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Axenic Batch Cultures in Artificial Human Faeces and Pig Manure

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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
$SE^{(n-r)}$	Standard error = error on mean value $\bar{x}$

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

n = number of repetitions

1 INTRODUCTION

The results of the breakdown of rat faeces by the strain *Coprothermobacter proteolyticus* I8 has been published in TN 15.4. It was proved that only 3 to 5 % of the total added carbon was converted to fatty acids and only 15% of the Kjeldahl-N was converted to NH_4^+-N.
TN 15.5 also stated that there was a problem with the availability of the substrate. Rat faeces are not very similar to human faeces and in order to obtain more realistic results, research has been done on the breakdown of synthetic human faeces by the strains *Coprothermobacter proteolyticus* I8, *Coprothermobacter proteolyticus, Clostridium thermocellum* and *Clostridium thermosaccharolyticum.*

2 MATERIALS AND METHODS

2.1 Human artificial faeces

The artificial human faeces used in the experiment were produced by the SHIME (Simulated Human Intestinal Microbial Ecosystem) - reactor (MOLLY et al., 1993).
The SHIME-reactor is a five-stage reactor developed to simulate the gastro-intestinal microbial ecosystem of humans. The small intestine is simulated by a two-step "fill and draw" system, the large intestine by a three-step reactor (see Figure 2.1).

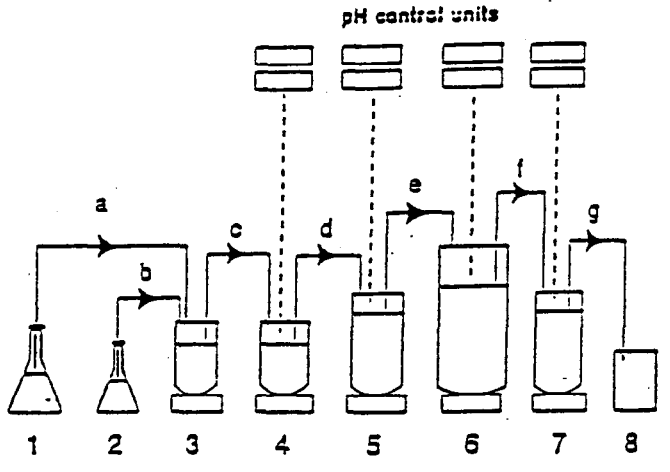

Figure 2.1 Set-up of the simulated human intestinal microbial ecosystem reactor: 1, feed; 2, pancreas acetone powder; 3, reactor 1 (duodenum and jejenum); 4, reactor 2 (ileum); 5, reactor 3 (caecum and ascending colon); 6, reactor 4 (transverse colon); 7, reactor 5 (descending colon); 8, effluent. Pumps a-d were operated semi-continuously; pumps e-g were operated continuously

The SHIME reactor was inoculated by adding 50 ml of a 20% faecal suspension to vessels 3,4 and 5. The slurry was prepared in an anaerobic sodium phosphate buffer (0.1 M, pH 7.0). Vessels 1 and 2 were inoculated over eight successive days with 10 ml of the supernatant of a human

western diet suspension. The diet contained 15% protein, 20% fat and 45% carbohydrate. The suspension was made by weighing 20 g of an average meal in 80 ml physiological solution and shaking for 1 h.

2.2 Pig manure

Pig manure used in the second degradation test was obtained from a pig farm.

2.3 Inoculum

Table 2.1 shows the four strains used for the degradation of artificial human faeces. The strains were grown up in the cultivation media during five days.

Table 2.1 Medium and substrate used to grow up the strains

Strain	Reference	Medium	Substrate
Clostridium thermocellum	ATCC 27405	MS-medium	Cellobiose (3 g/l)
Clostridium thermosaccharolyticum	LMG 2811	MS-medium	Cellobiose (3 g/l)
Coprothermobacter proteolyticus	ATCC 35245	MS-medium	Gelatine (3 g/l)
Coprothermobacter proteolyticus I8	DRANCO-isolate	MS-medium	Gelatine (3 g/l)

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

2.4 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 ionisation 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 oxydation 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 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.
Total organic carbon (TOC) was determined by measuring the CO_2 - production due to combustion of the sample.

2.5 Description of the experiment

Before the degradation tests were started, the artificial human faeces and the pig manure were Characterised.

In a first degradation test, the breakdown of the faeces by axenic cultures of *Clostridium thermocellum, Clostridium thermosaccharolyticum, Coprothermobacter proteolyticus* and *Coprothermobacter proteolyticus* I8 was examined.
Bottles of 250 ml were filled with 100 ml artificial human faeces and flushed with nitrogen gas. 0.8 ml of a 2.5% Na_2S solution was added to assure anaerobic conditions. The pH was set at 7.5 and the bottles were autoclaved during 20 minutes at 121°C. For each strain, four bottles were inoculated with 10 ml inoculum. In the four blank bottles 10 ml of autoclaved distilled water was added. Out of three bottles a subsample was taken on regularly times to analyse the concentration of VFA and NH_4^+-N in order to follow the breakdown of the artificial human faeces. At the end of the experiment the Kjehdahl-N, COD_{tot}, DM and SS were also determined on a mixed sample. In the fourth bottle the pressure evolution was measured.
The bottles were incubated at 60°C. It wasn't possible to shake the bottles continuously, but they were shaken manually several times per day.

After 5 days no production of VFA and NH_4^+-N was noticed. A 10 ml gelatine solution, containing 3g nitrogen/l, was added to the fourth bottle of each strain to investigate if this would stimulate the breakdown of the faeces.

Because of the negative results obtained during the degradation test of artificial human faeces, in a second experiment pig manure was used as substrate.
Bottles with medium were prepared as in the previous test. For each strain, one bottle was inoculated with 10 ml inoculum.

3 RESULTS

3.1 Composition of the artificial human faeces

The composition of the artificial human faeces used in the degradation experiment is represented in Table 3.1.

The dry matter content of the artificial human faeces was rather low, about 14 g/l.

About 50% of the dry matter was organic matter. Seventy per cent of the organic matter was organic carbon and it appeared that a third of the organic carbon was present as volatile fatty acids. Acetic acid, propionic acid and butyric acid were the major fatty acids present in the artificial human faeces (see Table 3.2).

By comparing the COD_{sol} to the COD_{tot} , it can be concluded that most of the organic matter present in the artificial human faeces was soluble.

The nitrogen content of the artificial human faeces was low. 75% of the total nitrogen was ammonia nitrogen. Only 64 mg proteins per gram dry matter were found. Based on the measured values, it was possible to calculate following ratios:

- protein/protein nitrogen - ratio : 7.1
- C/N_{tot}-ratio : 10
- COD_{tot}/N_{tot} - ratio :100/2.7

Table 3.1 Composition of the artificial human faeces

Parameter	Mean value mg/l	Standard error$^{(n=3)}$ mg/l	Mean value mg/g DM	Standard error$^{(n=3)}$ mg/g DM
Dry Matter	14393	68	-	-
Organic Matter	7533	113	523	73
Ash	6860	91	476	7
SS	1046	12	73	1
VSS	966	20	67	1
COD_{tot}	18761	47	1303	7
COD_{sol}	17509	291	1216	21
TOC	5145	-	357	-
Kjeldahl-N	523	28	36	2
NH_4^+-N	394	7	27	1
NO_3^--N	0	0	0	0
Organic-N	129	29	9	2
Protein content	921	142	64	10
VFA	3563	42	248	3
	1673*	10	116	1
pH	7.8**	0.1	-	-

* expressed in mg C/l

** no unit

Table 3.2 Composition of the volatile fatty acids detected in the artificial human faeces

Volatile fatty acids	Concentration		
	mg acid/l	mg acid-C/l	relative (% on acid basis)
Total VFA	3562	1679	100
Acetic acid	1654	661	46.4
Propionic acid	727	353	20.4
Iso-butyric acid	89	48	2.5
Butyric acid	658	359	18.5
Iso-valeric acid	167	98	4.7
Valeric acid	169	99	4.8
Capronic acid	98	61	2.8

3.2 Composition of the pig manure

Table 3.3 presents the composition of pig manure used in the second degradation test.
The pig manure had a dry matter content of about 35 g/l. 54% of the dry matter was organic material. Only a quarter of the organic matter was soluble.
The nitrogen content was low. More than two third of the total nitrogen was present as ammonia. The protein-content was not determined, but based on the value of the organic nitrogen it could be calculated that about 25 x 6.25 = 156 mg protein per gram dry matter was present.
The main fatty acid present was acetic acid (see Table 3.4).

Table 3.3 Composition of the pig manure

Parameter	Mean value	Standard error$^{(n-3)}$	Mean value	Standard error$^{(n-3)}$
	mg/l	mg/l	mg/g DM	mg/g DM
Dry matter	35402	1539	-	-
Organic matter	19294	1646	545	52
Ash	16108	586	455	27
SS	24647	2696	696	82
VSS	15564	1832	440	55
COD_{tot}	24359	1643	688	55
COD_{sol}	6401	865	181	26
TOC	ND	-	-	-
Kjeldahl-N	3202	246	90	8
NH_4^+-N	2316	104	65	4
NO_3^--N	0	0	-	-
Organic N	882	321	25	9
Protein-content	5512[c]	-	156[c]	-
VFA	1117	110	32	3
pH**	7.9	-	-	-

c calculated

** no unit

ND not determined

Table 3.4 Composition of the volatile fatty acids detected in pig manure

Volatile acid	Concentration	
	mg acid/l	relative(%)
Total VFA	1117 ± 110	100
Acetic acid	877 ± 100	78.6
Propionic acid	92 ± 9	8.3
Iso-butyric acid	37 ± 17	3.4
Butyric acid	51 ± 12	4.6
Iso-valeric acid	51 ± 18	4.6
Valeric acid	7 ± 2	1.1
Capronic acid	-	-

3.3 Degradation of the artificial human faeces

During the degradation test the concentration of the fatty acids and ammonia was measured.
The evolution of the volatile fatty acids concentration in the artificial human faeces is represented in Figure 3.1.
The results of the analysis of variance on the fatty acid concentration are shown in Table 3.5. It can be concluded that there was no significant increase of the fatty acid concentration during the experiment.

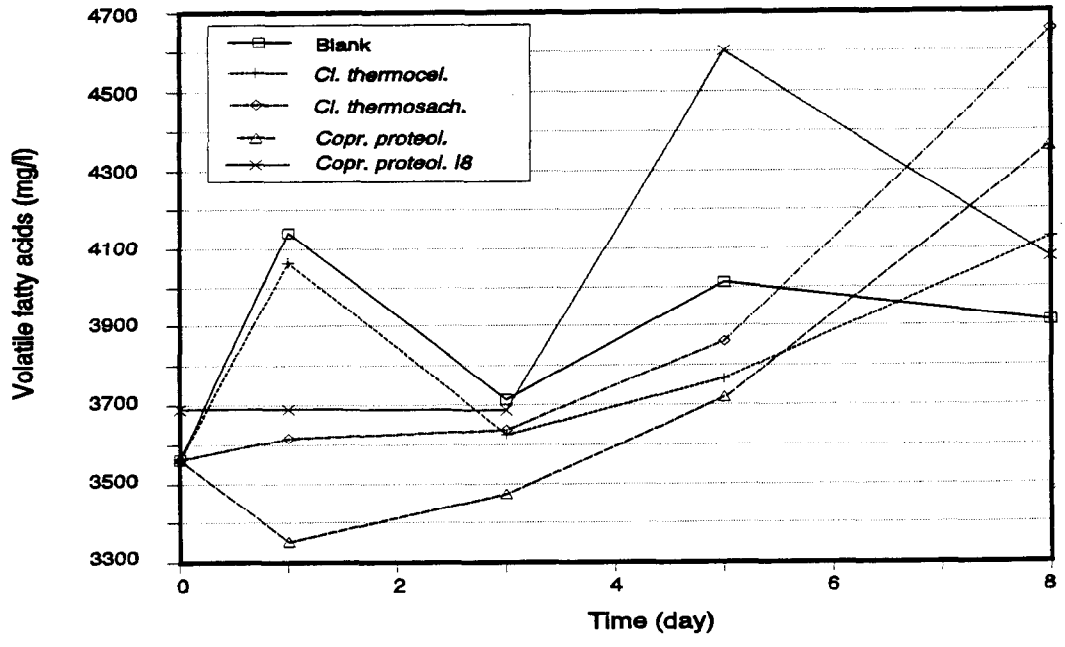

Figure 3.1 Evolution of the fatty acids concentration during the breakdown of the artificial human faeces

Table 3.5 , Evolution of the VFA concentration (mg/l) during the breakdown of the artificial human faeces

Strain	Time (day)				
	0	1	3	5	8
Blank	3563[a]	4142[c]	3713[a]	4013[ab]	3911[a]
Cl. thermocel.	3563[a]	4065[bc]	3627[a]	3765[a]	4128[a]
Cl. thermosach	3563[a]	3617[a]	3638[a]	3860[a]	4654[a]
Copr. Prot.	3563[a]	3352[a]	3475[a]	3718[a]	4467[a]
Copr. Prot. I8	3563[a]	3689[ab]	3687[a]	4601[b]	4076[a]
pooled standard error	42	130	250	220	680

Letters: indication of significant differences (0.05 significance level) between the different strains on a certain time.

Figure 3.2 shows the evolution of the ammonia nitrogen concentration in the artificial human faeces. Analyses of variance (see Table 3.6) showed that no significant (95% significance level) amount of ammonia was produced during the experiment.

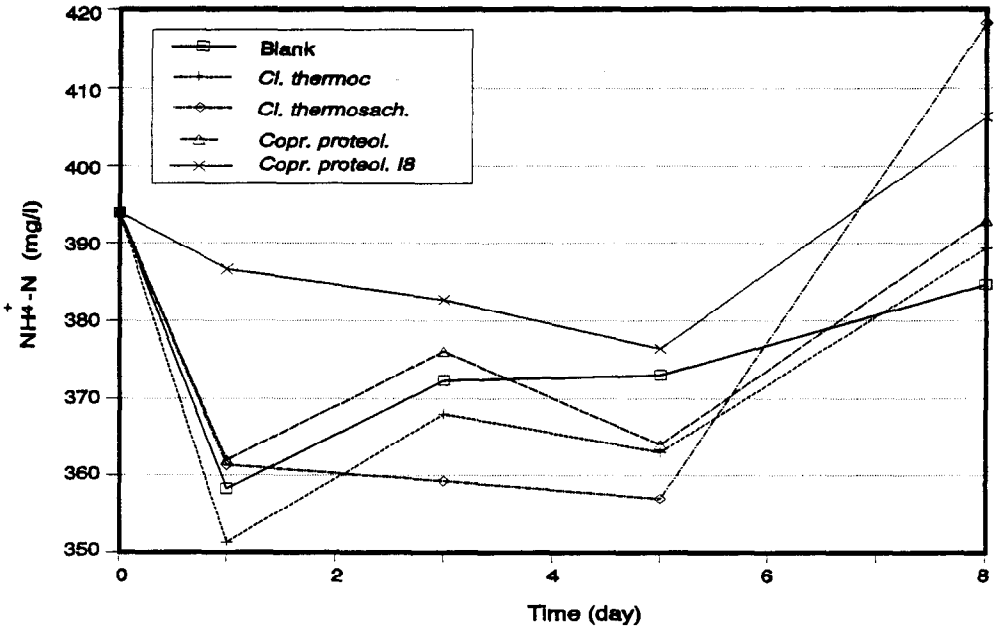

Figure 3.2 Evolution of the concentration of NH_4^+-N during the breakdown of the artificial human faeces

Table 3.6 Evolution of the ammonia nitrogen concentration (mg/l) during the breakdown of the artificial human faeces

Strain	Time (day)				
	0	1	3	5	8
Blank	394[a]	358[a]	372[ab]	373[a]	384[a]
Cl. thermocel.	394[a]	351[a]	368[ab]	363[a]	389[a]
Cl. thermosach.	394[a]	358[a]	359[a]	357[a]	418[a]
Copr. Prot.	394[a]	362[a]	376[ab]	364[a]	393[a]
Copr. Prot I8	394[a]	386[b]	382[b]	376[a]	406[a]
pooled standard error	7	7	7	11	15

Letters: indication of significant differences (0.05 significance level) between the different strains on a certain time.

The values of the dry matter content, the suspended solids, the total chemical oxygen demand and the Kjeldahl nitrogen didn't change during the experiment (see Table 3.7).

Table 3.7 Values of the measured parameters at the beginning and at the end of the biodegradation test of the artificial human faeces

	pH	DM g/l	SS g/l	COD_{tot} mg/l	Kj-N mg/l
t = 0 days					
	7.5	14.39	1.04	18761	523
t = 8 days					
Blank	7.6	14.51	1.07	18740	521
Cl. thermocel.	7.7	14.55	1.01	18732	556
Cl. thermosach.	7.7	14.42	1.06	18770	566
Copr. prot.	7.7	14.49	1.03	18782	573
Copr. prot. I8	7.6	14.36	1.05	19783	569

3.4 Effect of the addition of gelatine

In the different applications, addition of gelatine had no effect on the production of ammonia and VFA (see Table 3.8). The concentration of suspended solids didn't change. This indicates that no liquefaction of insoluble material occurred.

The concentration of the dry matter, COD_{tot} and Kjeldahl nitrogen remained constant. This implies that no gasses were formed.

Addition of extra proteins did not stimulate the breakdown of the artificial human faeces.

Table 3.8 Values of the measured parameters at the beginning and at the end of the biodegradation test of the artificial human faeces enriched with gelatine

	pH	DM	SS	COD_{tot}	Kj-N	NH_4^+-N	VFA
		g/l	g/l	mg/l	mg/l	mg/l	mg/l
t = 5 days							
	7.7	15.42	1.09	19153	850	363	3542
t = 12 days							
Blank	7.8	15.63	1.14	19172	825	349	3540
Cl. thermocel.	7.7	15.60	1.11	19326	866	360	3189
Cl. thermosach.	7.8	15.47	1.13	18980	850	348	3166
Copr. prot.	7.7	15.45	1.43	19288	881	356	3292
Copr. prot. I8	7.8	15.09	1.08	19018	874	378	3513

3.5 Degradation of pig manure

In none of the applications a significant change in ammonia concentration was noticed. The volatile fatty acid production was only significant at a 0.05 significance level in the bottle inoculated with the strain *Clostridium thermocellum.*
The change of the other measured parameters was not different at a 0.10 significance level.
Based on the values of the measured parameters it couldn't be concluded that breakdown of pig manure occurred.

Table 3.9 Values of the measured parameters at the beginning and at the end of the biodegradation test of pig manure

	pH	DM	COD_{tot}	Kj-N	NH_4^+-N	VFA
		g/l	g/l	mg/l	mg/l	mg/l
t = O days						
	7.5	35.4±1.5	24.3±1.6	3202±346	2316±104	1117±110
t = 14 days						
Blank	7.6	34.4±1.1	21.9	3318	2380±12	1234
Cl. thermocellum	7.5	34.3±1.8	24.2	3188	2296± 7	1596
Cl. thermosaccharolyticum	7.5	32.8±1.1	21.4	3367	2353±16	1290
Copr. proteolyticus	7.6	33.1±1.2	23.5	3365	2433±10	1249
Copr. proteolyticus I8	7.6	31.7±2.6	25.1	3348	2361±15	1190

4 DISCUSSION

Neither the artificial human faeces, nor the pig manure were broken down by axenic cultures of *Coprothermobacter proteolyticus* I8, *Coprothermobacter proteolyticus*, *Clostridium thermocellum* and *Clostridium thermosaccharolyticum*.
In order to find an explanation, the composition of the artificial human faeces and the pig manure used in the experiments were compared to literature data with regards to the composition of human faeces (see Table 4.1).

Table 4.1 Comparison of the composition of artificial human faeces and the pig manure used in the experiments to literature data on the composition of human faeces

Parameter		Human faeces*	Artificial human faeces**	Pig manure***
Dry matter	%	13 - 23	1.4	3.5
Ash	mg/g DM	133 - 266	476	455
NH_4^+-N	mg/g DM	1 - 4	27	65
Kjeldahl-N	mg/g DM	33 - 106	36	90
Organic N	mg/g DM	32 - 102	9	25
Protein content	mg/g DM	177 - 222	63	156
VFA	mg/g DM	11- 108	247	32

* calculated values based on literature (Passmore 1973; Wollager et al. 1947; Hawk et al. 1947)
** produced by the SHIME-reactor
*** obtained from a pig farmery

Compared to human faeces, the artificial human faeces contained about ten times more ammonia, expressed on dry matter. On the other hand, the protein content, expressed on dry matter, of the artificial human faeces was three to four times lower than the protein content of human faeces (see Table 4.1). This can be explained by the fact that proteins were probably already more digested in the SHIME-reactor compared to the digestion in the human intestinal tract.
The absence of production of ammonia during the breakdown experiment, especially in the bottles inoculated with *Coprothermobacter proteolyticus* and *Coprothermobacter proteolyticus* I8, can possibly be explained by the lack of available organic nitrogen.
The VFA content of the artificial human faeces was much higher compared with the content present in human faeces. One thirth of the total carbon present in the artificial faeces, was volatile fatty acid carbon. This is an indication that the conversion of carbohydrates in the SHIME-reactor compares favorably with the conversion in the human intestinal tract.
Consequently, the human artificial faeces probably didn't contain enough easy biodegradable substances to encourage the growth of the inoculated strains.
Although, addition of gelatine at the artificial human faeces did not stimulate the breakdown of the artificial faeces.

Pig manure was used as a substrate for the second experiment. The organic nitrogen content, expressed on dry matter, of pig manure was two an a half times higher than the nitrogen content in artificial human faeces. Compared to human faeces, the organic nitrogen present in the pig manure was still low (see Table 4.1).

The concentration of organic nitrogen (mg N/l) in pig manure was seven times higher than the concentration of nitrogen present in the artificial human faeces (see Table 3.2 and Table 3.3). Although, no production of ammonia was noticed by one of the four strains.
The dry matter content of the pig faeces was two and a half times higher than the dry matter content of the human artificial species (see Table 3.2 and Table 3.3). Probably the pig faeces contained more fibrous material than the artificial human faeces, but only in the bottle inoculated with *Clostridium thermocellum* a slight production of fatty acids was noticed.

Faecal material is a product of anaerobic digestion of organic matter by a diverse microbial community. In order to have a further breakdown of the faeces, probably consortia of different bacteria will be needed.
It is important to investigate if a co-culture of the strains *Coprothermobacter proteolyticus* I8, *Coprothermobacter proteolyticus*, *Clostridium thermocellum* and *Clostridium thermosaccharolyticum*, will be able to breakdown the artificial human faeces.
Also the effect of the addition of supplementary nitrogen sources on the breakdown of the artificial human faeces has to be examined profoundly.

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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