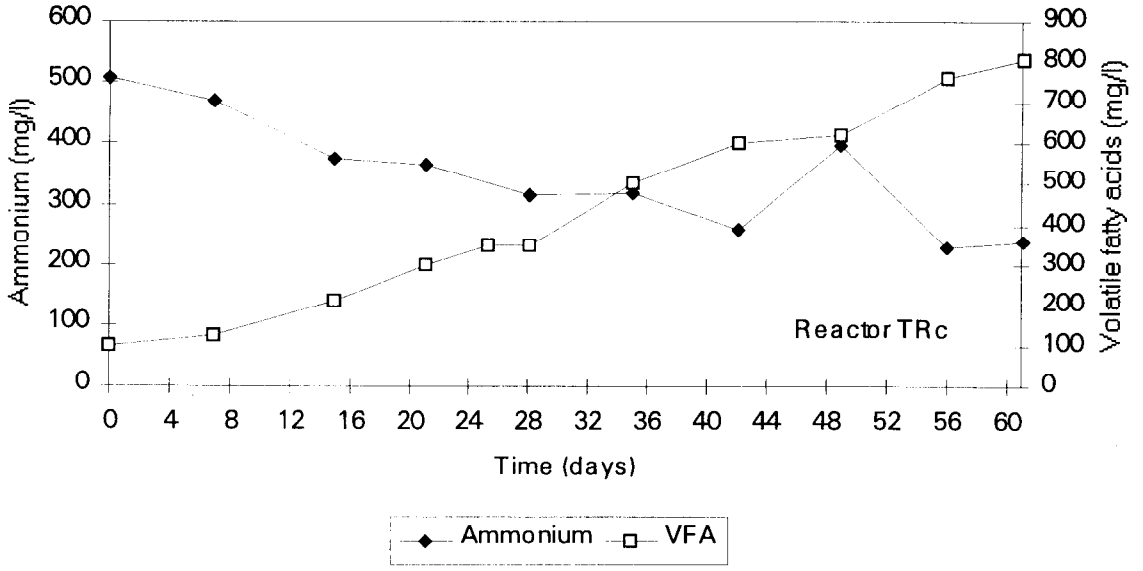


Figure 3-16. Cumulative amount of substrate fed to the reactor TRc, cumulative amount of converted substrate and the calculated conversion efficiency

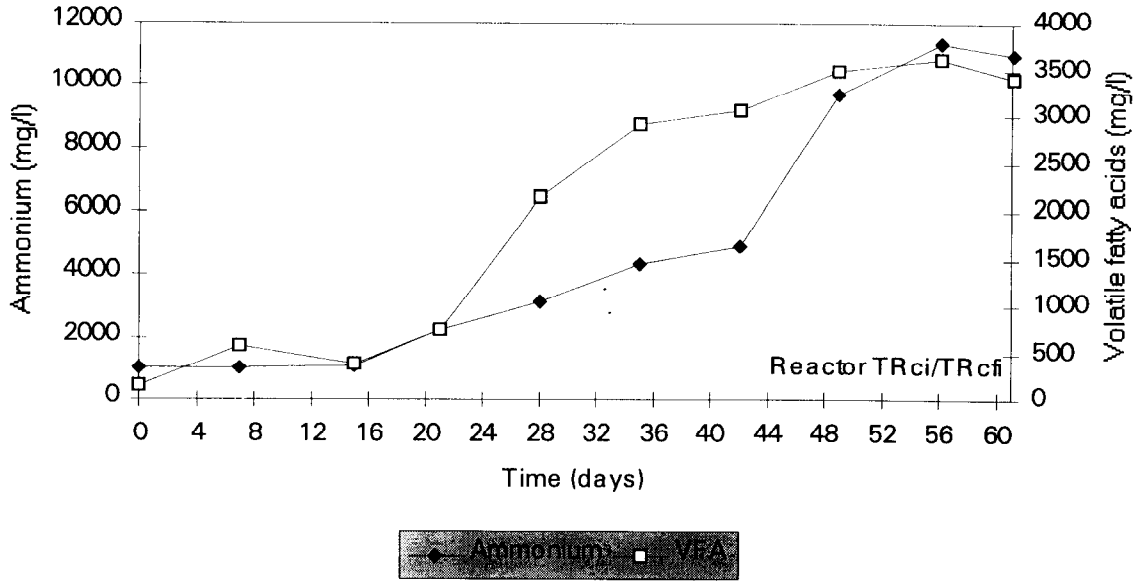


Figure 3-17. Evolution of the ammonium concentration and volatile fatty acid concentration in the reactor TRci/TRcfi.

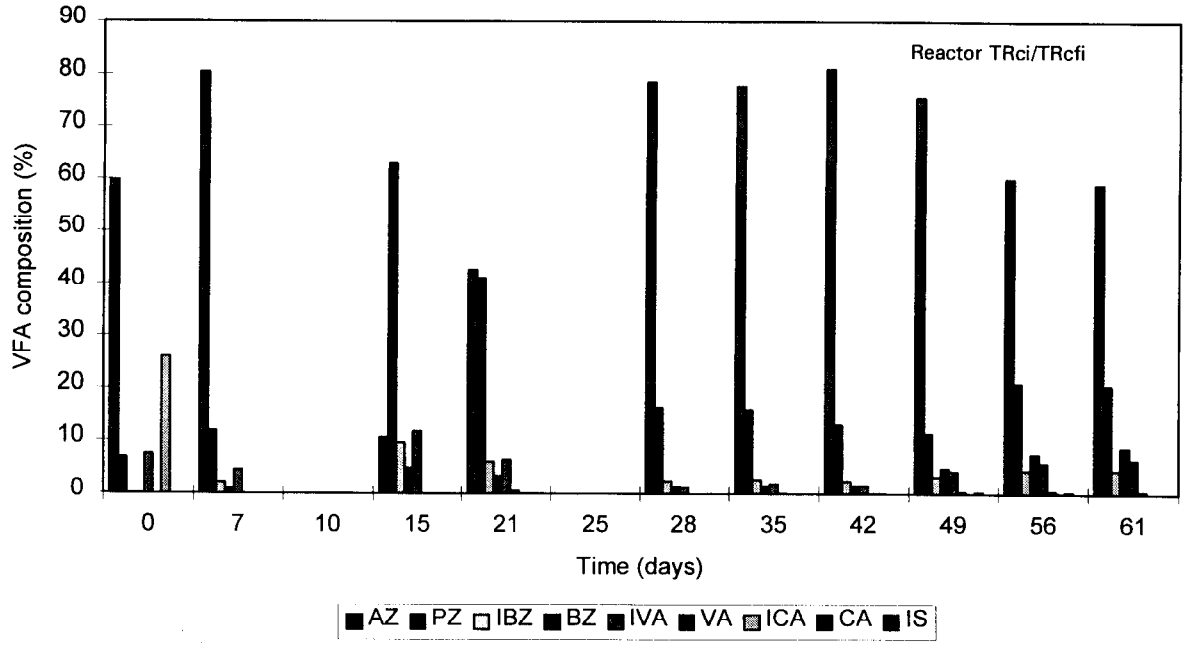


Figure 3-18. Composition of the volatile fatty acids produced in reactor TRci/TRcfi.

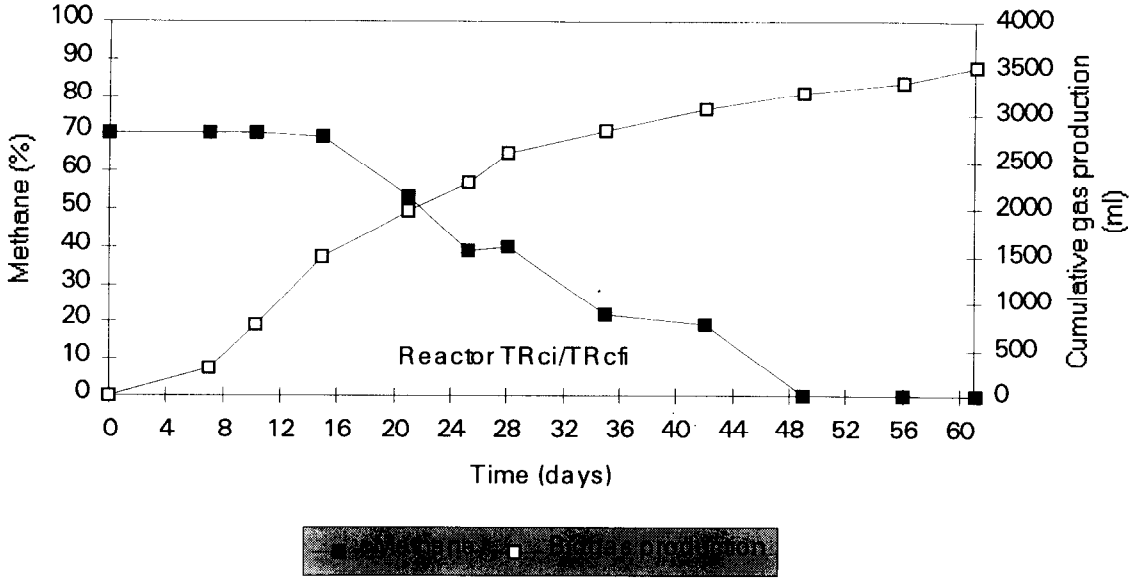


Figure 3-19. Evolution of the ammonium concentration and volatile fatty acid concentration in the reactor TRci/TRcfi

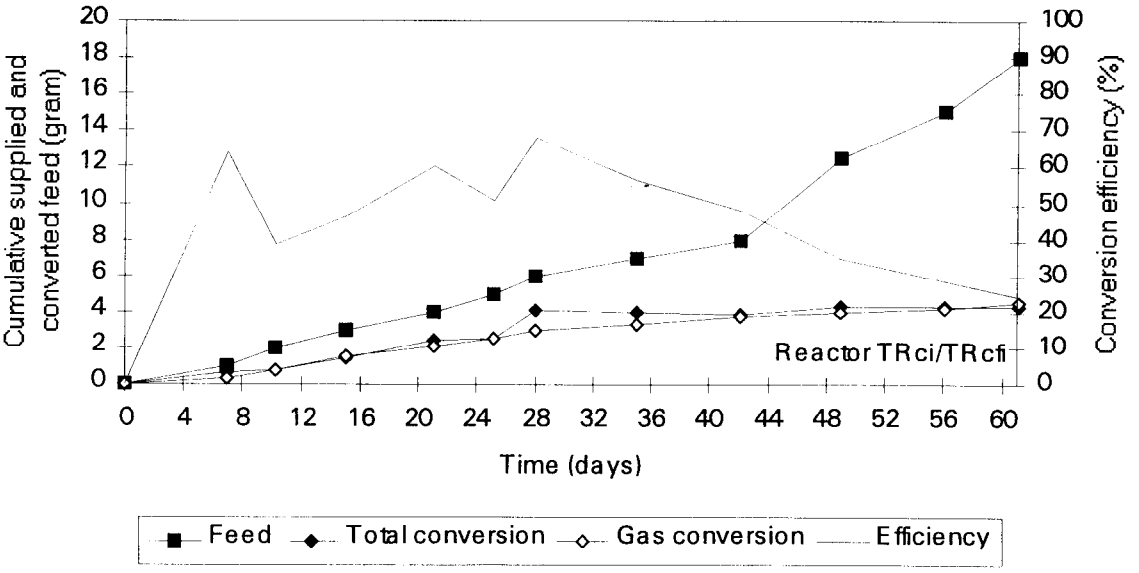


Figure 3-20. Cumulative amount of substrate fed to the reactor TRci/TRcfi, cumulative amount of converted substrate and the calculated conversion efficiency

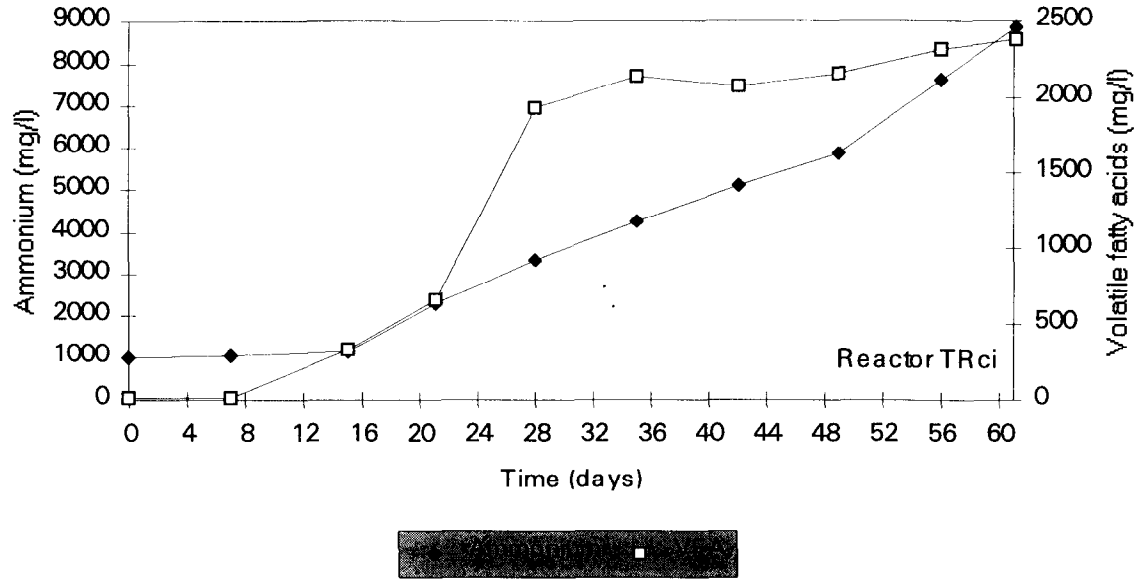


Figure 3-21. Evolution of the ammonium concentration and volatile fatty acid concentration in the reactor TRci

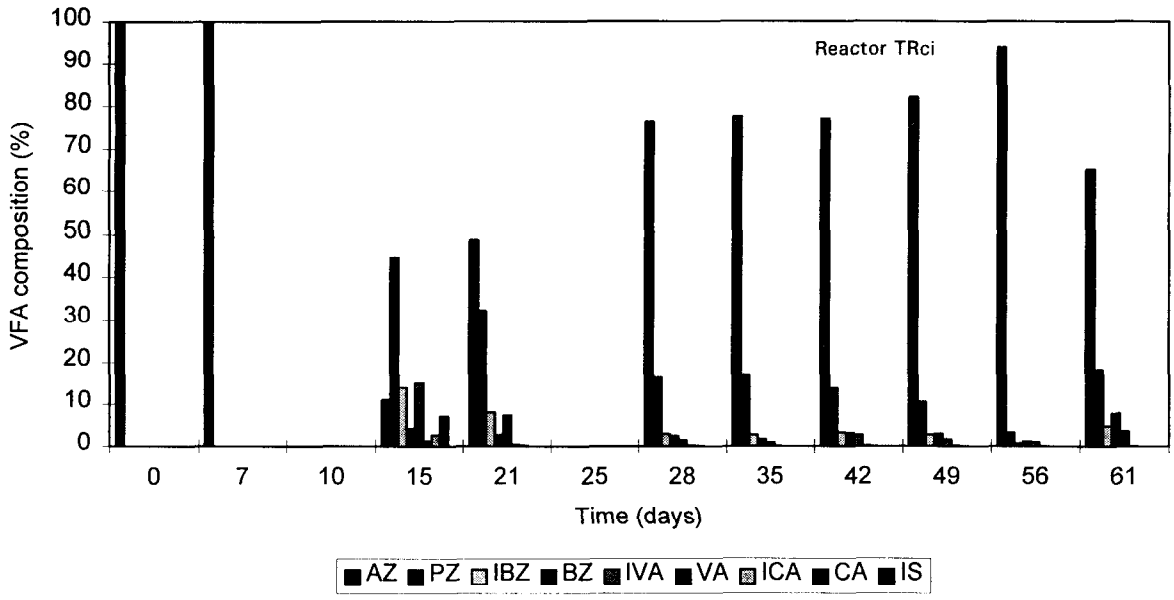


Figure 3-22. Composition of the volatile fatty acids produced in reactor TRci

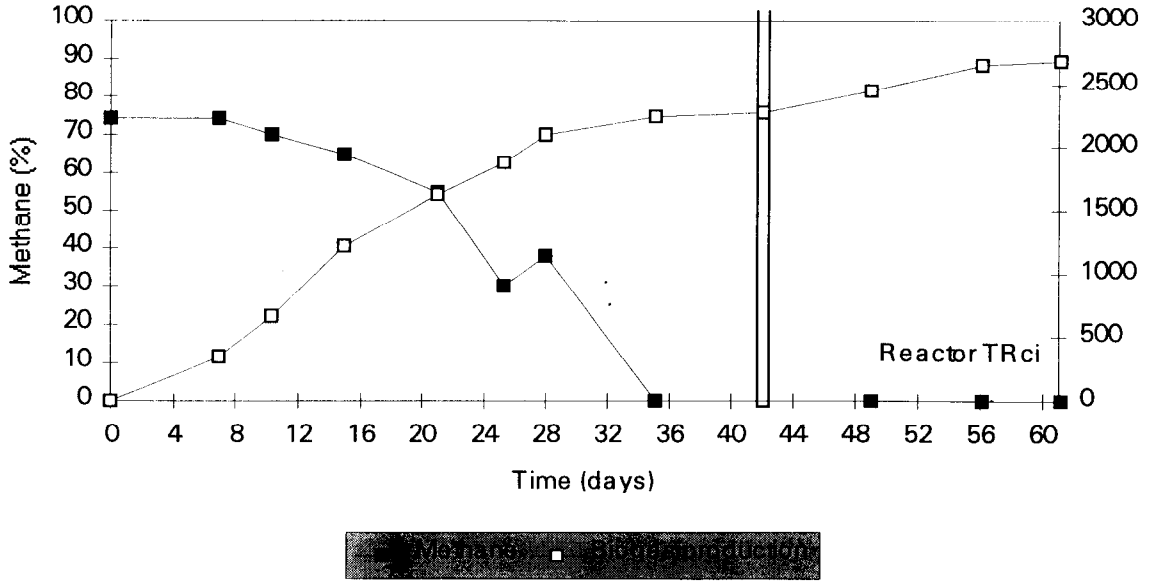


Figure 3-23. Evolution of the ammonium concentration and volatile fatty acid concentration in the reactor TRci

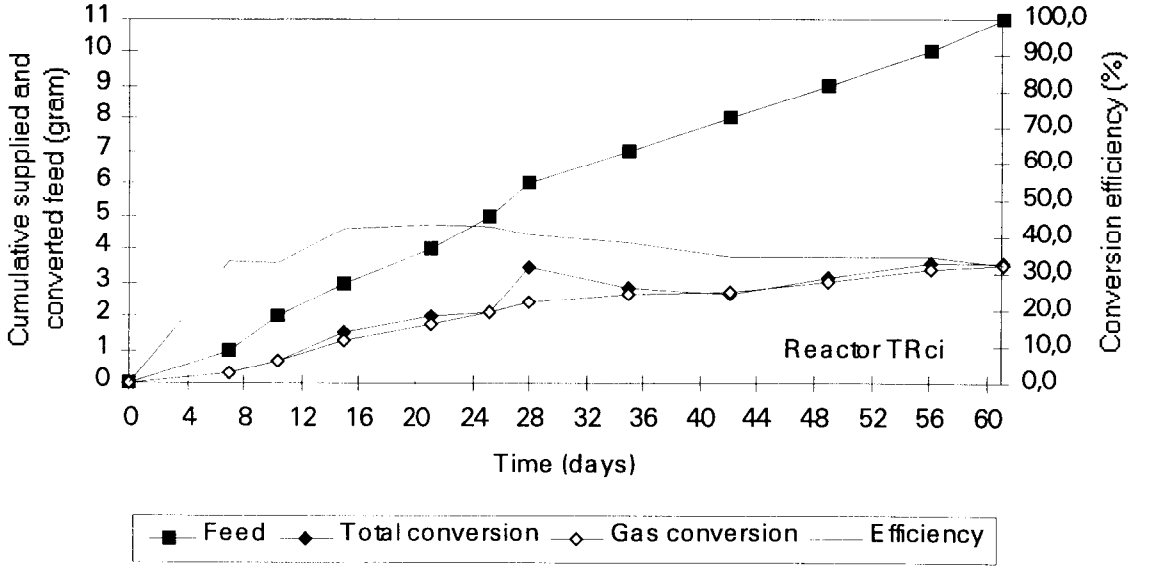


Figure 3-24. Cumulative amount of substrate fed to the reactor TRci, cumulative amount of converted substrate and the calculated conversion efficiency

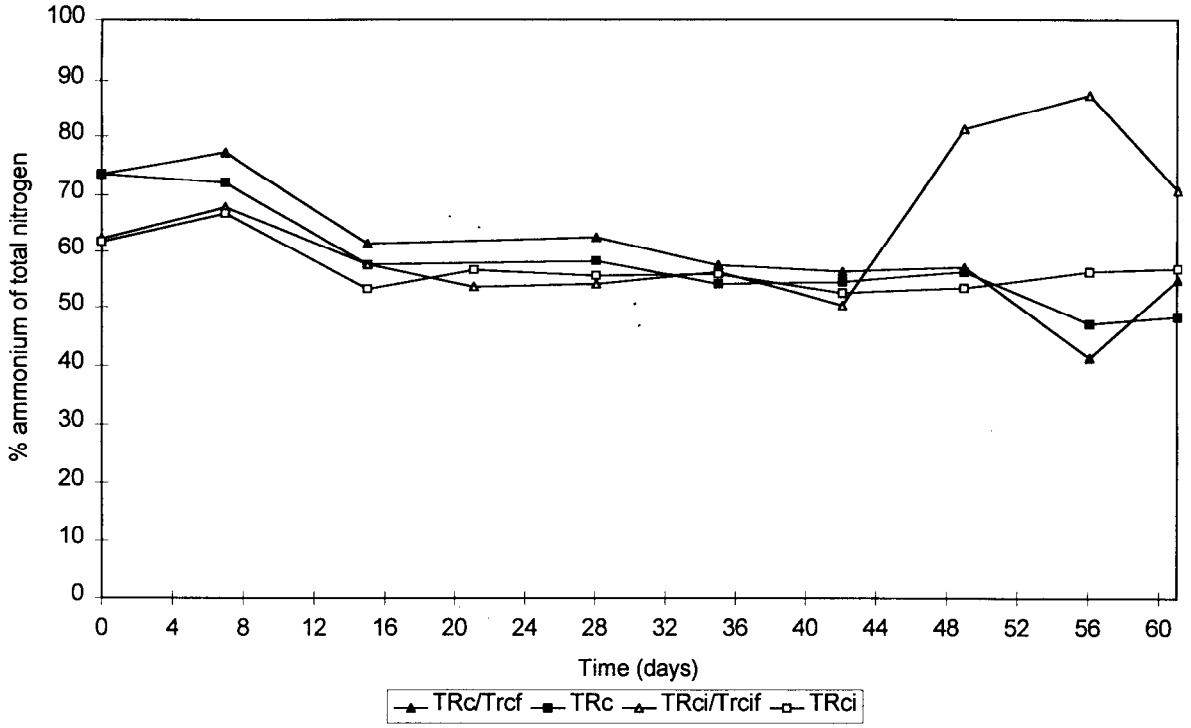


Figure 3-25. Overview for the different reactors of the ratio of the amount of ammonium to the total amount of nitrogen.

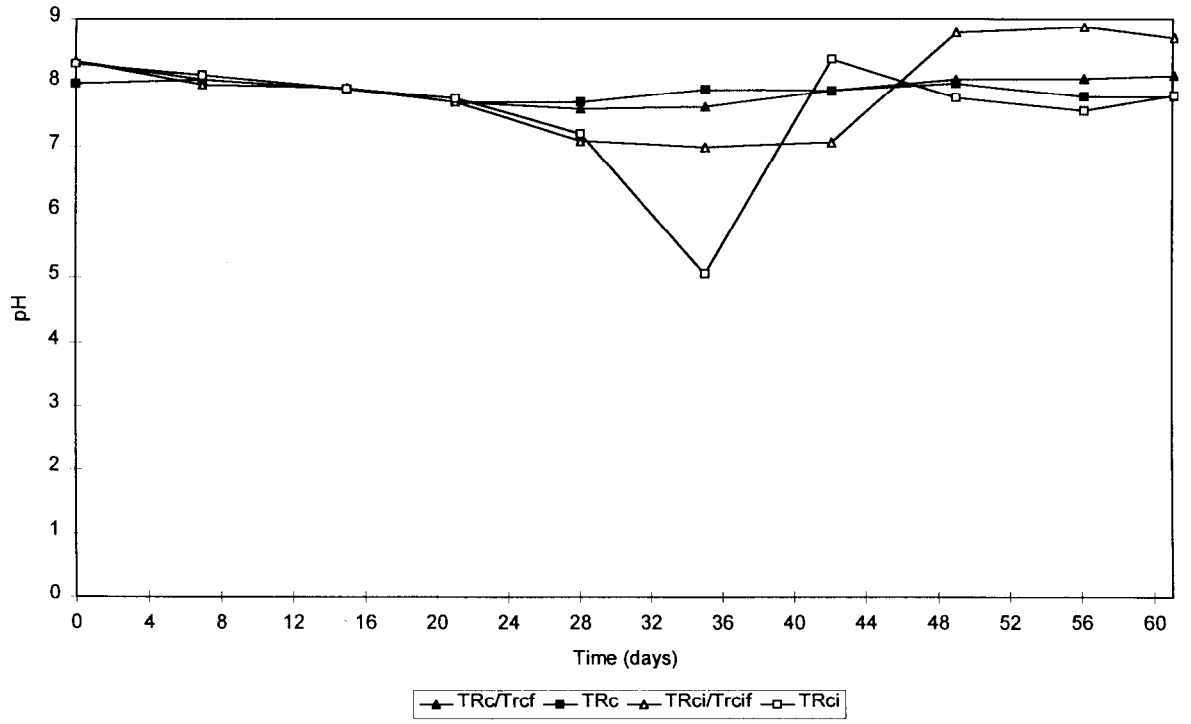


Figure 3-26. Overview for the different reactors of pH

3.3.4 Results of the pre-acidification test

The concentration of volatile fatty acids present in the solution containing the faecal material increased from 500 mg/l to 9500 mg/l during the incubation period of 4 days at a temperature of 55 °C. The intitial pH was equal to 7.24 and final pH equal to 7.14. Figure 3.27 is presenting the mass-balance. Based on the data of the pre-acidification tests it can be calculated that 3.48 g of organic material was converted into 0.238 g biogas and 0.69 g volatile fatty acids. This means that about 26 % of the organic matter was converted during the pre-acidification. The cake remainign after centrifuging was fed to the reactors "TRc/TRcf" and "Trci/Trcif".

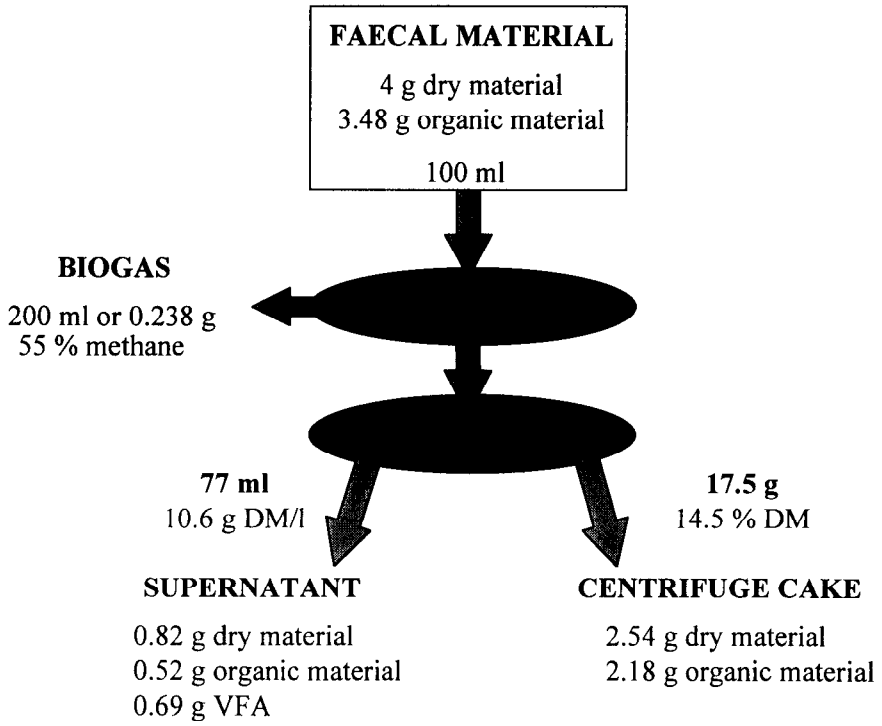


Figure 3-27. Mass-balance calculated on basis of results obtained during the pre-acidification tests.

3.3.5 Discussion

Figure 3.28 gives an overview of the calculated conversion efficiencies for the different reactors. The conversion of the material fed to the reactors was the highest in reactors TRc/TRcf and TRc. During the first period (day 0 - 42) the two reactors were identically fed with cellulose. The conversion of the powdered cellulose was relative constant during the test period. At the end of the first feeding period the about 69 % of the cellulose was converted in reactor TRc/TRcf and about 61 % in reactor TRc. During the second test period the conversion efficiency in reactor decreased significant to a value of 54 %. There was no clear explanation for this decrease. The conversion efficiency of the human faces fed to reactor TRc/TRcf during the second period was calculated. The cellulose and human faeces fed to the reactor where completely converted into biogas. The cumulative conversion efficiency of the powdered cellulose was equal to about 60 %. Taking this information into account it could be calculated which part of the biogas was generated by the biodegradation of the human faeces. The results of the calculation are presented in Table 3.3. The calculated conversion efficiency of fresh faecal material (FM) was equal to 69 %. The centrifuged cake from pre-acidified human faeces was only converted for 25 % during the first feeding cycle and for 50 % during the following feeding cycle. The global conversion efficiency of the human faeces could be calculated by taking into account that the faecal material was biodegraded for 25 % during the pre-acidification. The global conversion efficiency was equal to 44 % and 62 %.

Table 3.3. Overview of the conversion efficiency of the human faeces fed to reactor TRc/TRcf

Feed (gram)			**Converted (gram)**		**Conversion of FM (%)**	
			Total (1)	Faecal material	Fed-batch (2)	Total (3)
1 c +	3.48 FM	4.48	3.0	2.4	69	-
1 c +	1.55 CAFM	2.55	1.0	0.4	25	44
1 c +	2.00 CAFM	3.00	1.6	1.0	50	62

c : cellulose
FM : faecal organic material
CAFM : centrifuged acidified faecal organic material
(1): total cellulose + faecal material converted
(2): conversion efficiency of the faeces in the fed-batch reactors
(3): conversion efficiency of the faeces by pre-acidification and in the fed-batch reactors

In reactor TRc/TRcf the cellulose and volatile fatty acids were converted into biogas. The volatile acid produced during the acetogenesis were not accumulated in the reactor. The concentration of volatile fatty acids increased slowly from about 100 mg/l to 800 mg/l in the reactor TRc. The major part of the cellulose fed to the reactor was also converted into methane.

The MELISSA-concept aims the maximising of the volatile fatty acid production during the biodegradation of organic waste material and not the production of biogas. Urea was fed to the reactors TRci and TRci/TRcfi in order to increase the ammonia content. Ammonia, which is the free form of ammonium, inhibits the methanogenesis. The inhibition of the methanogenesis during digestion of cattle manure was investigated by Angelidaki I. & Ahring B.K. (1993). A preliminary batch reactor test was also performed with Melissa substrate. This test is described in TN 26.3. The results of this test are summarised in Table 3.4 and compared to the results obtained during the fed batch experiments. The fed-batch experiments confirmed the fact that at an ammonium concentration lower than 2 g/l no inhibition of the methanogenesis occurred. The critical range were methanogenesis was affected seemed to be at an ammonium concentration of at least 4 g/l. An ammonium concentration higher than 8 g/l inhibited completely the methanogenesis. The results of the closed-bottle experiments seemed to be confirmed. An increase of the volatile fatty acid

concentration was noticed when the ammonium concentration in the fed-batch reactors TRci/TRcfi and TRci was increased. The volatile fatty acid concentration in reactor TRci increased from 150 mg/l at the start to 3000 mg/l at day 42. After this period faecal material was added but this didn't resulted in a further increase of the volatile fatty acid concentration. Only an increase in ammonium concentration was noticed due to the hydrolysis of urea. The sudden increase of the pH from 7 to 8.8 could have also had a negative effect on the acetogenesis. The conversion efficiency of cellulose in reactor TRci at day 28 was equal to 68%. After this period the conversion efficiency decreased due to the inhibition of the acetogenesis and methanogenesis. The volatile fatty acid concentration in reactor TRci increased from 10 mg/l to 2000 mg/l. At the moment that the ammonium concentration was higher than 4 g/l increase of volatile fatty acids concentration was noticed. Hence, that the inhibition in this case could also be due to the abrupt decrease in pH of the reactor from 7.2 to 5.

Table 3.4. Effect of the ammonia concentration on the inhibition of the methanogenesis. Results of the fed-batch experiments compared to previous results from closed-bottle experiments (see TN 26.3).

Ammonium concentration (g/l)	Effect
< 2	no inhibition
2 - 6	50 % inhibition
6 - 8	50 - 100 % inhibition
> 8	100 % inhibition
< 2	no inhibition
2 - 3	40 % inhibition
3 - 4	70 % inhibition
> 8	100 % inhibition
< 2	no inhibition
3	20 % inhibition
> 4	100 % inhibition

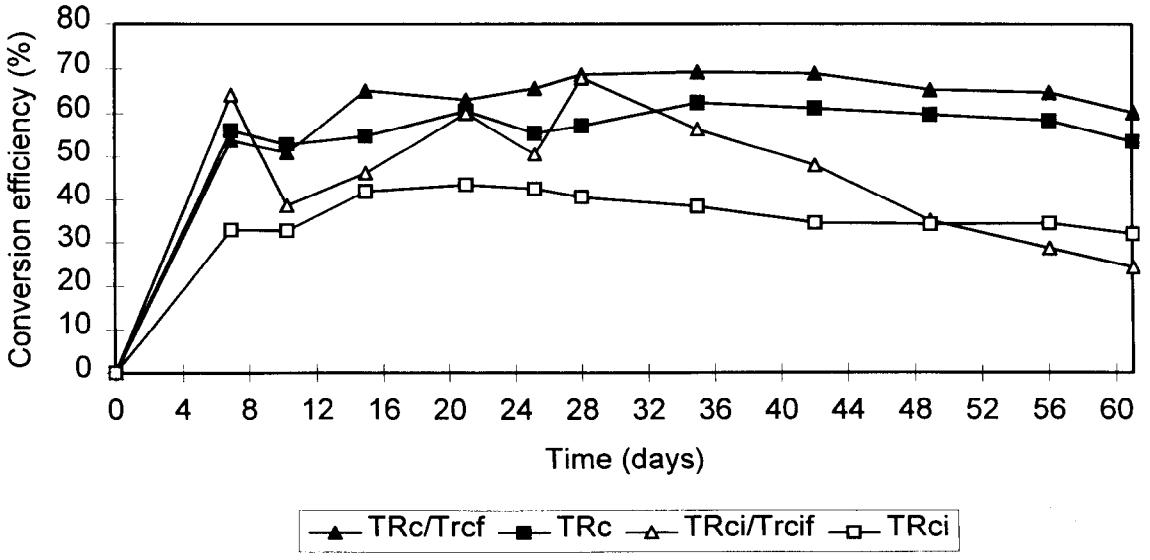


Figure 3-28. Overview of the conversion efficiency of the reactor feed into biogas and or volatile fatty acids for the different reactors (expressed as gram biogas and volatile fatty acids formed per gram of organic matter converted x 100)

4. GENERAL CONCLUSIONS

Batch reactor experiments

The batch reactor experiments showed that the maintaining of a constant pH during the experiments was a major problem. Because of the high buffer capacity of the inoculum used in "batch experiment 1" was it not possible to keep the fixed pH. Using mineral medium in "batch experiment 2" was not a solution to keep the pH constant because the buffer capacity of synthetic medium with different buffer concentrations was to low to prevent a significant pH drop when volatile fatty acids were produced.

Fed batch experiments

The addition of *Clostridium thermocellum* and *Clostridium thermosaccharolyticum* in the batch experiment "Batch experiment 1" resulted in a higher volatile fatty acid production. Based on this result a fed batch experiment (FB Experiment I) was performed to test the biodegradation of cellulose by a thermophilic inoculum with and without additional Clostridia strains. It appeared that the final biodegradation efficiency of the added cellulose of about 60 % was the same in the set-up with thermophilic inoculum and the set-up with thermophilic inoculum and additional Clostridia strains. Addition of Clostridia strains did not improve the biodegradation efficiency of cellulose.

During the first period of FB Experiment II the cellulose in non-inhibited reactors ranged from 55 % to 70 % and was comparable with the results of FB Experiment II. When the ammonia concentration of the reactor content ranged between 2 and 3 g N/l about 500 mg VFA and 250 gram of biogas containing 40 vol% methane was produced per gram of cellulose fed to the reactor. When the ammonia concentration was higher than 4 g N/l no methane was produced anymore but also the production of volatile fatty acids decreased to only 150 mg VFA per gram of cellulose fed. The free ammonia inhibited the methanogenesis but also the volatile fatty acid production was inhibited. Yet, it is not clear that the decrease in volatile fatty acid production was only induced by the high ammonium concentration.

Faecal material was biodegraded in conditions at which methanogenesis was not inhibited. Non pre-acidified faecal material was converted for 69 % into biogas and volatile fatty acids. The acidification of faecal material before feeding to the reactor did not increase the biodegradation of the material. Only 44 to 62 % of the faecal material was converted in the case that anaerobic digestion in the reactors was combined with pre-acidification. When the ammonium content of the reactor was equal to 8 g N/l no biodegradation of faecal material was noticed.

It can be stated that in conditions at which methanogenesis is not inhibited about 60 % of the amount of powdered cellulose fed to the reactor was biodegraded. Yet, the results indicated that a high ammonium concentration did not only result in methanogenesis inhibition but also in a general destabilisation of the anaerobic biodegradation process and a decrease of the conversion efficiency.

In previous experiments the biodegradation efficiency of the non-bacterial carbohydrates was also equal to 60 % (final reports of 1994 & 1995). This indicates that in order to reach a biological degradation of carbohydrate polymers with an efficiency higher than 60 %, the material needs to be pretreated in a physical or biochemical way. The biodegradtion efficiency of sonicated faecal material will be determined during the following experiments. The results will be publisched in the next technical note (TN 34.3).

Addendum 2. General overview of the results obtained during the fed batch biodegradation experiment (weight units are expressed per reactor).

Period	Feed			VFA			Gas	Biodegraded feed	
	Type	Amount	Cum. amount	Initial	Final	Difference	produc-tion	Cumulative	Relative amount*
day		g	g	mg	mg	mg	g	g	%
0 - 14	cl + HF	1 + 0.6	2.6	2715	3209	494	0.96	1.46	56
14 - 18	cb	0.5	3.1	3209	3463	254	0.39	2.10	68
18 - 21	cb	1	4.1	3463	2659	-804	0.87	2.17	53
21 - 26	cb	1	5.1	2659	1348	-1311	1.03	2.17	37
26 - 29	cl	1	6.1	1348	1044	-304	0.95	2.81	42
29 - 32	cl	1	7.1	1044	795	-249	1.09	3.65	48
32 - 35	cl	1	8.1	795	419	-376	1.02	4.30	50
35 - 39	cl	1	9.1	419	339	-80	1.02	5.24	54
39 - 43	cl	1	10.1	339	185	-154	0.78	5.86	55
43 - 46	cl	0.6	10.7	185	19	-166	0.43	6.13	55
46 - 50	cl	1	11.7	19	201	182	0.61	6.92	57
[illegible]									
0 - 14	cl + HF	1 + 0.6	2.6	1356	1689	333	1.21	1.55	59
14 - 18	cb	0.5	3.1	1689	1700	11	0.36	1.92	62
18 - 21	cb	1	4.1	1700	1260	-440	0.68	2.16	53
21 - 26	cb	1	5.1	1260	1070	-190	0.83	2.80	55
26 - 29	cl	1	6.1	1070	707	-363	0.57	3.00	49
29 - 32	cl	1	7.1	707	1089	382	0.75	4.13	58
32 - 35	cl	1	8.1	1089	1041	-48	0.86	4.94	61
35 - 39	cl	1	9.1	1041	1515	474	0.85	6.27	69
39 - 43	cl	1	10.1	1515	838	-677	0.74	6.33	63
43 - 46	cl	0.6	10.7	838	875	37	0.82	7.18	67
46 - 50	cl	1	11.7	875	167	-708	0.70	7.18	61

HF: human faeces - cl: cellulose - cb: cellobiose

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