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Effect of sonication on the biodegradation of faecal material

Pathogen removal in the thermophilic reactors

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1. INTRODUCTION

An overview of possible methods to improve the biodegradation of faecal material was reported in technical note TN 34.1. Sonication was selected as the first physical method to improve the biodegradation of human faecal material. This technical note reports the results of the sonication experiments. The effect of sonication on the biodegradability of faecal material in thermophilic fed-batch reactors was tested.

The presence of pathogenic organisms in the Melissa-loop is of major concern. Some basic principles of microbial die-off in thermophilic systems are reported in this technical note together with results obtained in the fed batch experiments. The destruction of pathogenic indicator organisms was monitored in reactors fed with faecal material and sonicated faecal material.

2. SONICATION

2.1 Introduction

The effects of sonication of activated sludge were reported by King & Forster (1990). Sonication with power levels of 7.5 - 75 W caused disruption of the sludge flocs which increased with the intensity of the power. There was a relationship between the mean particle size and the sonic power. Sonication also released soluble carbohydrate and protein from the sludge. It appeared that there was a sequential release of different biopolymers from the sludge as the power was increased. Sonication is a relative simple method to destruct organic material and was for this reason chosen as a first method to improve the biodegradability of faecal material.

2.2 Experimental set-up

2.2.1 Sonication

A Branson sonifier model 250 was used in the sonication tests. The sonicator is especially developed to disrupt cells, bacteria and tissues. The main components are a power supply, a converter and a horn. The power supply sends 20 kHz electrical energy to the converter, which changes this energy into 20 kHz mechanical vibrations. The horn intensifies and focuses these mechanical vibrations on the material to be processed.

The faecal material was diluted in water in a ratio of 20 g wet weight faecal material / 200 ml water. The dilution was sonicated during 6 minutes at a power level of 110 W. At day 21 of the experiments the sonication time was doubled (12 minutes). This settings are normally used for the destruction of microbial cells.

The sonicated material was fed to the fed batch reactors.

2.2.2 Fed-batch reactor experiment

The reactors had a wet volume of 800 ml and were operated at thermophilic conditions (55 °C). Two reactors were taken in operation by dividing the reactor content of reactor "TRc/TRcf " (see TN34.2) into reactor "TR_FM" and reactor "TR_FMs". TR_FM was fed with non-sonicated faecal material and reactor TR_FMs with sonicated faecal material. Figures $$ and $$ give a schematic presentation of the feeding operations of the reactors. The reactors were fed with a dilution of faecal material or sonicated faecal material and the centrifuge cake. The cake was obtained by centrifuging a volume of 200 ml reactor content after the digestion period. The reactor content was centrifuged to separate the produced ammonium and volatile fatty acids. Table 2.1 gives the feeding regime of the reactors. Before the start of the feeding period with recycled cake, already two times an amount of faecal material (25 g wet weight / liter reactor wet volume) and sonicated faecal material was added in reactor "TR_FM" and "TR_FMs" respectively.

Table 2.1. Feeding regime of the reactors

Time	**Non sonicated** reactor "TR_FM"		**Sonicated** reactor "TR_FMs"	
	Faecal material (g OM*/reactor)	**Recycle (g OM*/reactor)**	**Faecal material (g OM*/reactor)**	**Recycle (g OM*/reactor)**
Initial period				
-6	3.4		3.4	
-3	3.4		3.4	
Test period				
0	2.49	5.12	2.49	2.62
3	3.20	5.83	3.20	6.01
7	3.12	6.05	3.12	6.65
11	3.12	6.50	3.12	6.74
14	4.05	7.40	4.05	7.01
17	4.05	6.64	4.05	6.91
21	4.11	6.97	4.05	8.83
24	3.92	8.15	3.92	8.15
28	3.92	10.16	3.92	10.16

* diluted in 200 ml

2.2.3 Calculation of the conversion efficiencies

The percentage of conversion of nitrogen compounds and non-nitrogen compounds were calculated based on the produced gas volume, the gas composition and the evolution of the volatile fatty acid and ammonia concentration in the reactors. Considerations about the calculation method were published in the technical note TN34.1. Because the methanogenesis is not inhibited in the experiments, the conversion efficiency must be interpreted as efficiencies obtained in non-stressed and optimal conditions. The conversion of organic nitrogen into ammonium is considered as the efficiency of protein conversion. The amount of proteins was calculated by multiplying the organic nitrogen with a factor 6.25. The amount of carbohydrates and other products was obtained by subtracting the amount of proteins from the total amount of organic matter. In previous calculations of the mass-balances it was assumed that the breakdown of organic matter was mainly related with the non-bacterial organic matter present in faecal material. Therefore the conversion efficiencies were calculated based on the total organic matter and also on the non-bacterial organic matter. It was assumed that one third of the organic matter were proteins and one third of the total amount of organic faecal material were bacteria.

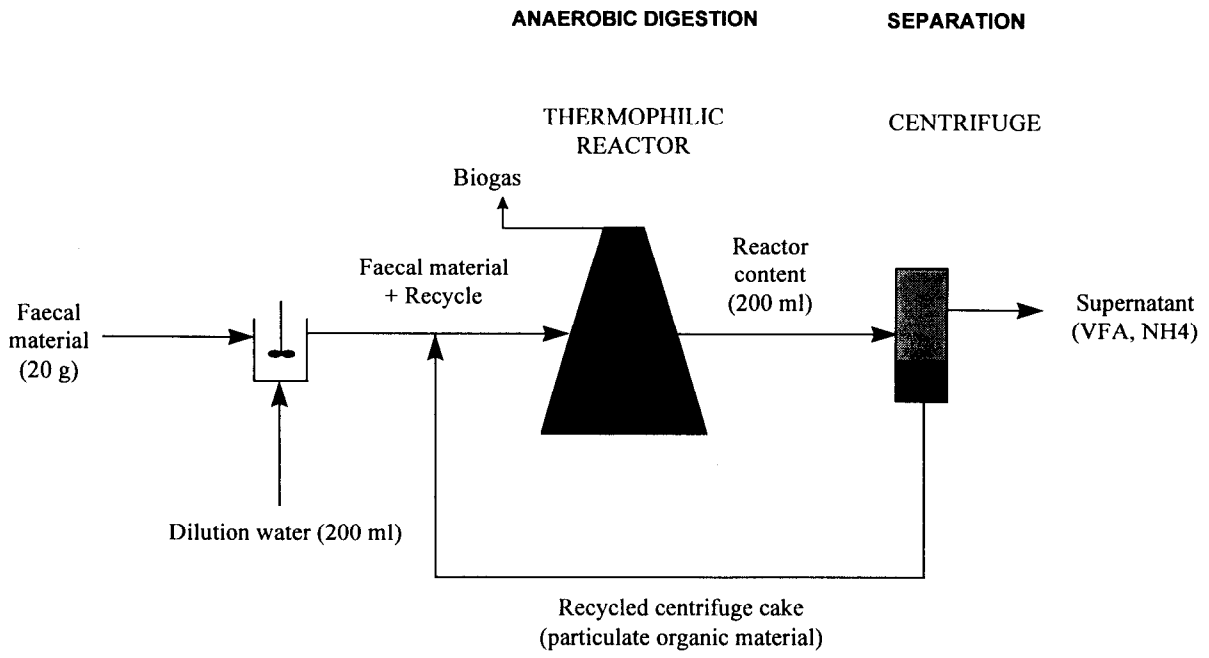

Figure 2-1. Feeding regime of reactor " TR_FM" with human faecal material.

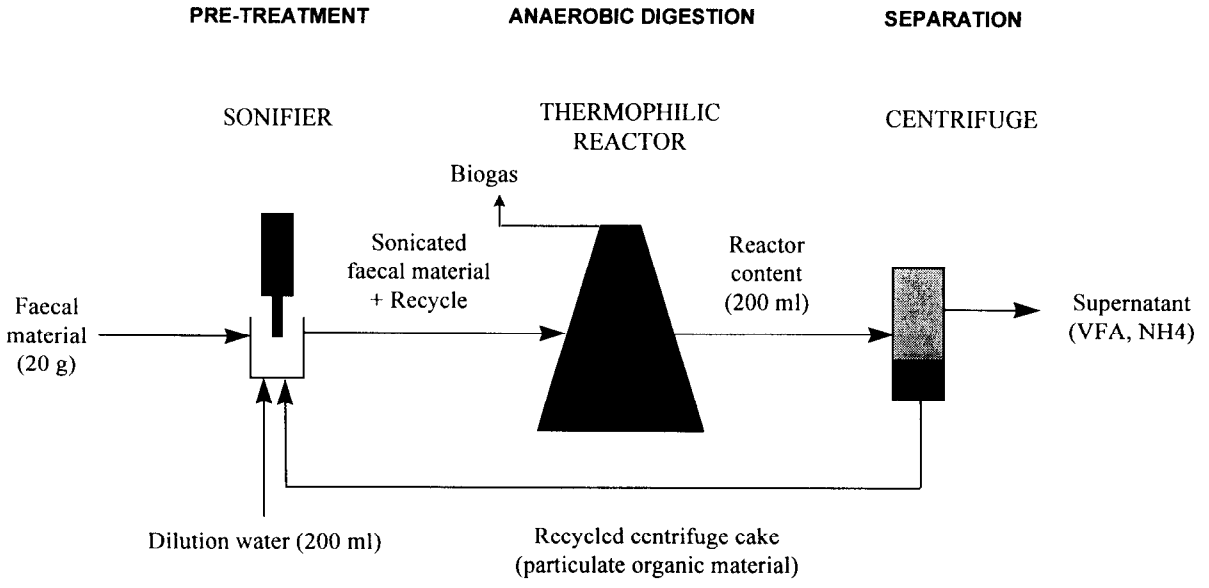

Figure 2-2. Feeding regime of reactor "TR_FMs" with sonicated human faecal material

2.3 Results

2.3.1 Reactor performance

Figure 2.3 gives the cumulative gas production in the two reactors during the experiment. During the first three days the gas production in the two reactors was identical. In the period after day 3 was the gas production higher in reactor "TR_FM" than in reactor "TR_FMs". This could be explained by the fact that in the beginning of the feeding period the volatile fatty acid concentration in the reactor "TR_FM" was higher then in reactor "TR_FMs" (see Figure 2.5). Between day 3 and day 7 decreased the volatile fatty acid concentration in the reactor "TR_FM" and resulted in an increased biogas production. During the rest of the experimental period was the gas production in the two reactors comparable. The composition of the biogas is plotted in Figure 2.4 and varied between 65 and 80 % methane. There were no significant differences between the two reactors.

The volatile fatty acid concentration in reactor "TR_FM" at the beginning of the test was 1600 mg/l and in reactor "TR_FMs" about 350 mg/l. Yet, the volatile fatty acid concentration in the reactor "TR_FM" decreased during the test period to a comparable concentration present in reactor "TR_FMs".

The ammonium concentration in both reactors was between day 0 and day 16 about 520 mg NH_4-N/l. The ammonia concentration in both reactors increased to about 650 mg NH_4-N/l after day 17. The relative constant ammonia level indicates that the ammonia produced during the hydrolysis of nitrogen containing materials was removed for 90 % by centrifuging.

The pH in the both reactors varied between 8.0 and 8.2 and can be considered as stable.

2.3.2 Conversion efficiencies

The conversion efficiencies were calculated using the method described in Point 2.2.3.

The conversion efficiency of the total faecal material and non-bacterial biomass into the final product (biogas, volatile fatty acids and ammonia) is plotted in Figure 2.6. At the beginning of the test the total conversion efficiency of sonicated faecal material was 48 % and for non-sonicated faecal material 41 %. Up to day 14 the conversion efficiency of sonicated faecal material was about 5 % higher than non-sonicated faecal material. At day 14 was the total conversion equal for both types of feed. The total conversion efficiency for sonicated and non-sonicated faecal material was during the rest of the test period equal to about 38 %. It was clear from the experiments that sonication of the faecal material did not result in an increase of the biodegradation efficiency. The conversion efficiency of the non-bacterial organic matter was at the beginning of the test period equal to 72 % and 61 % for respectively sonicated and non-sonicated faecal material. The conversion efficiency of the non-bacterial biomass equilibrated at about 55 % for both reactors.

The biodegradation efficiency of the proteins present in the non-sonicated human faeces was equal to about 50 % during the whole test period. The protein biodegradation in sonicated human faeces varied between 50 % and 55 %. The conversion of non-bacterial proteins was about 77 % for the non-sonicated faeces as well for the sonicated faeces (see Figure 2.7, Figure 2.8 and Figure 2.9).

The conversion of carbohydrates and the rest of the non-protein components in non-sonicated faecal material was equal to about 30 % (Figure 2.7 & 2.10). Taking only the non-bacterial components in consideration, the conversion efficiency during the first 14 days of the test period was equal to 35 % and at the end equal to 30 % (Figure 2.10). Sonication of the faecal material did not result in a

significant increase of the biodegradation efficiency of carbohydrates and the rest of the non-protein components (Figure 2.10).

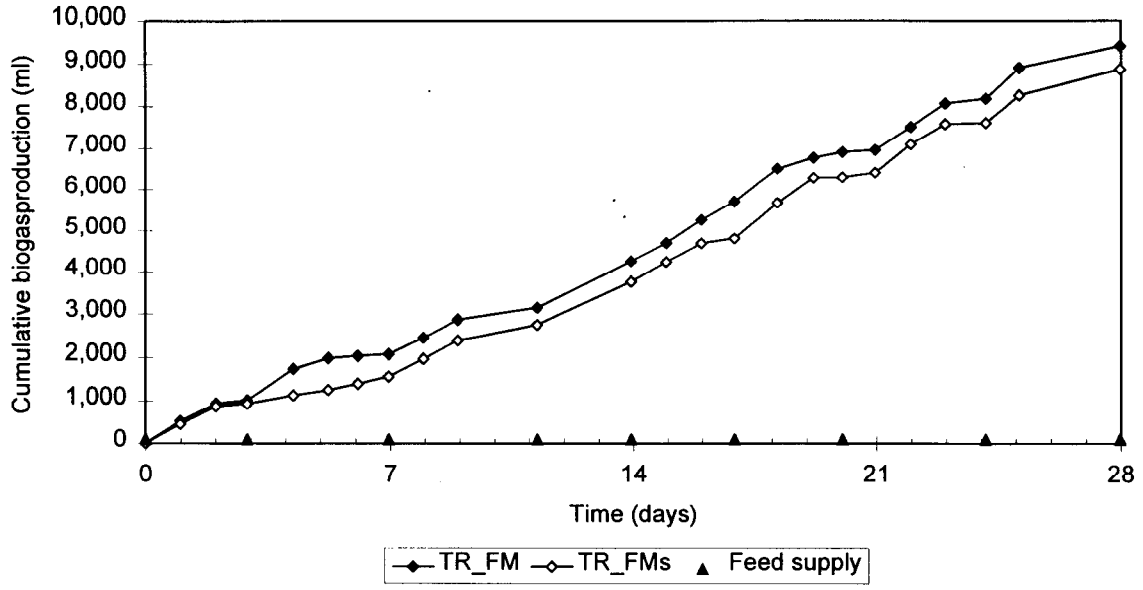

Figure 2-3. Cumulative biogas production in the reactors "TR_FM" and "TR_FMs"

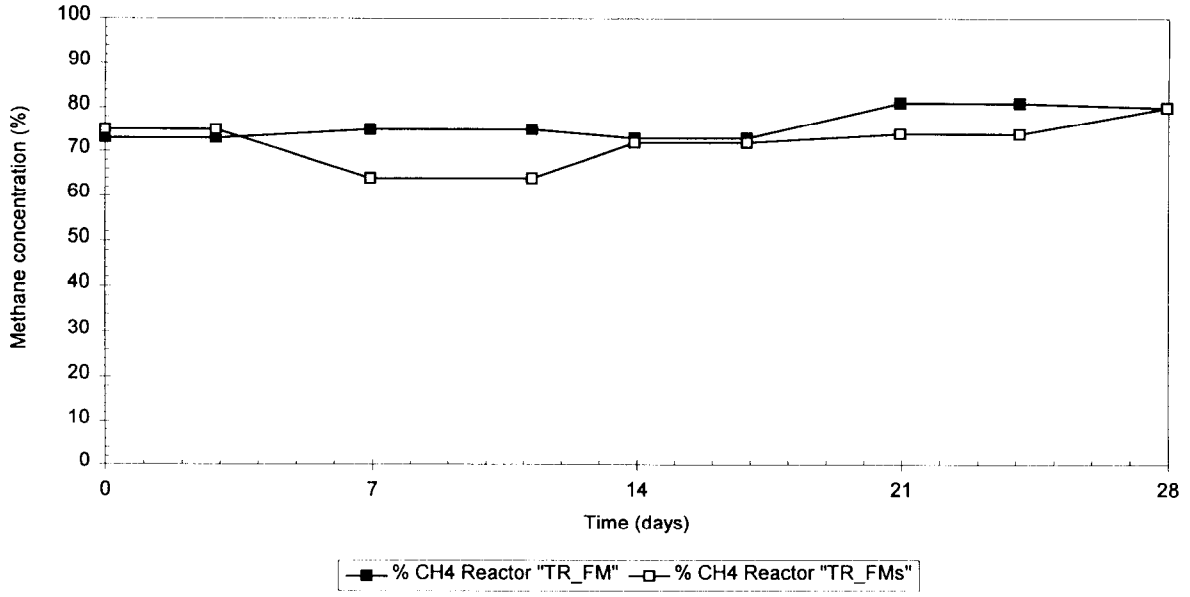

Figure 2-4. Biogas composition in the reactors "TR_FM" and "TR_FMs"

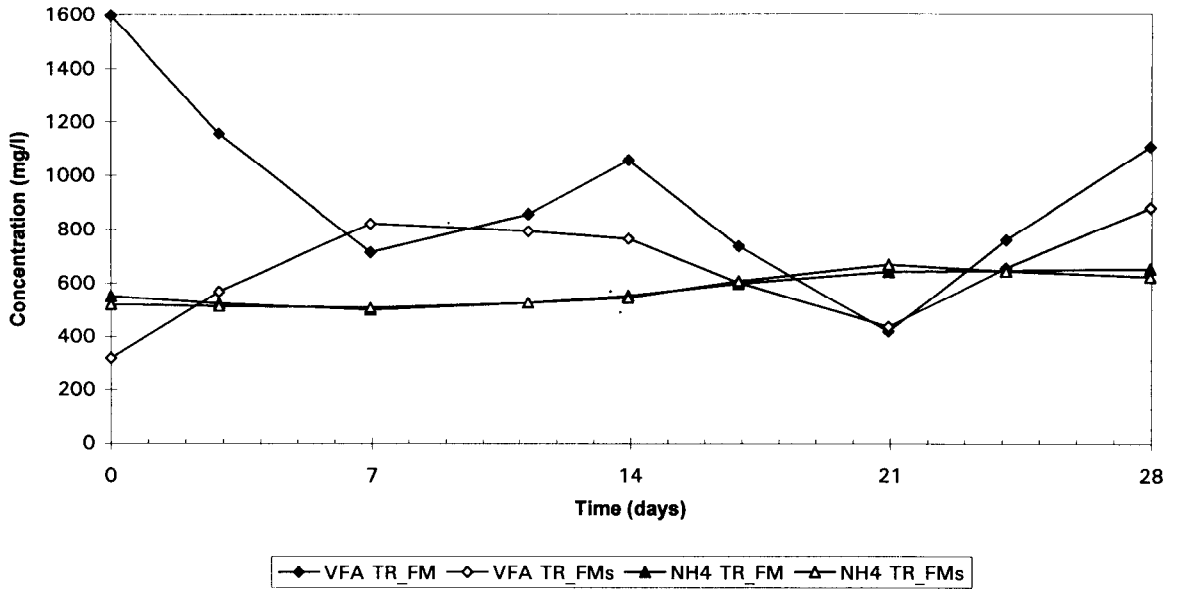

Figure 2-5. Evolution of the concentration of volatile fatty acids and ammonia in the reactors "TR_FM" and "TR_FMs"

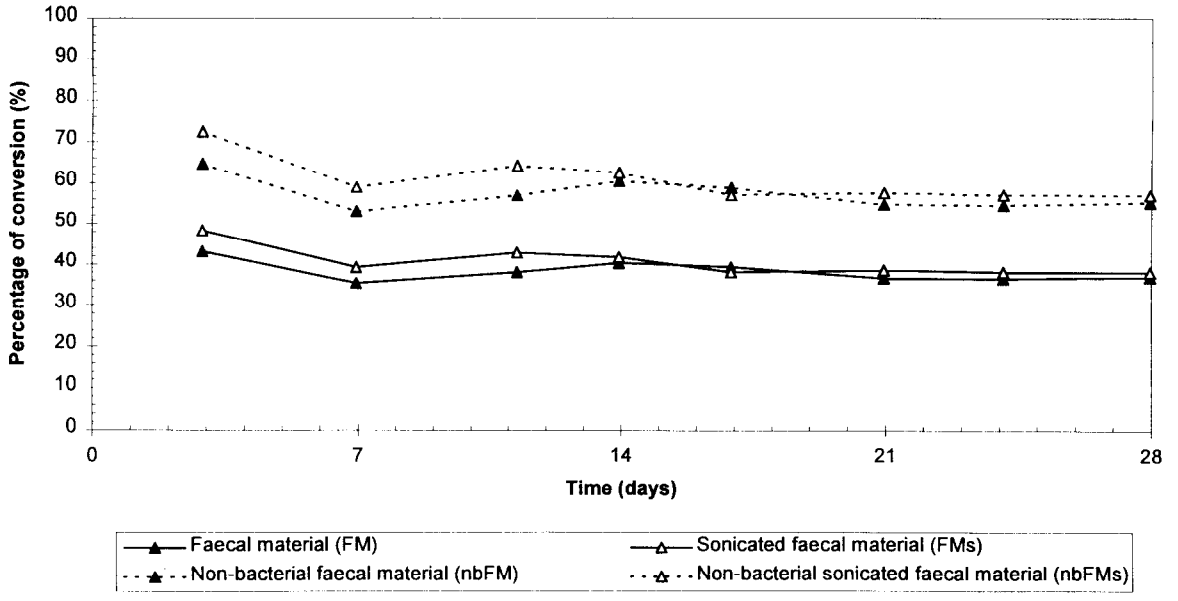

Figure 2-6. Conversion efficiencies of faecal material and the non-bacterial faecal material in reactors "TR_FM" and "TR_FMs"

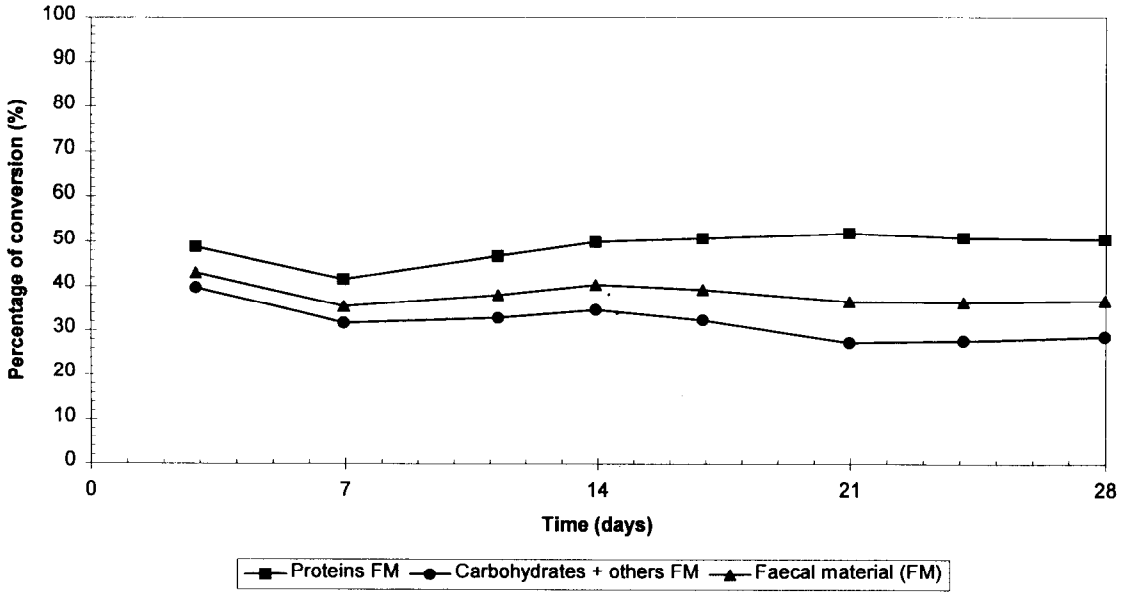

Figure 2-7. Conversion efficiencies of proteins and carbohydrates + other products present in faecal material and global conversion efficiency of non-sonicated human faeces

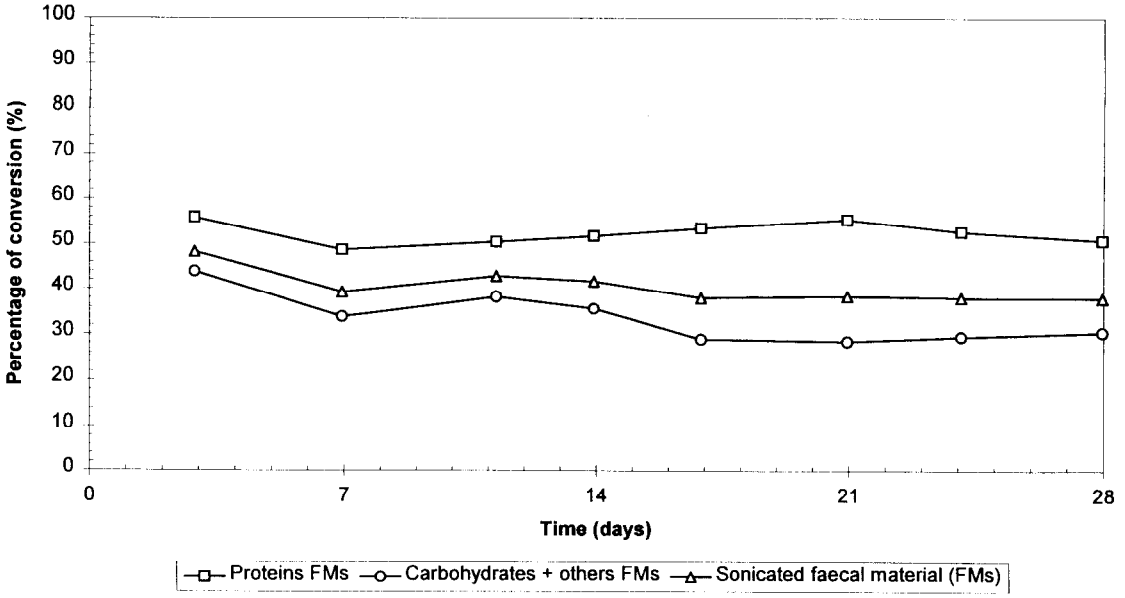

Figure 2-8. Conversion efficiencies of proteins and carbohydrates + other products present in faecal material and global conversion efficiency of sonicated human faeces

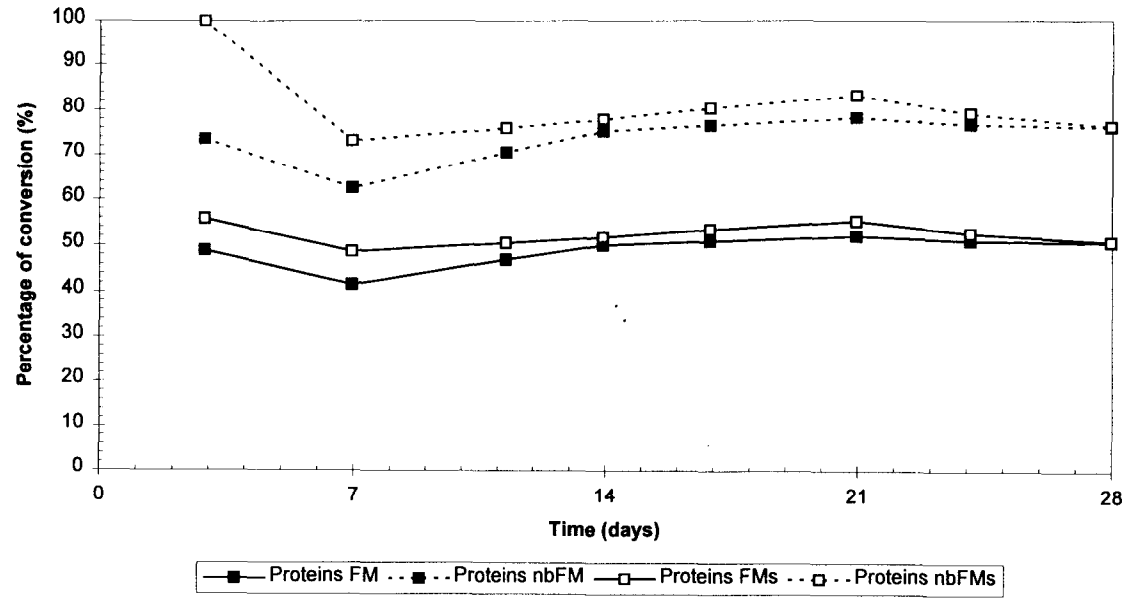

Figure 2-9. Conversion efficiencies of total proteins and non-bacterial proteins in faecal material and sonicated faecal material.

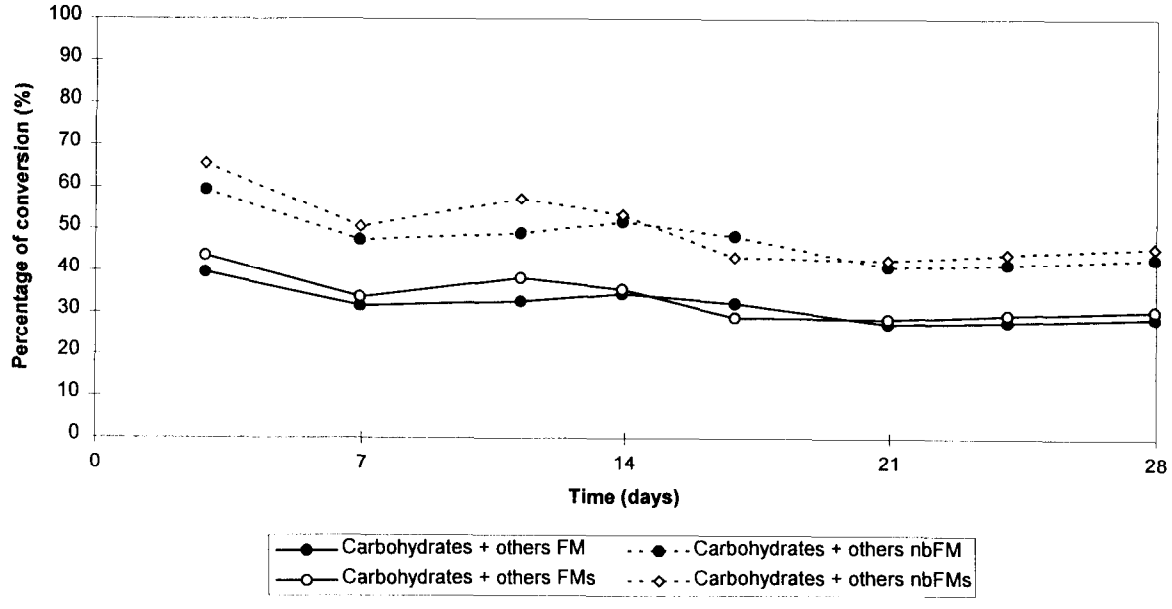

Figure 2-10. Conversion efficiencies of total carbohydrates and non-bacterial carbohydrates in faecal material and sonicated faecal material.

2.4 Conclusions

Sonication of the faecal material during 6 minutes at a power level of 110 W did not result in an increase of the conversion efficiency of the faecal material. Increasing the sonication time to 12 minutes had no positive effect on the conversion efficiency. The conversion efficiency of the faeces was in both cases equal to about 37 %. Protein and carbohydrate biodegradation were in both cases respectively equal to about 50 % and 30 %. Non-bacterial proteins and carbohydrates were biodegraded for respectively 76 % and 44 %. Yet, based on the results obtained in this experiment the hypothesis whereby it is assumed that bacterial cells are the difficult part to biodegrade can be taken in question, because it was expected that sonication of the faecal material would destruct bacterial cells and make them more accessible for biodegradation.

The conversion efficiencies obtained during the different experiments are compared in Table 2.2. In technical note TN 34.2 a preliminary experiment was performed to determine the biodegradation efficiency of pre-acidified human faecal material. It appeared that the efficiency was equal to 44 % and 62 % after respectively the first and the second feeding time. This conversion efficiency was calculated from data obtained from an experiment whereby the reactor was fed with pre-acidified faecal material and cellulose. More experiments over a longer term need to be done with only pre-acidified faecal material before we can conclude that pre-acidification has a better effect on the biodegradation than sonication.

The conversion efficiency from proteins and carbohydrates present in non-diluted faecal material whereby the dry matter concentration in the reactor content was equal to 13 % was about 10 % higher than in the reactors fed with pre-acidified faecal material and sonicated and non-sonicated human faecal material.

Table 2.2. Overview of the conversion efficiencies of the human faecal material determined during different experiments under thermophilic conditions

	Diluted faeces (1 - 2 % DM_{RC})			Non-diluted faeces (13 % DM_{RC})
	Non-sonicated	Sonicated	Pre-acidification (TN 34.2)	(Final report 1995)
Organic matter				
Total	37	38	44 - 62	46
Non-bacterial	55	57	-	70
Proteins				
Total	51	51	-	60
Non-bacterial	76	76	-	88
Carbohydrates + others				
Total	29	31	-	40
Non-bacterial	43	46	-	60

DM_{RC} : Dry matter content of the reactor

3. PATHOGENIC ORGANISMS

3.1 Introduction

The presence of pathogenic organism is of great concern in the MELISSA-cycle. Potential pathogenic organisms can be present in human faeces. The liquefying compartment is the first compartment of the Melissa-cycle. It is important that during this first step possible pathogenic organisms are eliminated. In this perspective a literature study was performed concerning the elimination of pathogenic organisms during anaerobic digestion.

3.2 Basic principles of heat inactivation of microorganisms

Heat death of a cell results in part from the thermal inactivation of enzymes. At higher temperatures, enzymes can be irreversibly inactivated. Micro-organisms can not withstand long periods with enzymes inactivity and die. The fraction of active enzymes decreases significantly over a narrow temperature range. In this part, the general concept of the kinetics of heat inactivation is explained in brief.

Time and temperature are the two basic parameters which drive the dying off of the micro-organisms. The combined effect of these two parameters is shown in Figure 1 for faecal *Streptococcus* and *Salmonella Enteritidis*. The slope of the curves is a measure for the speed of inactivation. It is shown that for *Salmonella*, the reduction rate increases significantly in the temperature range of 50 to 54°C. An important factor affecting the time of inactivation of enzymes is the availability of water. Enzyme denaturation is faster under moist conditions.

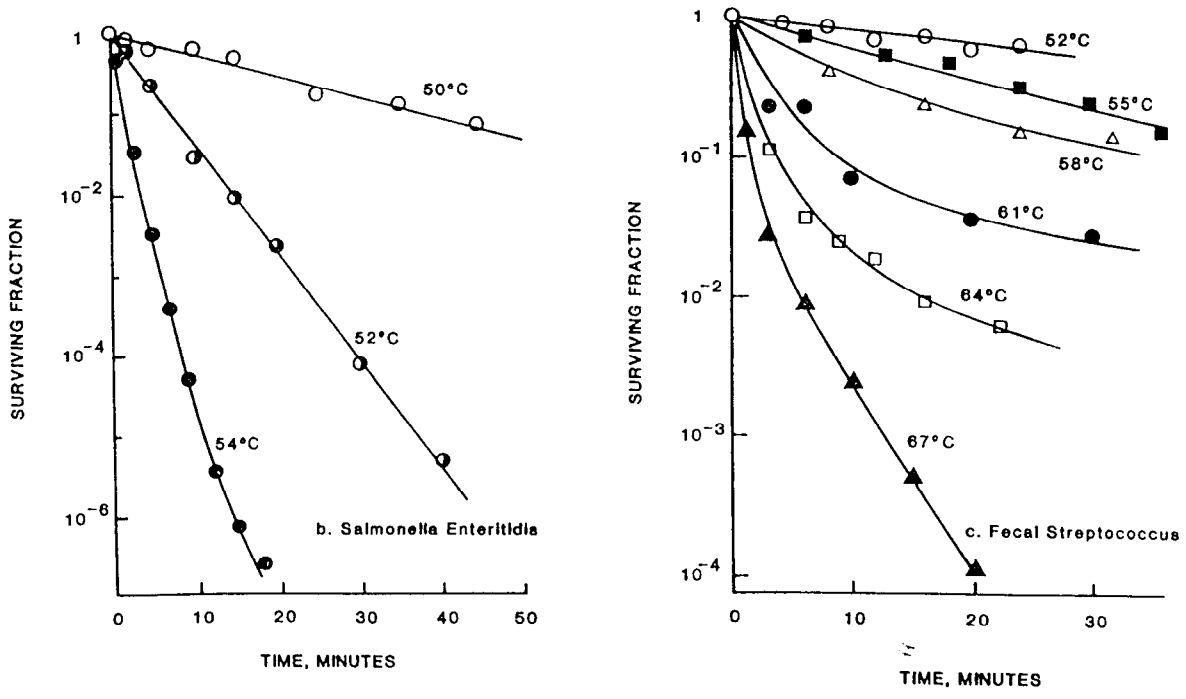

Figure 3-1. Heat inactivation of *Salmonella enteritidis* and faecal *Streptococcus* (after Ward and Brandon, 1977)

Figure 3.1 shows that the decay over time is more or less a straight line when the surviving fraction is plotted on a log scale. This means that the inactivation kinetics can be assumed following a first-order decay :

$$\frac{dn}{dt} = -k_d.n \quad (1)$$

If k_d is constant, integration of Equation 1 from an initial cell population n_0 to a later population n_t at time t yields :

$$n_t = n_0 e^{-k_d t} \quad (2)$$

Taking the log of both sides and rearranging :

$$t = \frac{(\ln \frac{n_0}{n_t})}{k_d} \quad (3)$$

Converting to base 10 logs and considering a one log reduction in cell concentration (i.e. a reduction of 90 %),

$$t_{90} = Dr = \frac{2.303}{k_d} \quad (4)$$

The term Dr is called the decimal reduction time and is the time required for a tenfold reduction in cell population.

The effect of the temperature on K_d is often modelled by an Arrhenius form :

$$k_d = C.e^{-Ed/RTk} \quad (5)$$

where Tk is the temperature (expressed in K). Ed is the inactivation energy, which is between 50 and 100 kcal./mol for many spores and vegetative cells. Logaritmic transformation of equation 5 yields :

$$\log_{10} k_d = \log_{10} C - \frac{E_d}{R}(\frac{1}{T_k}) \quad (6)$$

A plot of the logarithm of k_d versus $1/T_k$ allows the determination of the constant C and the inactivation energy E_d.

3.3 Die-off of pathogens

The removal of pathogens in thermophilic aerobic and anaerobic waste processing systems is reported in literature. Most reported data concern the removal of pathogens in processed pig manure and sludge from waste water treatment plants. Yet, wasted sludge originating from municipal waste water treatment plants can be contaminated with potential pathogenic bacteria or viruses of human origin. Pathogenic organisms present in pig manure may also be representative for human faeces because of the fact that the gastroinstestinal tract of the pig is similar to the human gastrointestinal tract. Data reported for this types of waste material can be directed to a situation were faecal material is processed.

The reduction of some pathogenic bacteria in function of the temperature expressed as Dr values is represented in Table 3.1. The table illustrates that the temperature of the reactor has an important influence on the inactivation time. In thermophilic systems operated at a temperature of 53 °C the Dr value is in the magnitude of hours. The use of a thermophilic system in the MELISSA-cycle is strongly advised concerning the elimination capacity of pathogens. Destruction of worm eggs is also much more efficient in thermophilic systems than mesophilic systems. Future research will provide more data on the elimination of pathogenic organisms.

Table 3.1. Dr values at different temperatures and for different types of pathogenic bacteria and indicator bacteria in sludge with biogas digestion and conventional storage. (ND : no decay observed) (Larsen and Munch, 1981 ; Olsen et al., 1985).

System	Biogas system		Slurry system	
Temperature (°C)	53	35	18-21	6-15
Time units	Hours	Days	Weeks	Weeks
Salmonella typhimurium	0.7	2.4	2.0	5.9
Salmonella dublin	0.6	2.1		
Escherichia coli	0.4	1.8	2.0	8.8
Clostridium perfringens, C	ND	ND	ND	ND
Bacillus cereus	ND	ND		
Erysipelotrix rhusiopathiae	1.2	1.8		
Staphylococcus aureus	0.5	0.9	0.9	7.1
Mycobacterium paratuberculosis	0.7	6.0		
Coliform bacteria		3.1	2.1	9.3
Group of D-Streptococci		7.1	5.7	21.4
Streptococcus faecalis	1.0	2.0		

Table 3.2. Inactivation of some current parasite eggs and larvae during biogas digestion (Olsen et al. 1985).

Temperature (°C)	Loss of Viability max. time	Parasite
53	1-4 hours	Eggs of gastrointestinal worms Eggs of nodular worms Eggs of roundworms
35	2 days < 2 days 6 to 8 days 21, <35 days <7 days	Eggs of gastrointestinal worms (cattle) Eggs of tapeworm (cat) Eggs of nodular worms (pig) Eggs of ascaris (pig) Larvae of lungworms (cattle)

3.4 Removal of pathogens in the Melissa reactors

3.4.1 Introduction

The use of the method whereby indicator organisms are detected to determine the hygienisation capacity of treatment processes for organic wastes has proven its practical value (Bendixen, 1994; Farrell, 1993). Normally used indicator organisms for faecal pollution are faecal coliforms and streptococci. The presence of indicator organisms is well correlated with the presence of other pathogenic organisms. This method was also used in the experiments to evaluate the pathogen removal efficiency of the thermophilic Melissa-reactors.

3.4.2 Experiment

Sampling

Samples were taken from the reactor immediately after feeding (samples "AF") of the reactor and also after the digesting period before feeding (samples "ADP"). The decrease of faecal coliforms and faecal streptococci after the digestion period (3 days) is a measure for the die-off of potential pathogenic organisms present in the reactor.

Determination of faecal coliforms

A sample of the 10 ml reactor content was diluted in 90 ml physiological solution. A serial dilution of 1/10 was made next. One ml of each dilution was brought on petri-dishes and mixed with 20 ml of Mc Conkey agar. After solidification of the Agar, the plates were incubated at 43 °C. The colonies were counted after one day. The results are expressed as colonyforming units per ml (CFU/ml).

Determination of 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. The results are expressed as colonyforming units per ml (CFU/ml).

3.4.3 Results and conclusions

Table 3.3 gives an overview of the amount of faecal streptococci and coli present in the reactor content from reactors "TR_FM" and "TR_FMs" immediately after feeding (sample "AF") and after the digestion period (sample "ADP"). After feeding are faecal streptococci and faecal coli present in a magnitude of 10^3 to 10^4 CFU per ml. About three to five days after the feeding period the amount of faecal coli decreased below the detection limit. Also the amount of faecal streptococci decreased under the detection limit or was of the magnitude of 10 CFU/ml. Sonication of the faecal material did not have a stimulating effect on the die-off of pathogenic organisms.

The results supports the data cited in literature. Pathogens are significantly reduced in thermophilic (55 C°) anaerobic reactors. Yet, this does not guarantee an effluent free of pathogens. Additional treatment to eliminate pathogenic organisms is necessary. This may be possible with an additional heat treatment combined with filtration techniques. The results obtained in the experiment are determined in a system with a hydraulic residence time of 14 days. Increasing the hydraulic residence time will enhance the elimination of pathogenic organisms.

Table 3.3. Overview of the bacterial counts (CFU/ml reactor content) present in the reactors "RC_FM" and "TR_FMs" before and after the digestion period

	Faecal streptococci	Faecal coli
Reactor "RC_FM"		
Day 7		
ADP	$2.1\ 10^1$	<dl
AF	$5.9\ 10^2$	$7.0\ 10^2$
Day 14		
ADP	<dl	<dl
AF	$2.1\ 10^4$	$2.5\ 10^4$
Day 21		
ADP	<dl	<dl
AF	$1.2\ 10^3$	$1.0\ 10^5$
Reactor "TR_FMs"		
Day 7		
ADP	$2.0\ 10^1$	<dl
AF	$1.9\ 10^3$	$4.2\ 10^3$
Day 14		
ADP	$1.0\ 10^1$	<dl
AF	$3.0\ 10^3$	$1.1\ 10^3$
Day 21		
ADP	<dl	<dl
AF	<dl	$1.0\ 10^3$

ADP : After digesting period
AF : After feeding
dl: detection limit

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