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TN 22.5

Breakdown of human faeces by autochtonous strains.

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1. INTRODUCTION

Previous experiments showed that no efficient biodegradation of artificial human faeces and pig manure by axenic strains of *Clostridium thermocellum*, *Clostridium thermosaccharolyticum* and *Coprothermobacter proteolyticus* I8 nor by a co-culture of these strains occurred (TN 22.1, TN 22.2, TN 22.3).

The next step was to investigate if a co-culture of *Clostridium thermocellum*, *Clostridium thermosaccharolyticum* and *Coprothermobacter proteolyticus* I8 could biodegrade human faeces. During a fermentation for 21 days, 1520 mg/l volatile fatty acids and 250 mg/l NH_4^+-N were formed (TN 22.4).

A literature study on the microbiota present in human faeces was carried out (TN 22.4) to prepare the following experiment whereby the autochtonous strains present in human faeces would be used as inoculum.The test was performed in batch reactors incubated at 37°C and 55°C. The production of volatile fatty acids and ammonia was measured.

2. MATERIALS AND METHODS

2.1. Human faeces

Table 2.1 shows the characteristics of the faeces used in the experiments. These were the same faeces, collected from a panel of five healthy persons (age 20-30 years: 4 persons and 50 years: one person), that were used for the experiments described in TN 22.4. The faeces had a dry matter content of 20 to 35%. They were immediately stored at a temperature of minus 18°C.

At the start of the experiments, the faeces were defrosted and diluted with demineralised water. The dilution was necessary to determine the composition in an accurate way and to obtain a liquid medium for the biodegradation tests. The dilution of the faeces was about twice as high than in the experiments described in TN 22.4 in order to determine the composition in an even more accurate way.

Table 2.1. Composition of the diluted faeces used in the biodegradation test

Parameter	Value mg/l	$SE^{(n=3)}$ mg/l	Value mg/g DM	$SE^{(n=3)}$ mg/g DM
Dry matter	18533	356	-	-
Organic matter	15270	360	823	464
Ash	3263	51	176	9
Suspended solids	11400	256	615	18
Volatile suspended solids	9793	201	528	15
COD_{tot}	27309	1241	1472	73
COD_{sol}	8871	152	479	12
TOC	ND	-	-	-
NH_4^+-N	63	2	3	
NO_3^--N	0	0	0	0
Kjeldahl N	1138	31	61	2
Organic N	1075	31	58	2
Protein content*	6717	192	362	12
VFA	748	33	40	49
pH	6.8	0	-	-

* calculated
ND not determined

2.2. Inoculum

The ability of the autochtonous bacteria in the human faeces to biodegrade the faeces was tested. No supplementary strains were added.

2.3. Analytical techniques

The *dry matter (DM)* of the sample was determined after 24 hours drying at 105°C. The *ash content* was determined after incineration at 450°C for 3 hours.

A sample was filtered and the residue was dried for 24 hours at 105°C to determine the *suspended solids* (SS). The *volatile suspended solids (VSS)* were determined by incinerating the dried residue at 450°C for 3 hours.

Volatile fatty acids (VFA) were extracted with diethylether from acidified samples and determined by gas chromatography using a flame ionization detector coupled to a glass column containing chromosorb 101.

Total protein concentrations were determined by acid hydrolysis (decomposition into amino acids) and a colorimetric measurement (Hattingh et al., 1967).

The NH_4^+-N content was determined by steam distillation in a Kjeltec 1002 apparatus under alkaline conditions. *(NO_3^- + NO_2^-)-N* was determined by steam distillation in a Kjeltec 1002 after reduction to NH_3 by the addition of Devarda alloy. *Kjeldahl nitrogen* was determined similarly after complete destruction of the sample in strong acid.

The *chemical oxygen demand (COD)* corresponds to the amount of oxygen necessary for complete oxidation of all organic matter present in a given volume of sample. The organic content of the sample is subjected to oxidation by potassium dichromate in a strong acid medium (sulphuric acid plus silver sulphate) at a temperature of 150° C for two hours. The excess dichromate is then measured by back titration with ferrous ammonium sulphate. The *total COD* (COD_{tot}) is determined on the total sample, whereas *soluble COD* (COD_{sol}) is determined on a centrifuged sample.

2.4. Description of the experiments

Bottles of 250 ml were filled with 120 ml human faeces and flushed with nitrogen gas. The pH was set at 6.8 and 0.8ml of a 2.5% Na_2S-solution was added to ensure anaerobic conditions. The bottles were incubated at 55°C and 37°C and shaken manually several times per day. Table 2.2 gives an overview of the experimental set-ups.

Table 2.2. Scheme of the experimental set-up

Set-up		Autoclavation	Incubation temperature
Name	description		
B55	Blank of set-up A55	40 minutes at 121°C	55°C
A55		not autoclaved	55°C
B37	Blank of set-up A37	40 minutes at 121°C	37°C
A37		not autoclaved	37°C

At the end of the experiment (after 14 days) the volatile fatty acids, ammonia, Kjeldahl-nitrogen, the COD_{tot}, the COD_{sol}, the dry matter (DM), the ash-content, suspended solids and volatile suspended solids were determined.

3. RESULTS

Table 3.1 shows the results of the biodegradation experiment. About half of the organic nitrogen was broken down in set-ups A37 and A55 after 14 days.

At the end of the test, a significantly higher amount of volatile fatty acids was produced in the bottles incubated at 37°C as well as in the bottles incubated at 55°C compared with the blanks. The concentration of VFA in set-up A37 was not significantly different (significance level 0.05) from the concentration in set-up A55. Table 3.2 presents the procentual composition of the fatty acids. Acetic acid, propionic acid, butyric acid and iso-valeric acid were the most important fractions of the total volatile fatty acids. Due to the VFA production a decrease of the pH was noticed.

The evolution of the ammonia and VFA concentration is presented in Figure 3.1 and Figure 3.2. After a period of 7 days the production of VFA and ammonia reached a maximum.

The soluble COD content increased in both cases. This confirms that a part of the insoluble organic matter was liquified.

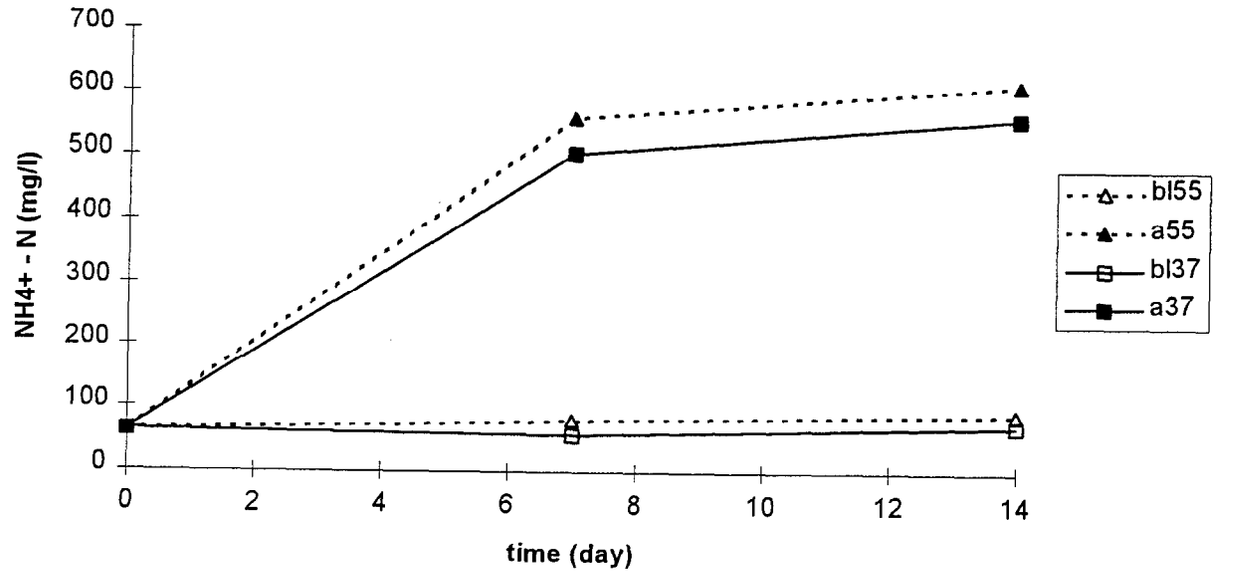

Figure 3.1. Evolution of the NH_4^+-N content during the experiment

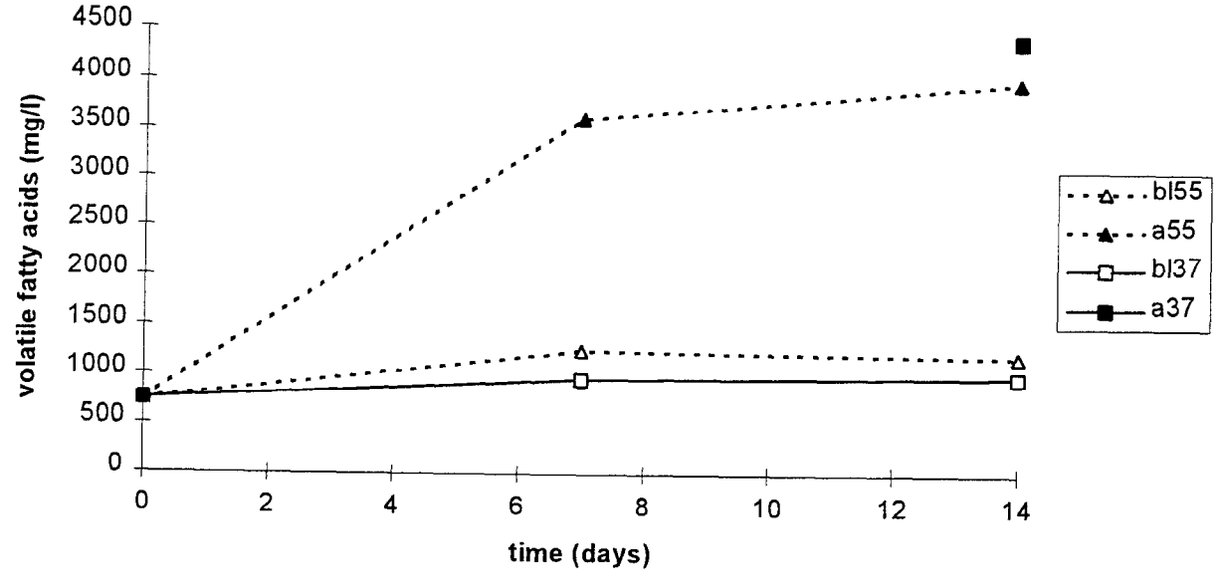

Figure 3.2. Evolution of the VFA concentration during the experiment

A decrease of organic matter of about 20% in both cases indicates that a significant amount of organic matter was converted to gaseous compounds. This statement can be confirmed by the fact that also the total COD content decreased with 7% for application A55 and with 9% for application A37.

Table 3.1. Values and standard error$^{n=3}$ of the measured parameters at the beginning and at the end of the biodegradation test (after 14 days)

Parameter	Set-up				
	t=0	Blank 55°C	55°C	Blank 37°C	37°C
	mg/l	mg/l	mg/l	mg/l	mg/l
Dry matter	18533± 356	18117±402	15295±428	19110±460	15797±429
Organic matter	15270± 375	14810±454	12318±1486	15600±730	12285±433
Ash	3263± 51	3307±70	3582±204	3510±141	3512 ± 79
SS	11400± 256	11870±721	10075±544	12380±930	11322±1618
VSS	9793± 201	10100±655	8390±455	9458±153	9925±1485
COD_{tot}	27309±1241	23580±1300	21953±975	25120±1710	22757±2825
COD_{sol}	8871±152	7841±303	9302±127	7334±477	9968±894
NH_4^+-N	63± 2	91±11	611±2	72±16	558±15
NO_3^--N	0	0	0	0	0
Kjeldahl-N	1138±31	1161±53	1174±7	1175±36	1158±49
Organic N	1075±31	1069±46	562±2	1103±39	600±44
Protein-content*	6717±192	6684±287	3515±37	6894±246	3750±278
VFA	748±33	1190±229	3966±253	987±37	4390±328
pH**	6.8	6.70	6.20	6.75	5.90

* calculated

** no unit

Table 3.2. Procentual composition of the volatile fatty acids (mean value ± standard error$^{n=3}$)

	Set-up		
	Initial (t = 0)	A55 (t = 14 d)	A37 (t = 14 d)
Acetic acid	50.0	41.8±1.9	44.3±5.3
Propionic acid	16.0	14.7±0.5	11.1±6.0
Iso-butyric acid	2.5	5.9 ± 0.2	4.5±0.6
Butyric acid	18.3	22.3 ± 1.3	15.1±5.5
Iso-valeric acid	2.3	13.3 ± 0.1	9.9±1.0
Valeric acid	4.9	1.3	6.1±0.3
Iso-capronic acid	0.7	0.1	<DL
Capronic acid	4.2	0.7	8.9±8.1

<LD: lower than detection limit

4. CONCLUSIONS

The test shows that the autochtonous bacteria of human faeces are capable to biodegrade human faeces as well under mesophilic conditions (37°C) as under thermophylic (55°C) conditions.

After seven days the biodegradation reached a maximum. In both cases the organic matter decreased with about 20% and half of the proteins were broken down. The following seven days practically no further breakdown was noticed.

Based on the results, shown in Table 3.1, a mass balance can be calculated. The results are presented in Table 4.1. It appears that the decrease of organic matter is equal to the decrease of proteins. Thus, mainly the proteins were biodegraded during the test.

Table 4.1. Decrease in dry matter, organic matter and proteins during the biodegradation test (mean value ± standard error$^{n=3}$)

Parameter	Decrease (mg/l)	
	55°C	37°C
Dry matter	2822 ± 587	3313 ± 629
Organic matter	2492 ±1553	3315 ± 848
Proteins	3169 ± 289	3144 ± 371

Figure 4.1 shows the composition of human faeces. One third of the organic material consists out of bacteria, one third out of undigested material and one third out of epithelium of the gastro-intestinal tract. At the beginning about 6700 mg of proteins per liter are present. Taking into account the composition of the organic material, it can be calculated that 2230 mg proteins/l are incorporated in bacterial biomass and 4470 mg proteins/l are non-bacterial proteins. The cell-yield factor of acetogenic bacteria is equal to 0.2 mg biomass formed per mg organic matter biodegraded. When 4470 mg proteins nitrogen is broken down, 143 mg N or 894 mg proteins are assimilated in new bacteria. The proteins, incorporated in the bacterial biomass at the end of the experiment, equalled 3124 mg N/l. When this value is compared with the organic nitrogen content present at the end of the test, it can be concluded that the main part of non-bacterial nitrogen was broken down.

To increase the degradation of the proteins, bacterial proteins need first to be set free by destroying the bacterial cells. This can for example be done by autoclaving the faeces, followed by an inoculation with autochtonous bacteria present in non-autoclaved faeces.

The volatile fatty acids produced during the test were also generated by the biodegradation of proteins.

A chemical equation for the degradation of proteins can be written as follows :

$$2\ C_2H_5NO_2 + 4\ H_2O \rightarrow CH_3COO^- + 2\ HCO_3^- + H^+ + 2\ NH_4^+ + 2\ H_2$$

When proteins are broken down, ammonia and volatile fatty acids are formed.

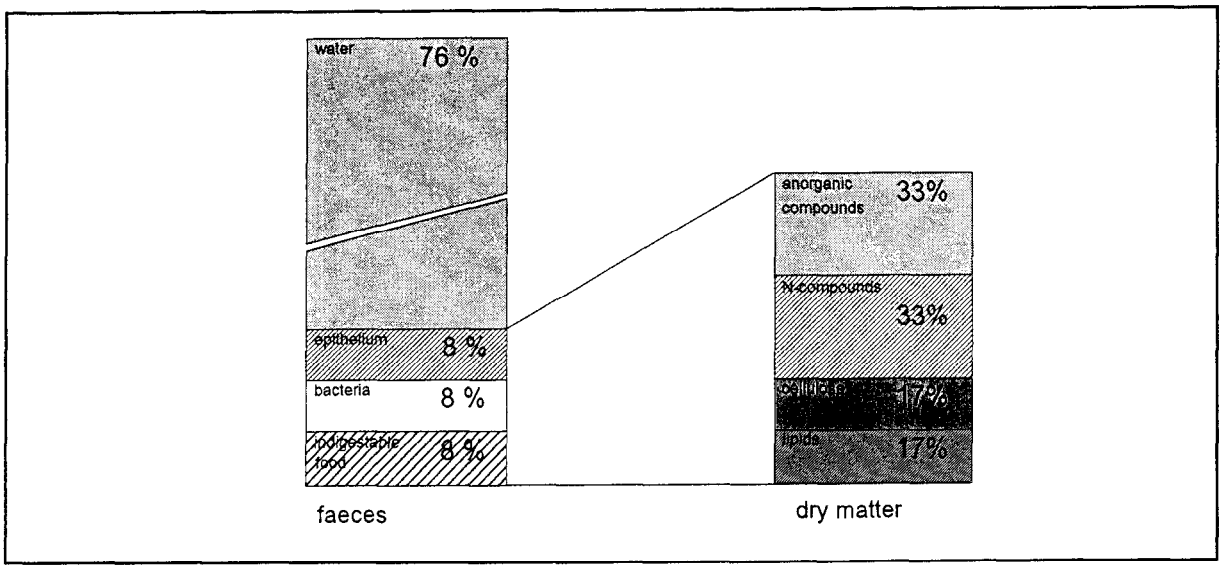

Figure 4.1. Composition of human faeces

The mass balance indicated that mainly the non-bacterial proteins were broken down. It was not clear if carbohydates such as cellulose and lignin were broken down during the test.

In anaerobic digestion, the hydrolysis of particulates to soluble substrates is generally slow and incomplete. It is optimal at pH 5.0 to 6.0 for carbohydrates, but at 7.0 for proteins. During the experiment the pH dropped from 6.8 to 6.2 for the set-up at 55℃ and from 6.8 to 5.9 for the set-up at 37°C. Probably, at these pH-values, the hydrolysis of the proteins was more efficient than the hydrolysis of long chain carbohydrates.

Lipids only degrade provided the hydrolysis is directly connected to methanogenesis. When no methanogenesis occurs, no lipids are degraded. Because no quantitative and qualitative gas analyses could be done during the experiment, no information on the composition of the biogas can be given.

In further experiments the optimal process for a complete biodegradation of human faeces mixed with paper will be studied by semi-continuous fed-batch experiments.

5. REFERENCES

Hattingh, W.H.J, Thiel, P.G. & Sievert, M.L. (1967). Determination of protein content of anaerobic digesting sludge. Wat.Res., p. 185 - 189.