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TN 22.2

Batch Co-cultures in Artificial Human Faeces

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DM	Dry Matter
SS	Suspended solids
VSS	Volatile suspended solids
Kj-N	Kjeldahl nitrogen (= organic nitrogen + NH_4^+-N)
NH_4^+-N	Ammonia nitrogen
NO_3^--N	Nitrate nitrogen
NO_2^--N	Nitrite nitrogen
N_{tot}	Total nitrogen (= Kjeldahl-N + (NO_3^- + NO_2^-)-N)
VFA	Volatile fatty acids
COD_{sol}	Soluble chemical oxygen demand
COD_{tot}	Total chemical oxygen demand
TOC	Total organic carbon
2S	Application 2S: see Table 2.4, page 3
3S	Application 3S: see Table 2.4, page 3
$SE^{(n-r)}$	Standard error = error on mean value $\bar{x}$

$$s_{\bar{X}} = \frac{s_X}{\sqrt{n}}$$

n = number of repetitions

1 INTRODUCTION

TN 22.1 reported that no breakdown of artificial human faeces by single cultures of *Clostridium thermocellum*, *Clostridium thermosaccharolyticum*, *Coprothermobacter proteolyticus* and *Coprothermobacter proteolyticus* I8 occurred. Addition of gelatine to the artificial human faeces to increase the organic nitrogen content had no effect.
The degradation of pig manure by these strains was also investigated, but no breakdown was noticed.
Because of the incapacity of axenic cultures to break down the artificial human faeces, a breakdown test of the artificial human faeces was carried out using a co-culture of *Clostridium thermocellum* and *Coprothermobacter proteolyticus* I8 and a co-culture of *Clostridium thermocellum*, *Clostridium thermosaccharolyticum* and *Coprothermobacter proteolyticus* I8.

2 MATERIALS AND METHODS

2.1 Human artificial faeces

The artificial human faeces were produced by the SHIME-reactor as described in TN22.1.
The composition of the artificial human faeces used in the experiment is represented in Table 2.1.
The detailed composition of the volatile fatty acids (VFA) is represented in Table 2.2.

Table 2.1 Composition of the artificial human faeces

Parameter	Mean value mg/l	$SE^{(n-3)}$ mg/l	Mean value mg/g DM	$SE^{(n-3)}$ mg/g DM
DM	19677	438	-	-
SS	600	51	31	3
COD_{tot}	16843	128	855	20
COD_{sol}	15799	89	802	18
Kjeldahl-N	424	4	22	1
NH_4^+-N	244	4	12.4	0.4
NO_3^--N	0	0	0	0
organic N	180	6	9.2	0.4
Protein-content	1279[C]	41	65	3
VFA	3177	131	162	8
pH	7.7	-	-	-

C: calculated, based on protein/protein-nitrogen ratio = 7.1 (TN 22.1)
SE: standard error$^{(n-3)}$

Table 2.2 Composition (mean value ± standard error$^{(n-3)}$) and relative composition of the volatile fatty acids detected in the human faeces

	mg/l	relative
Total VFA	3177 ± 131	100
Acetic acid	1548 ± 71	48.7
Propionic acid	645 ± 29	20.3
Iso-butyric acid	97 ± 5	3.0
Butyric acid	530 ± 19	16.7
Iso-valeric acid	165 ± 6	5.2
Valeric acid	184 ± 3	5.8
Iso-capronic acid	3.8 ± 1.5	0.1
Capronic acid	1.1 ± 0.7	0.03

2.2 Inoculum

Table 2.3 shows the three strains used for the degradation of the artificial human faeces. The strains were grown up in the cultivation media during five days.

Table 2.3 Medium and substrate used to grow up the strains

Strain	Reference	Medium	Substrate	Activity
Clostridium thermocellum	ATCC 27405	MS-medium	Cellobiose (3 g/l)	Proteolytic
Clostridium thermosaccharolyticum	LMG 2811	MS-medium	Cellobiose (3 g/l)	Cellulolytic
Coprothermobacter proteolyticus I8	DRANCO-isolate	MS-medium	Gelatine (3 g/l)	Cellulolytic

MS-medium (see addendum 1)
DRANCO-isolate (Kersters, 1992)

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 incineration of 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-N 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 the sample. The organic content of the sample is subjected to oxidation by potassium dichromate, in strong acid media (sulphuric acid plus silver sulphate) at a temperature of 150° C for two hours. The excess of 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 experiment

Bottles of 250 ml were filled with 120 ml artificial human faeces and flushed with nitrogen gas. 0.8 ml of a 2.5% Na_2S solution was injected to assure anaerobic conditions. The pH was set at 7.5 and the bottles were autoclaved during 20 minutes at 121° C.
Next, cultivated strains were injected in the bottles. Table 2.4 shows the three different applications. Each application was carried out in treble.

Table 2.4 Amount of inoculum (ml) injected in the 250 ml bottles for the different applications

Strains	Application		
	Blank	2S	3S
Clostridium thermocellum	-	5	5
Clostridium thermosaccharolyticum	-	-	5
Coprothermobacter proteolyticus I8	-	5	5
MS-medium (without strain)	15	5	-

Out of the bottles a subsample was taken on several times to analyse the volatile fatty acids and NH_4^+-N produced during the experiment. At the end of the experiment (after 21 days) the Kjeldahl-N, the COD_{tot} and the dry matter (DM) were determined.
The bottles were incubated at 60° C and shaken manually several times per day.

3 RESULTS

3.1 Production of volatile fatty acids

The evolution of the volatile fatty acids concentration during the breakdown experiment is represented in Figure 3.1.
Only at the end of the experiment (after 21 days), a significant production (0.10% significance level) of VFA compared to the blank was noticed by the applications 2S and 3S. At a 0.05% significance level, the VFA production was not significant.
Figure 3.2 shows the relative composition of the volatile fatty acids at the beginning and at the end of the test. The application 3S had a significant higher percentage (0.05% significance level) of acetic acid compared to the blank at the beginning and at the end of the test. The relative composition of the VFA of application 2S was not different compared to the blanks.

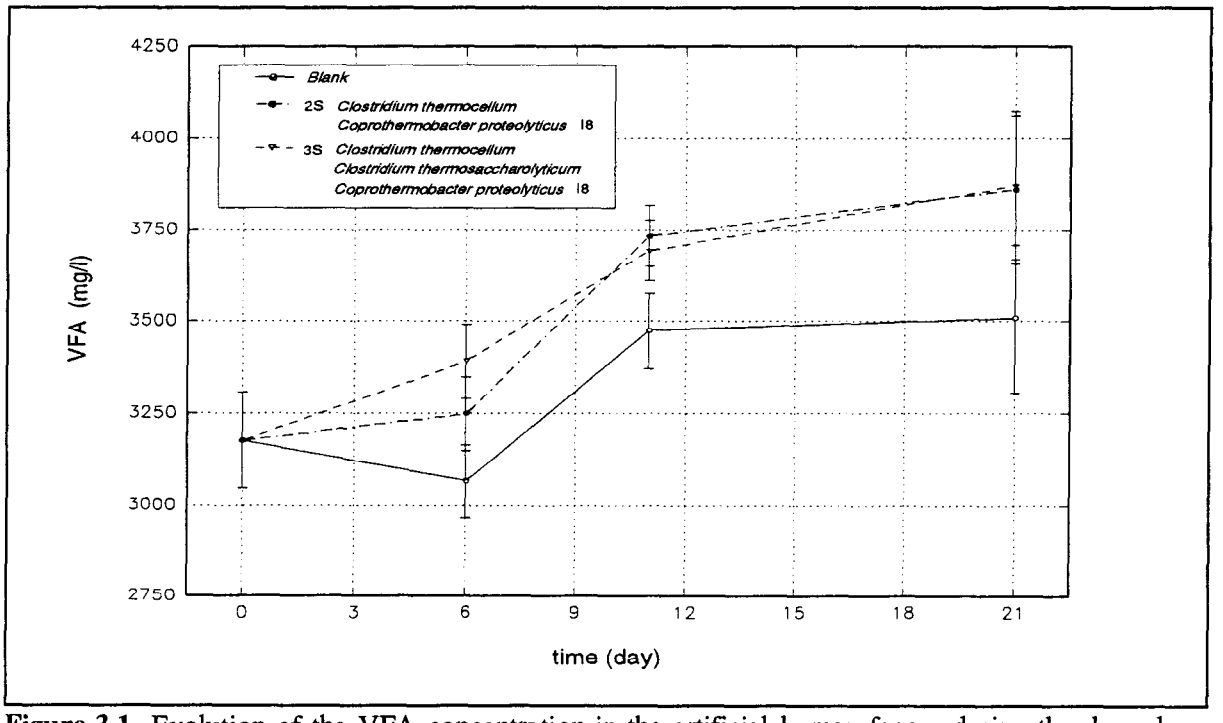

Figure 3.1 Evolution of the VFA concentration in the artificial human faeces during the degradation test (error bars: $SE^{(n-3)}$)

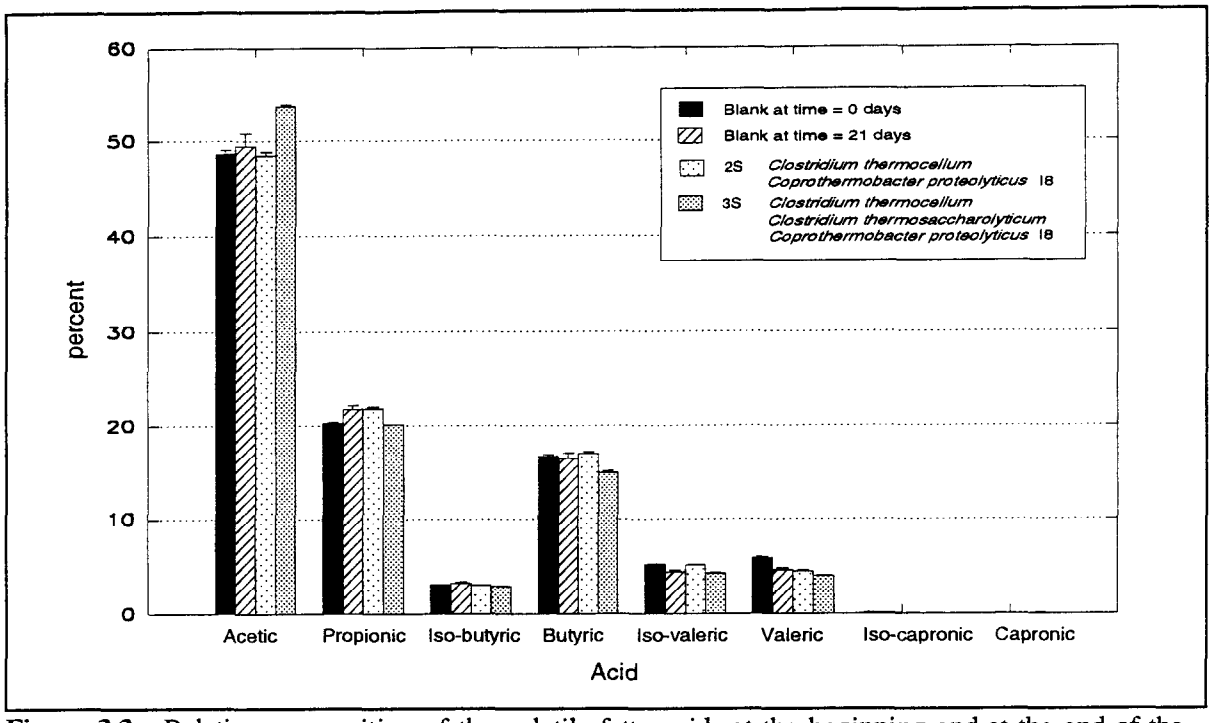

Figure 3.2 Relative composition of the volatile fatty acids at the beginning and at the end of the degradation test (error bars: $SE^{(n-3)}$)

3.2 Production of ammonia

At the end of the test a significant ammonia production compared to the blank was noticed for application 3S at a significance level of 0.05% and for application 2S at a significance level of 0.1%.

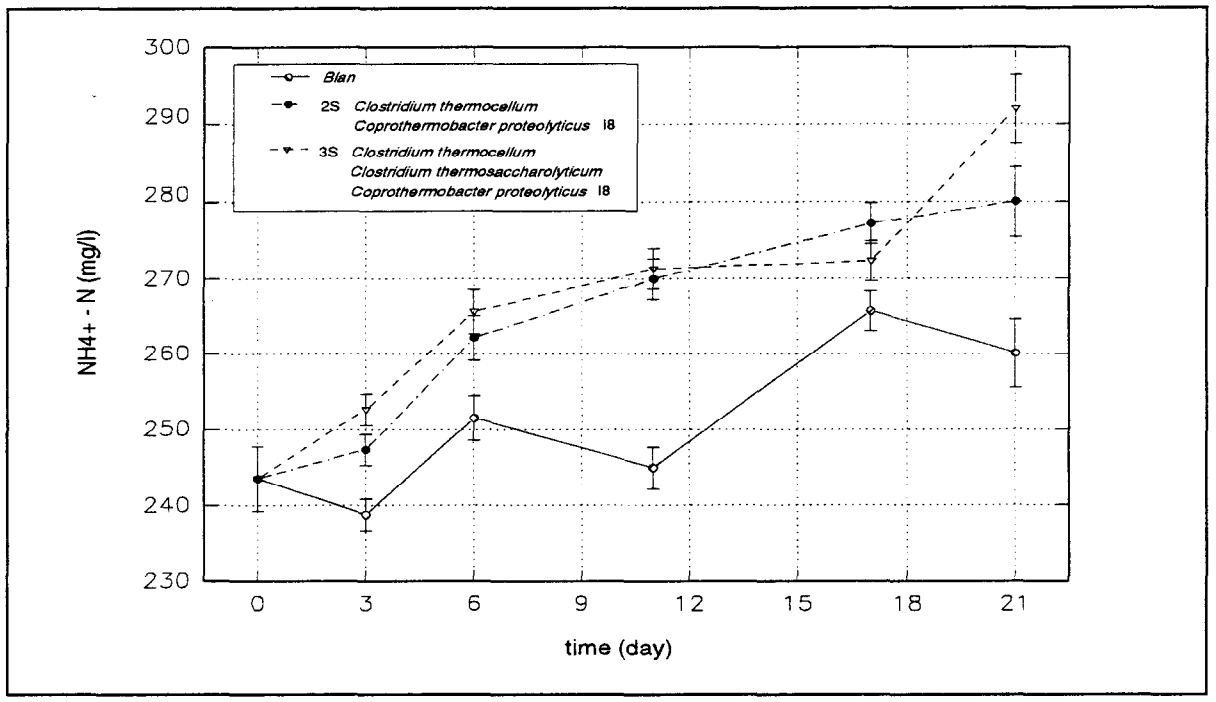

Figure 3.3 Evolution of the ammonia concentration during the degradation test of the artificial human faeces (error bars: $SE^{(n-3)}$)

3.3 Evolution of the measured parameters

In Table 3.1 the values of the measured parameters at the beginning of the test are compared to the parameters measured at the end of the test.
The suspended solids (SS) did not change significantly during the test. This indicates that no liquefaction of macro-molecular material occurred. Because of the fact that also the values of the dry matter (DM), the total COD (COD_{tot}) and the Kjeldahl-nitrogen did not change significantly, it can be concluded that no organic material was converted to CO_2 during the test.

Table 3.1 Values (± standard error$^{(n-3)}$) of the measured parameters at the beginning and at the end of the biodegradation test of the artificial human faeces

	pH	DM	SS	COD_{tot}	Kj-N	NH_4^+-N	VFA
		g/l	mg/l	mg/l	mg/l	mg/l	mg/l
t = O days							
	7.5	19.05±0.43	600±51	16843±128	423.7±4.1	243.5±4.2	3177±131
t = 21 days							
Blank	7.5	20.1±1.5	580±121	14415	448.7	260±5	3508±202
2S	7.6	19.8±1.5	528±148	16245	487.5	280±5	3860±202
3S	7.5	19.7±1.5	567±148	16482	478.5	292±5	3872±202

4 CONCLUSIONS

The result of the degradation test indicates that the breakdown of artificial human faeces by a mixed culture of *Clostridium thermocellum* and *Coprothermobacter proteolyticus* I8 and a co-culture of *Clostridium thermocellum*, *Clostridium thermosaccharolyticum* and *Coprothermobacter proteolyticus* I8 did not result in a significant production of volatile fatty acids, ammonia and gases. This can be due to the composition of the artificial human faeces which contain not enough ready biodegradable components anymore or to the fact that the used co-cultures are not capable to breakdown artificial human faeces.

References

HATTINGH, W.H.J., THIEL, P.G. & SIEVERT, M.L. (1967). Determination of protein content of anaerobic digesting sludge. Wat. Res., 185-189.

KERSTERS, I (1992). Melissa TN 15.3.ESA/YCL Contract 8152/NL/FG.

Addendum 1: Composition of MS-medium

NaOH		4 g/l
yeast extract		2 g/l
trypticase pepton		2 g/l
resazurin solution (0.2%)		0.5 ml/l
coenzyme M		0.5 g/l
Solution A		10 ml/l
NH_4Cl	100 g/l	
$MgCl_2.6H_2O$	100 g/l	
$CaCl_2.2H_2O$	40 g/l	
pH	4	
Solution B		2ml/l
$K_2HPO_4.3H_2O$	200 g/l	
Mineral solution		10ml/l
$Na_2EDTA.2H_2O$	500 mg/l	
$CoCl_2.6H_2O$	150 mg/l	
$MnCl_2.4H_2O$	100 mg/l	
$FeSO_4.7H_2O$	100 mg/l	
$ZnCl_2$	100 mg/l	
$AlCl_3.6H_2O$	40 mg/l	
$Na_2Mo_4.2H_2O$	30 mg/l	
$CuCl_2.2H_2O$	20 mg/l	
$NiSO_4.6H_2O$	20 mg/l	
H_2SeO_3	10 mg/l	
H_3BO_3	10 mg/l	
$NaMoO_4.2H_2O$	10 mg/l	
Na_2S (2.5%)		5 ml/l
final pH		7