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Characterisation of I8: determination of end products

TN 20.3

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1 INTRODUCTION

In the previous technical note (TN20.2) five proteins and ten carbohydrates were tested as substrate for Thermobacteroides proteolyticus I8. Note that this genus was recently renamed as Coprothermobacter proteolyticus (Rainey & Stackebrandt, 1993). In this technical note some more information is given about the products formed during fermentation of those proteins and carbohydrates.

2 MATERIALS AND METHODS

Inoculum

Coprothermobacter proteolyticus was grown under anaerobic conditions (T = 60 °C) in a medium with gelatin as substrate. The composition of the medium is presented in Table 1.
In the test the volume of the inoculum was 5 % of the cultivation volume.

Analytical techniques

Volatile fatty acids were extracted with diethylether from acidified samples and determined by gas chromatography using an flame ionisation detector coupled to a glass column containing chromosorb 101.
The NH_4^+-N-content was determined by steam destillation in a Kjeltec.1002 apparatus under alkaline conditions. Kjeldahl-N was determined similarly after complete destruction of the sample in strong acid.
Lactic acid , ethanol and ureum concentrations were measured using a Boehringer Mannheim test kit.

Description of the experiments

The experiments were set up identically to the one for the determination of growth of Coprothermobacter proteolyticus (TN20.2). Five ml of a culture of the strain was inoculated in 50 ml of medium. Each medium was prepared similarly as the gelatin medium, omitting gelatin and adding different substrates (proteins or carbohydrates ± 5 g/l). The initial pH was set at 7. The incubation period was 15 days (60 ° C). At several intervals the medium was analyzed (volatile fatty acids, lactic acid, ethanol, ureum and ammonium-nitrogen).

Table 1. Composition of the medium used for the cultivation of I8

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		2 ml/l
$K_2HPO_4.3H_2O$		
Mineral solution		10 ml/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	
$Na_2Wo_4.2H_2O$	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 %)		20 ml/l
gelatin (3 %)		100 ml/l
final pH		7

3 RESULTS

1 Fermentation of proteins

No detectable amounts of ethanol and ureum were produced during the fermentation of proteins. The production of lactic acid was low (16-29 mg/l). The production of fatty acids and ammonium-N versus time was presented in TN 20.2. In Table 2 the composition of each medium on day 15 is given.

Table 2. Composition of the protein media after 15 days incubation of Coprothermobacter proteolyticus I8 at 60 °C

	gelatin	BSA	casein	trypton	bacto-pep-ton
NH_4^+-N (mg/l)	672	405	420	454	554
lactic acid (mg/l)	29	16	23	25	23
VFA (mg/l)	1936	1627	1641	1451	1649
acetic acid (mg/l)	1369	826	706	640	1100
(meq/l)	22.8	13.8	11.8	10.7	18.3
propionic acid (mg/l)	119	257	367	199	192
(meq/l)	1.6	3.5	5.0	2.7	2.6
isobutyric acid (mg/l)	124	123	144	172	94
(meq/l)	1.4	1.4	1.6	2.0	1.1
isovaleric acid (mg/l)	324	421	424	440	263
(meq/l)	3.5	4.6	4.6	4.8	2.8

The ammoniumconcentration was higher in the gelatin and the bacto-pepton medium than in the other media, in which the concentration of ammonium-nitrogen was of the same level (400-450 mg/l). This corresponds with the results of TN20.2. The efficiency of breakdown of the proteins, the growth rate and the fatty acids production rate was highest for gelatin and bacto-pepton. Looking at the composition of the fatty acids, acetic acid was the most important end product. In the gelatin medium acetic acid amounted to 71 % of the total amount of fatty acids. In BSA, casein, trypton and bacto-pepton this was respectively 51, 43, 44, 66 %. There seemed to be a correlation between these figures and the initial growth rates on the different substrates. When a protein was degraded and assimilated fast, the fraction of acetic acid in the total amount of fatty acids was higher.

During the fermentation of casein there seemed to be a shift from acetic acid to propionic acid

production.

CO_2 and H_2 were detected in the gasphase (no quantitative results).

Biomass analysis was done on a gelatin-grown culture of Coprothermobacter proteolyticus I8. The biomass consisted of 47.4 % carbon, 6.8 % hydrogen, 12.3 % nitrogen, 21.3 % oxygen, 0.6 % sulfur and 0.8 % phosphor. The C/N ratio of this strain is 3.85, which is lower than the average (C/N = 5) but can be explained by the proteolytic activity of the strain.

2 Fermentation of carbohydrates

In comparison with protein degradation, more fatty acids were produced (higher efficiency). There was also a significant amount of lactic acid detected. Ethanol was produced in small amounts. The production of fatty acids, ethanol and lactic acid is presented in Figure 1-8. In the figures can be seen that during the degradation of most carbohydrates the ethanol production took place during the first three days. After that the concentration in the medium decreased, probably due to volatilization. In Table 3a the composition of the starch, glucose, cellobiose and maltose media after 15 days incubation is presented. For ethanol, the concentration on day 3 is given (maximum). Table 3b gives an overview (cfr. Table 3) of the results in the sucrose, xylose, fructose and mannose media.

Fig. 1 Fermentation of starch by *Coprothermobacter proteolyticus I8*

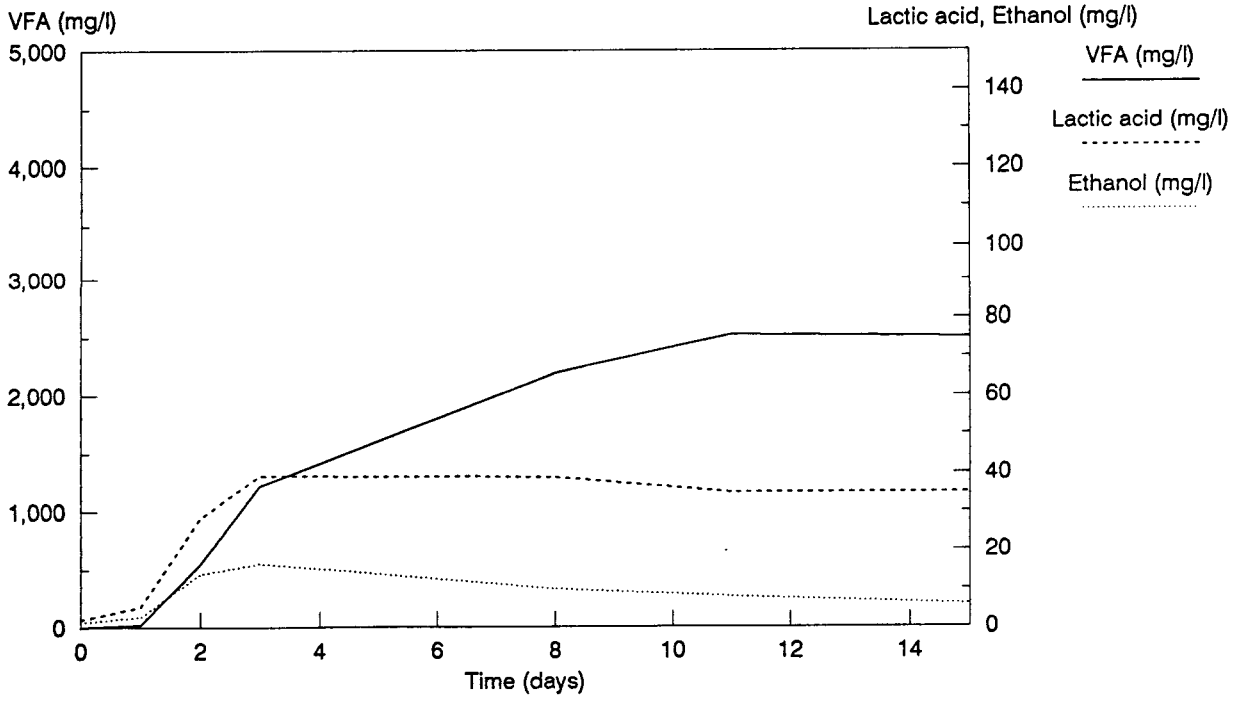

Fig. 2 Fermentation of glucose by ***Coprothermobacter proteolyticus I8***

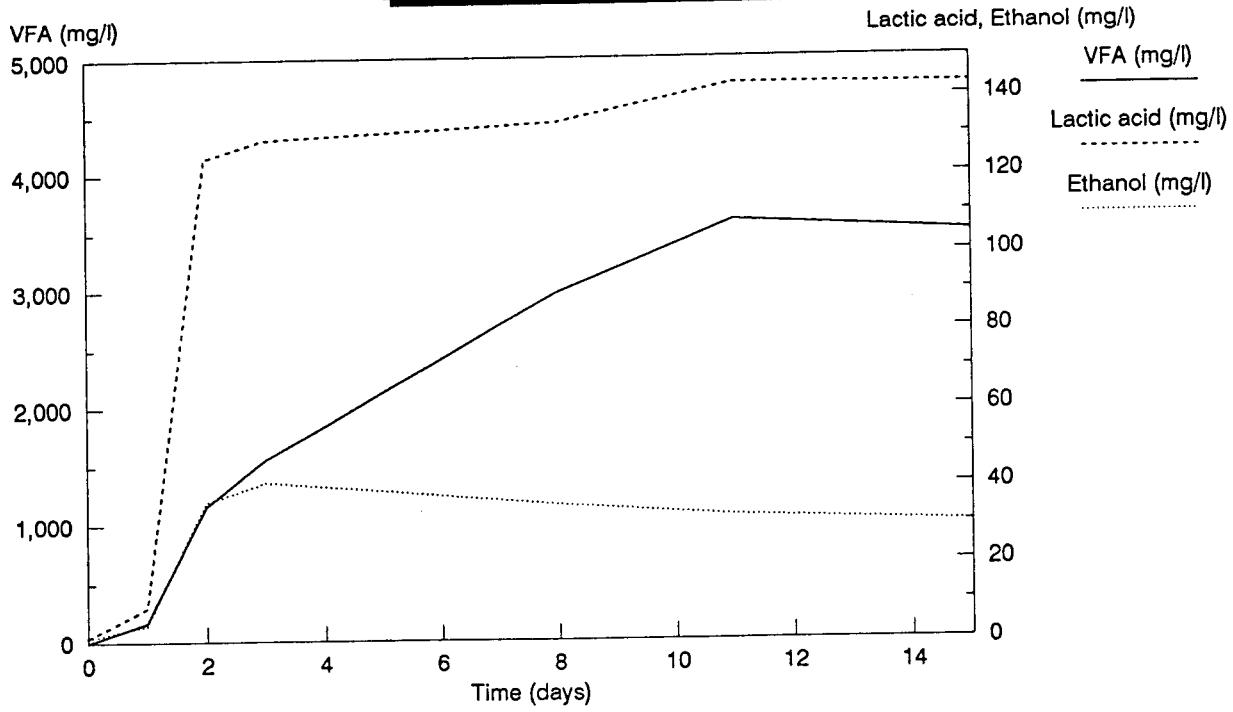

Fig. 3 Fermentation of cellobiose by ***Coprothermobacter proteolyticus I8***

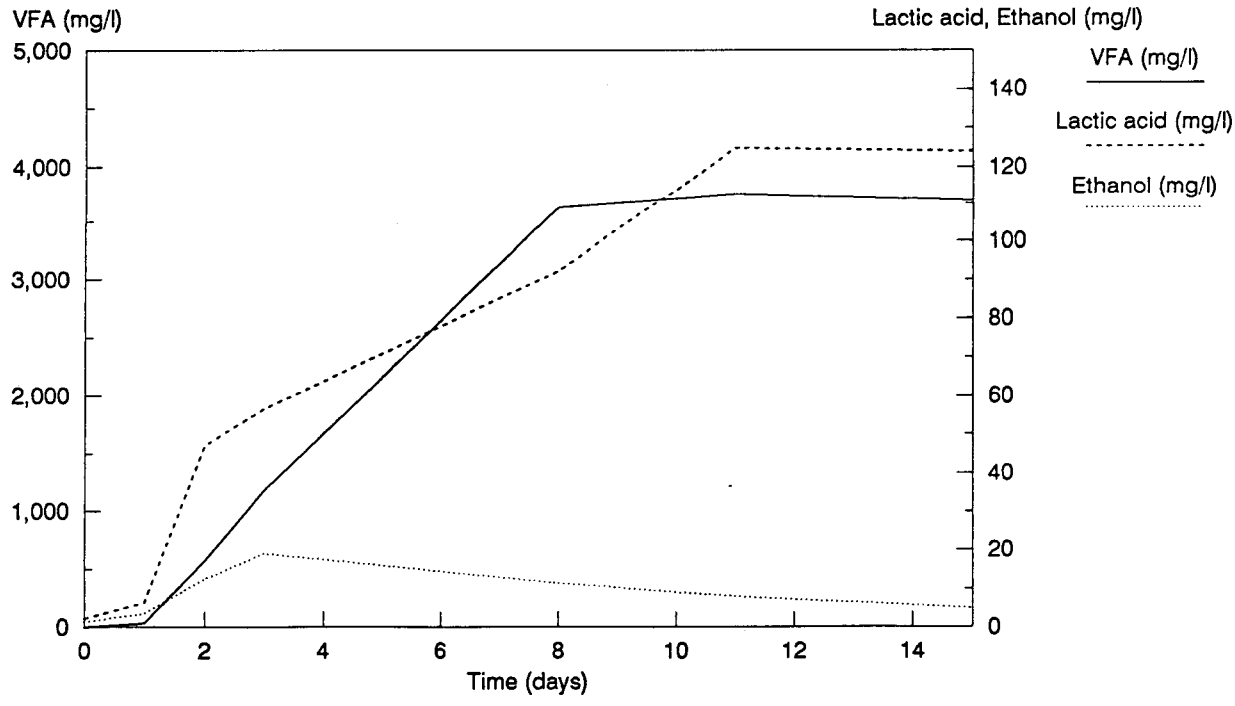

Fig. 4 Fermentation of maltose by
Coprothermobacter proteolyticus I8

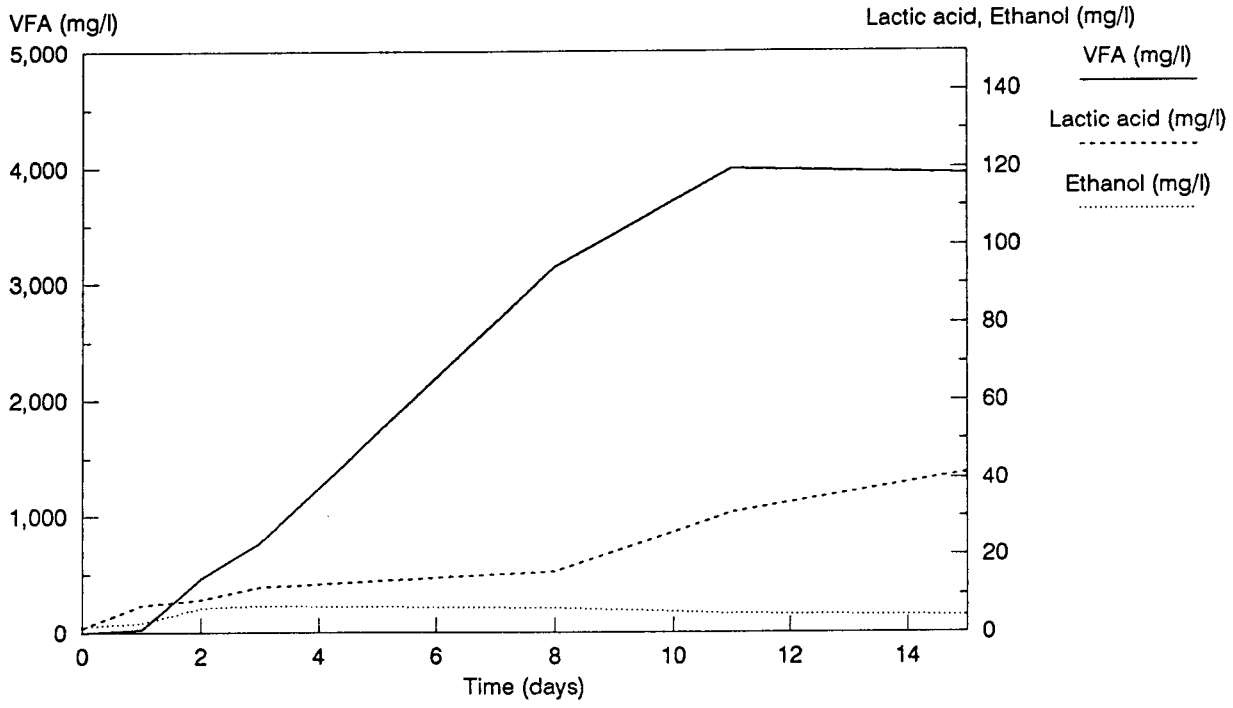

Fig 5 Fermentation of sucrose by
Coprothermobacter proteolyticus I8

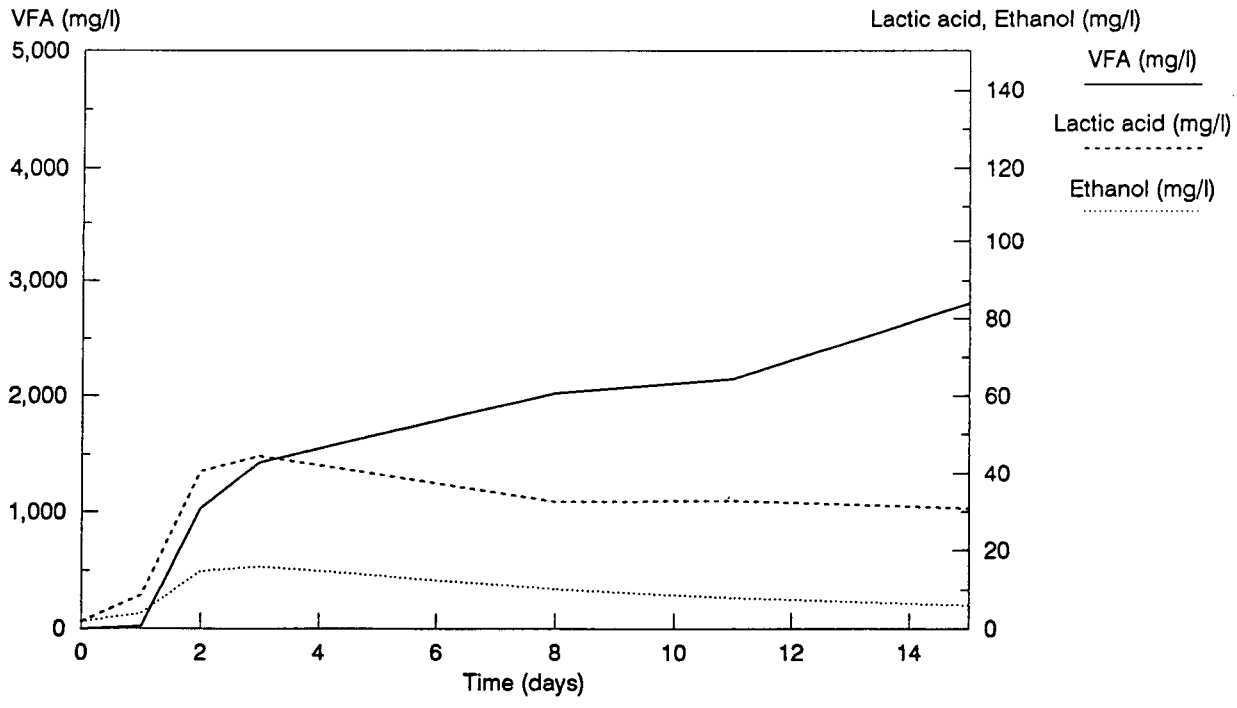

Fig. 6 Fermentation of xylose by
Coprothermobacter proteolyticus I8

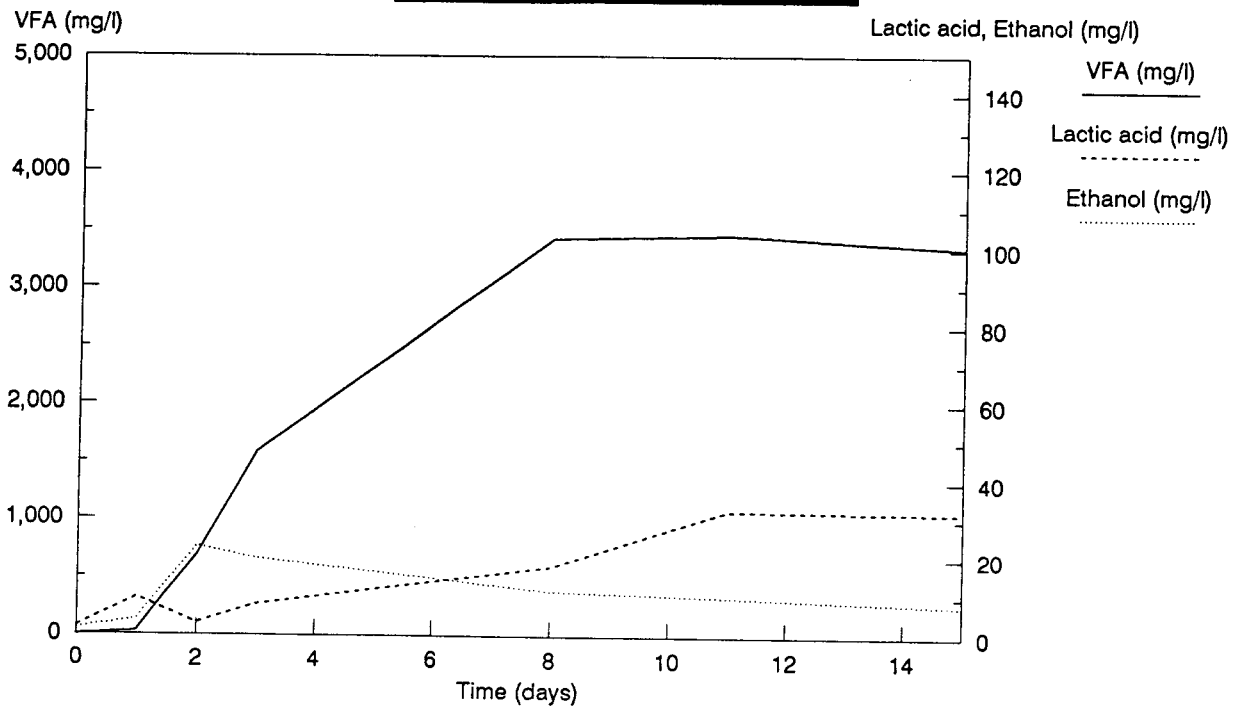

Fig 7 Fermentation of fructose by
Coprothermobacter proteolyticus I8

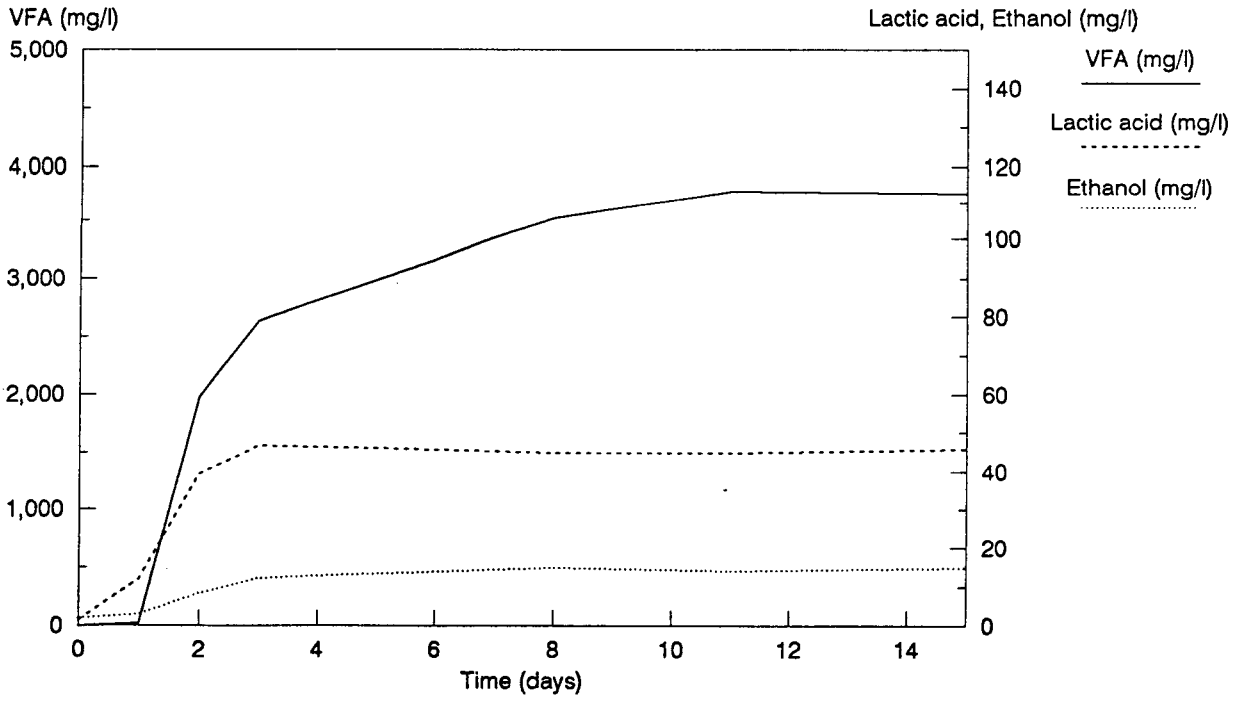

Fig. 8 Fermentation of mannose by ***Coprothermobacter proteolyticus I8***

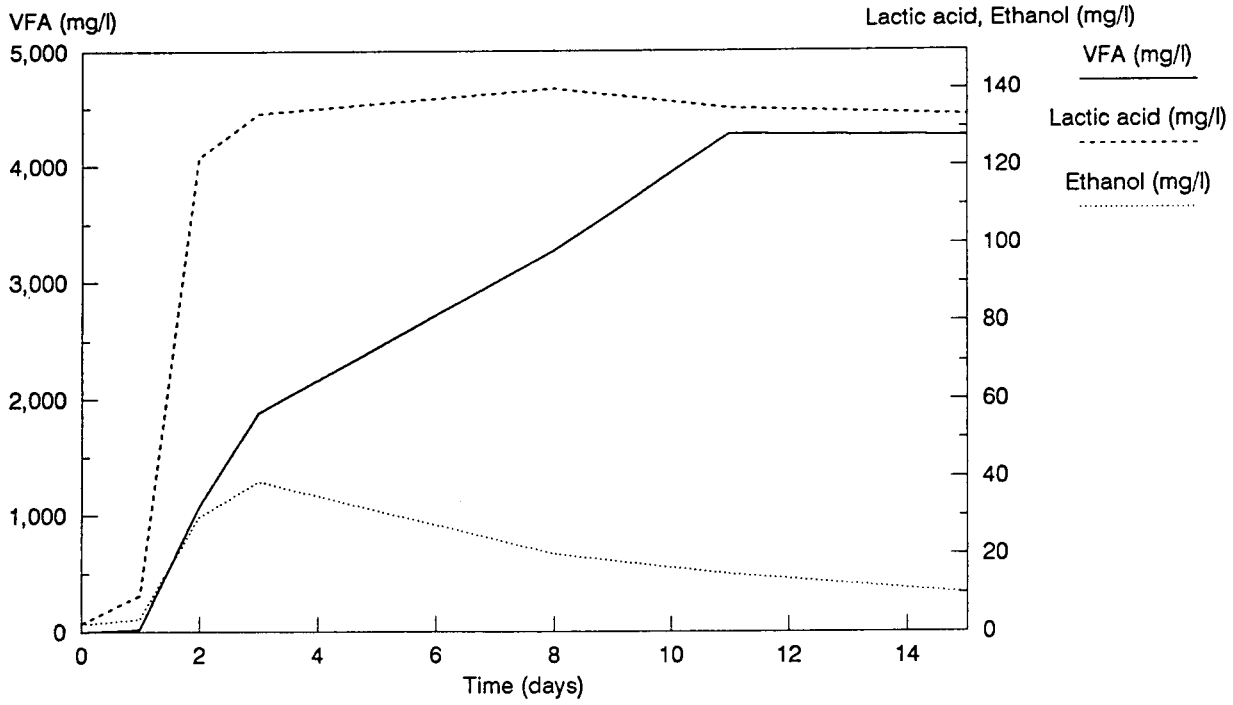

Table 3a Composition of 4 carbohydrate media after 15 days incubation of Coprothermobacter proteolyticus I8 at 60 °C

		starch	glucose	cellobiose	maltose
ethanol	(mg/l)	16	41	19	7
lactic acid	(mg/l)	39	143	124	41
VFA	(mg/l)	2525	3538	3483	3956
acetic acid	(mg/l)	2215	3259	3118	3550
	(meq/l)	36.9	54.3	52.0	59.1
propionic acid	(mg/l)	46	41	71	72
	(meq/l)	0.6	0.6	1.0	1.0
isobutyric acid	(mg/l)	65	53	71	79
	(meq/l)	0.7	0.6	0.8	0.9
butyric acid	(mg/l)	-	-	35	55
	(meq/l)	-	-	0.4	0.6
isovaleric acid	(mg/l)	200	185	188	201
	(meq/l)	1.9	1.8	1.8	2.0

For all carbohydrates acetic acid (AA) was the most important end product. Less than 1 meq/l was produced of propionic acid (PA), isobutyric acid (IBA) and butyric acid (BA), and between 1 and 2 meq/l of isovaleric acid (IVA) was produced. The molar ratio's of acetic acid and the other volatile fatty acids were much higher than the molar ratio's in the protein media. For example looking at the ratio AA/PA, a variation between 52.0 (cellobiose) and 91.9 (mannose) was calculated for the carbohydrate media compared to a variation between 2.4 (casein) and 14.3 (gelatin) in the protein media. The fermentation of cellobiose, maltose, xylose, fructose and mannose led to the production of butyric acid (< 1 meq/l).

From TN20.2 could be learned that the fastest growth occured on glucose, cellobiose, sucrose, fructose and mannose. This could not be linked with the amount of acetic acid produced (Cfr. protein degradation); more acetic acid was produced in maltose medium (growth rate 0.81 d^{-1}) than in cellobiose medium (growth rate 1.2 d^{-1}). The highest amount of lactic acid was produced in glucose, cellobiose and mannose medium.

Table 3b Composition of 4 carbohydrate media after 15 days incubation of Coprothermobacter proteolyticus I8 at 60 °C

		sucrose	xylose	fructose	mannose
ethanol	(mg/l)	16	20	28	39
lactic acid	(mg/l)	44	32	46	133
VFA	(mg/l)	2831	3457	3771	4231
acetic acid	(mg/l)	2503	3068	3429	3861
	(meq/l)	41.7	51.1	57.2	64.3
propionic acid	(mg/l)	60	56	53	51.3
	(meq/l)	0.8	0.8	0.7	0.7
isobutyric acid	(mg/l)	76	75	57	66
	(meq/l)	0.9	0.9	0.7	0.7
butyric acid	(mg/l)	-	62	78	59
	(meq/l)	-	0.7	0.9	0.7
isovaleric acid	(mg/l)	192	196	154	194
	(meq/l)	1.9	1.9	1.5	1.9

4 CONCLUSIONS

Protein degradation leads to the production of acetic acid, propionic acid, isobutyric acid (no butyric acid) and isovaleric acid. There is a production of ammonium-nitrogen. Lactic acid is formed in small amounts.
Carbohydrate degradation leads to the production of acetic acid, propionic acid, isobutyric acid, isovaleric acid and in some cases butyric acid. Ethanol and lactic acid are also formed.
Acetic acid is in all cases the main end product. The relative amount of acetic acid is much higher during carbohydrate degradation.
For the efficiencies of carbohydrate and protein degradation I refer to TN 20.2 (Table 4).

5 REFERENCES

Rainey, F. A. & Stackebrandt, E. (1993). Transfer of the type species of the genus Thermobacteroides to the genus Thermoanaerobacter as Thermoanaerobacter acetoethylicus (Ben-Bassat & Zeikus 1981) com. nov., description of Coprothermobacter gen. nov. and reclassification of Thermobacteroides proteolyticus as Coprothermobacter proteolyticus (Ollevier et al., 1985) comb. nov. Int. J. System. Bacteriol., **43**, p. 857-859.