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A computer-controlled system to simulate conditions of the large intestine with peristaltic mixing, water absorption and absorption of fermentation products

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Abstract This paper introduces a new type of system to simulate conditions in the large intestine. This system combines removal of metabolites and water with peristaltic mixing to obtain and handle physiological concentrations of microorganisms, dry matter and microbial metabolites. The system has been designed to be complementary to the dynamic multi-compartmental system that simulates conditions in the stomach and small intestine described by Minekus et al. [Minekus M, Marteau P, Havenaar R, Huis in't Veld JHJ (1995) ATLA 23:197–209]. High densities of microorganisms, comparable to those found in the colon in vivo, were achieved by absorption of water and dialysis of metabolites through hollow-fibre membranes inside the reactor compartments. The dense chyme was mixed and transported by peristaltic movements. The potential of the system as a tool to study fermentation was demonstrated in experiments with pectin, fructo-oligosaccharide, lactulose and lactitol as substrates. Parameters such as total acid production and short-chain fatty acid (SCFA) patterns were determined with time to characterize the fermentation. The stability of the microflora in the system was tested after inoculation with fresh fecal samples and after inoculation with a microflora that was maintained in a fermenter. Both approaches resulted in total anaerobic bacterial counts higher than 10^{10} colony-forming units/ml with physiological levels of *Bifidobacterium*, *Lactobacillus*, Enterobacteriaceae and *Clostridium*. The dry matter content was approximately 10%, while the total SCFA concentration was maintained at physiological concentrations with similar molar ratios for acetic acid, propionic acid and butyric acid as measured in vivo.

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Introduction

To evaluate the health claims for functional foods and the safety of novel foods, it is important to have systems available that mimic the conditions in the gastrointestinal tract. Systems have been designed to study the digestion of nutrients in the stomach and small intestine (Boisen and Eggum 1991; Savoie 1994). Recently, a multi-compartmental system has been developed that closely simulates the dynamic conditions in the lumen of the stomach and small intestine (Minekus et al. 1995). Culture systems are widely used to study the metabolic and ecological behaviour of colon microflora because experiments in human volunteers and animals are often hard to perform and have drawbacks with respect to ethics and costs (Rumney and Rowland 1992). The microbial cell densities in these systems are generally limited by the fact that water and microbial metabolites, such as short-chain fatty acids (SCFA), are not removed from the fermentation process separately from the microorganisms. In order to absorb water and metabolites, a tubular system has been designed containing hollow-fibre membranes. Furthermore, specific peristaltic mixing was achieved in order to handle physiological cell densities. The large intestinal system can be connected to the gastric/small intestinal system, which makes it possible to evaluate the effect of products or microorganisms on the large intestinal flora after simulated passage through the

stomach and small intestine. The aim of this paper is to introduce this new type of system and to evaluate its use to study ecological aspects and metabolic activity of the microflora under conditions approximating those in the colon. The stability and enzymatic and metabolic activities of the microflora were measured during feeding of a complex standard medium and several types of carbohydrates, and compared to in vivo values.

Materials and methods

The large intestinal system

The large intestinal system consists of units based on the same concept as that developed for the gastric/small intestinal system (Minekus et al. 1995; Minekus and Havenaar 1996, 1998). Four glass units (Fig. 1A), each with a flexible wall inside, were connected to each other. The system was kept at 37 °C by pumping water into the space between the glass jacket and the flexible wall. Peristaltic movements were achieved by changing the pressure on the water. A computer controlled the sequential squeezing of the walls, thus causing a peristaltic wave which forced the chyme to circulate through the loop-shaped system. The tubular shape of the lumen prevented 'obstipation'. The pH was measured with a pH electrode (Fig. 1B) and controlled by the addition of 5 M NaOH (Fig. 1C). The dialysis liquid used to maintain the appropriate electrolyte and metabolyte concentrations was pumped (Fig. 1D) from a bottle (Fig. 1E) through hollow-fibre membranes (molecular-mass cut-off 50,000 Da) positioned in the lumen of the reactor (Fig. 1F). Used dialysis liquid was collected in a waste bag inside the bottle, to obtain a closed system in order to prevent unintended flow of water across the hollow-fibre membrane. The amount of chyme in the reactor was monitored with a pressure sensor (Fig. 1G) and kept at a set level by the absorption of water with a pump (Fig. 1H) in the dialysis circuit. The feeding medium was mixed and kept anaerobic with nitrogen, and was introduced into the reactor with the peristaltic valve system (Fig. 1I) as described previously (Minekus et al. 1995) A peristaltic valve pump was used to remove chyme from the reactor. The system was kept anaerobic by flushing with nitrogen. Gas was allowed to leave through a water lock (Fig. 1J).

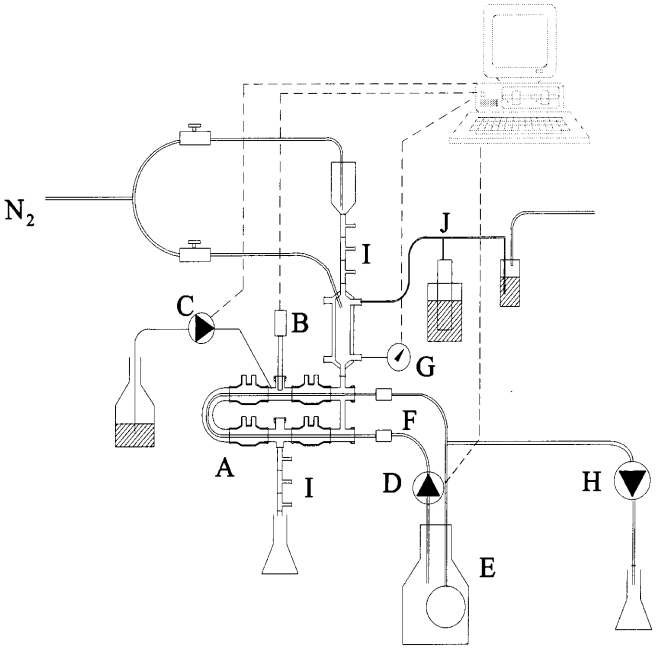

Fig. 1A–J Schematic presentation of the system to simulate conditions in the large intestine. **A** mixing units; **B** pH electrode; **C** alkali pump; **D** dialysis pump; **E** dialysis light; **F** dialysis circuit with hollow fibres; **G** level sensor; **H** water absorption pump; **I** peristaltic valve pump; **J** gas outlet with water lock

Experiments

Experiments were performed to assess the efficacy of the system in removing SCFA, and to study the activity and stability of the microflora with two different types of inocula: (1) a standard microflora that was maintained in a fermenter, and (2) freshly collected feces.

Efficacy of SCFA dialysis

To study the efficacy of the system in removing fermentation products such as SCFA, a test solution containing 20 mmol/l each of acetic acid, propionic acid and butyric acid was dialysed against water. During dialysis, the concentrations of SCFA in the system were measured at 1-h intervals. Samples for SCFA analysis were prepared by mixing 500 µl of a 10-fold diluted sample with 100 µl internal standard (diethylbutyric acid) and 100 µl formic acid and centrifuged at $9000 \times g$ for 5 min. A 0.2-µl aliquot of the supernatant was analysed on a gas chromatograph equipped with a Stabilwax-DA column (Restek, The Netherlands). The temperature of the injector and of the flame ionization detector was set on 200 °C. The oven temperature was 140 °C.

Experiments with a standard fermenter microflora

The reactor was inoculated with a standard microflora that was maintained under steady-state conditions in a 1600-ml fed-batch fermenter. The fermenter was inoculated with 80 g fresh feces, obtained from five healthy volunteers. The fermenter was flushed with nitrogen to maintain anaerobiosis, the pH was set to 5.8 and the incubation temperature was 37 °C. Selected groups of microorganisms were monitored on a weekly basis. Each day, 800 ml culture was taken from the fermenter and refilled during the following 24 h with a modified medium as described by Gibson et al. (1988). This medium contained per litre: 4.5 g NaCl, 2.5 g K_2HPO_4, 0.45 g $CaCl_2 \cdot 2H_2O$, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.005 g $FeSO_4 \cdot 7H_2O$, 0.05 g ox bile, 0.01 g haemin, 0.4 g cystein, 0.6 g pectin, 0.6 g xylan, 0.6 g arabinogalactan, 0.6 g amylopectin, 5 g starch, 2 ml Tween 80, 3 g bactopeptone and 3 g casein, plus 1 ml of a vitamin mixture containing per litre: 1 mg menadione, 2 mg D-biotin, 0.5 mg vitamin B-12, 10 mg pantothenate, 5 mg nicotinamide, 5 mg *para*-aminobenzoic acid, and 4 mg thiamine. An inoculum for the system was prepared by concentrating the effluent from the fermenter about five times with a hollow-fibre microfiltration cartridge (Microgon, USA). All handlings were performed under a flow of CO_2. The system was flushed with N_2 overnight prior to the introduction of 160 ml concentrated inoculum from the fermenter. The culture was fed with the same medium as described above for the fermenter, except that 10-fold concentrated carbohydrates, peptone, casein and Tween 80 were used. The feeding was set at 4 ml/h, while the chyme was removed at a flow rate of 2 ml/h and the flow of dialysis fluid was set at 6 ml/min. The dialysis fluid was the same medium as described above, without the carbohydrates, peptone, casein and Tween 80. The pH was kept constant at 5.8, acid production was monitored by recording the amount of dosed alkali to maintain the pH. The temperature was controlled at 37 °C.

Fermentation of carbohydrates

The fermentation experiments with carbohydrates in the system were performed with pectin (Sigma, St. Louis, Mass.), lactulose

(Sigma), lactitol (Purac, The Netherlands) and fructo-oligosaccharide (FOS, Orafti, Belgium). The carbohydrate test solutions were prepared by diluting 0.25 g carbohydrate in 10 ml reduced dialysis liquid in an anaerobic glove box. The pH of the test solutions was adjusted to 5.8.

After inoculation, the microflora was adapted to the conditions in the system for 12 h with both feeding and dialysis (Fig. 2). Then, the feeding was stopped and the microflora was allowed to ferment all available substrates. When no more acids were produced, monitored by the used alkali to keep the pH constant, dialysis was stopped to prevent small molecules from escaping during fermentation of the test solutions and a sample was taken from the chyme for SCFA analysis to determine initial values (Fig. 2, sample 1). The test solutions were introduced into the system in such amounts that the SCFA concentration did not exceed 150 mmol/l to avoid a nonphysiological situation (Cummings et al. 1987). When no more alkali was necessary to keep the pH constant, indicating that SCFA production has stopped, a sample was taken from the chyme for SCFA analysis (Fig. 2, sample 2). SCFA production was determined by calculating the difference between the levels before and after fermentation of the carbohydrate. The dialysis was then resumed to reduce the concentration of SCFA, in order to allow for the next test solution or to continue feeding.

Stability of the microflora

The stability of the microflora and its metabolic activity were evaluated during duplicate experiments with an incubation period of 5 d. Bacterial groups were enumerated on reduced media in an anaerobic glove box. Total anaerobic bacteria were counted on reinforced clostridium agar (Oxoid, UK), *Lactobacillus* on De Man Rogosa Sharp Agar (Oxoid), *Bifidobacterium* on a selective medium according to Beerens (1990), Enterobacteriaceae on violet red bile glucose agar (Oxoid), and *Clostridium* on perfringens agar base (Oxoid) with polymixin B as selective supplement. All plates were incubated at 37 °C under anaerobic conditions. Enzyme activities were determined with the API-zym test (API, France) according to the instructions of the manufacturer. The dry matter content was determined by drying 100 µl sample on a weighed filter in a microwave oven until no loss of mass could be detected.

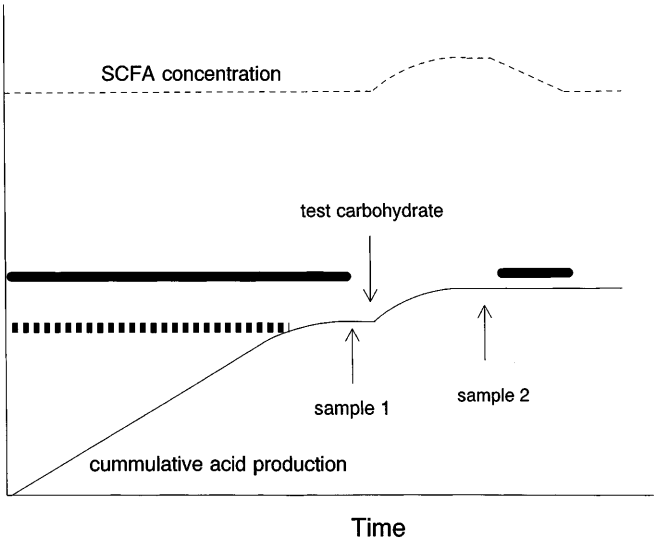

Fig. 2 Schematic presentation of the short-chain fatty acid (SCFA) concentration (*thin dotted line*) and the cummulative amount of produced acid (*thin line*), during feeding (*fat dotted line*) and dialysis (*fat line*). The difference between sample 1 and sample 2 is the amount of acid produced from the test carbohydrates. The method is explained in the text

Experiments with a fecal inoculum

An inoculum was prepared by mixing 200 g feces from a volunteer with 200 ml 0.1 M phosphate buffer, pH 6.5. After filtration through a gauze, the filtrate was centrifuged. The pellet from the filtrate was combined with the retained material on the filter and suspended in 200 ml buffer. All handlings were performed under a flow of nitrogen. The system was inoculated with 200 ml of this fecal inoculum. Three experiments were performed successively, using fecal samples from three different volunteers. The experimental conditions were similar to those described for the experiments with the standard fermenter flora, except for the feeding medium, which was supplemented with 2% mucin (Sigma) and a pH setpoint of 6.5. Samples for SCFA analysis were mixed with 10% phosphoric acid and kept at −20 °C for gas chromatographic analysis according to the method of Jouany (1982). Dialysis and nitrogen flow were stopped for 2 h. The gas produced was collected and analysed on a gas chromatograph for H_2, CO_2 and CH_4 (Jouany and Senaud 1978). Bacterial counts were carried out in the fecal inocula and in the system after 3, 6 and 9 d of incubation. Total anaerobes and methanogens were determined with the most probable number method according to Clarke and Owens (1983). Total anaerobes were incubated on a medium according to Leedle and Hespell (1980). Methanogens were cultivated in the medium described by Balch et al. (1979) supplemented with 20% (by vol) rumen fluid. This medium was selective for methanogen by the addition of clindamycin and cephalotin, and a mixture of 80% H_2/20% CO_2 as the only energy source. The other groups were enumerated on solid media. *Bacteriodes* was enumerated on brain heart infusion agar (BHI, Biokar Diagnostics, France), according to the method of Corthier et al. (1996), *Bifidobacterium* on MRS agar at pH 7 (Biokar Diagnostics), *Lactobacillus* on MRS agar at pH 5 (Biokar Diagnostics), Enterobacteriaceae on deoxycholate agar (DCA, Biokar Diagnostics), and facultative anaerobes were counted on G20 (Raibaud et al. 1966). All media were incubated at 37 °C for 48 h and 72 h. Facultative anaerobes, *Lactobacillus* and Enterobacteriaceae were counted after aerobic incubation, whereas *Bacteroides* and *Bifidobacterium* were enumerated after anaerobic incubation in a jar.

Results

Efficacy of SCFA dialysis

The concentrations of SCFA during dialysis in the system are shown in Table 1. The data could be well fitted with an exponential equation (Eq. 1) to describe the efficacy of the dialysis process.

$$f = 100 \times 2^{-(t/t_{1/2})} \quad (1)$$

where f presents the percentage of SCFA remaining in the lumen of the system, t the time (h) and $t_{1/2}$ (h) the time needed to dialyse 50% of the SCFA. The results show that the individual SCFAs were removed from the

Table 1 Percentage of short-chain fatty acids (SCFA) during dialysis of a 20-mmol/l solution of each SCFA in the system. Mean of triplicate experiments (±SEM)

Time (h)	Acetic acid (%)	Propionic acid	Butyric acid
0	100	100	100
1	60.0 ± 3.8	61.6 ± 3.5	61.4 ± 6.6
2	36.6 ± 5.8	40.3 ± 5.4	42.7 ± 4.9
3	22.3 ± 2.2	24.8 ± 2.8	26.3 ± 3.0
4	10.3 ± 3.0	13.1 ± 3.4	13.8 ± 3.3

lumen of the system at rates proportional to the molecular mass of the product, as is demonstrated by a $t_{1/2}$ of 1.35, 1.46 and 1.52 h for acetic acid, propionic acid and butyric acid, respectively. The efficacy of dialysis during fermentation experiments was demonstrated by the increasing concentration of SCFA after stopping the dialysis (Fig. 3).

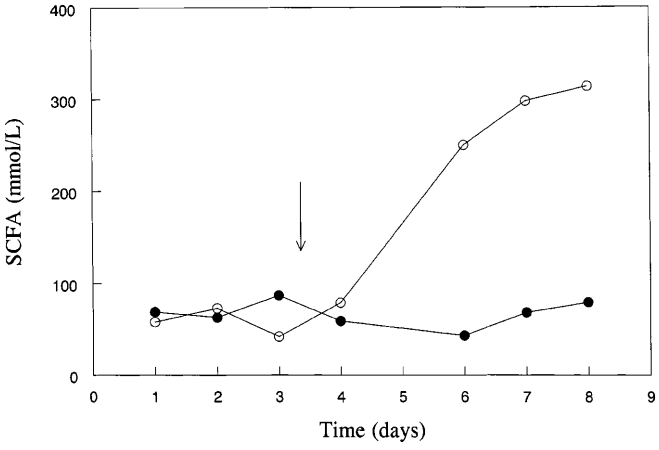

Fig. 3 Total concentration of short-chain fatty acid (SCFA) during an experiment in the system with continuous dialysis (●) and during an experiment with dialysis until the time indicated by the arrow (○)

Studies with standard fermenter flora

During an 8-week period in the fermenter, the enumerated bacterial groups showed little variation. Enterobacteriacea, *Lactobacillus*, *Clostridium* and total anaraerobes were maintained at 5.2 ± 0.4, 5.2 ± 0.6, 6.7 ± 0.8, and 9.7 ± 0.3 $\log_{10}$ colony-forming units/ml (± SEM), respectively.

During the experiments in the system, the numbers of enumerated bacterial groups were similar to those in the inoculum (Table 2). The concentration of SCFA was kept at 130 ± 50 mmol/l during the experiments, with an average molar ratio of 72 ± 6.6%, 14 ± 3% and 14 ± 2.7% (± SEM) for acetic acid, propionic acid and butyric acid, respectively. Enzyme activities showed little variation throughout the experiment, except for a decrease of cystine arylmidase and ucosidase after the first day (Table 3). The dry matter content increased from 4.8 ± 0.7% to 9.9 ± 1.5% during the 5-day experiments.

Pectin, lactulose and FOS showed a rapid fermentation pattern (Fig. 4). The amount of acid produced in 2 h was 1.55, 1.70 and 1.75 mmol, respectively. Lactitol showed a slower pattern of fermentation, with 0.6 mmol acid produced in 2 h. The molar distributions of acetic acid, propionic acid and butyric acid after the fermentation of pectin, lactulose, FOS and lactitol are shown in Table 4.

Studies with fecal inoculum

Three successive experiments were carried out using fecal inocula from three different methane-excreting volunteers. In each experiment, the number of the different groups of microorganisms established in the system was shown to be close to the initial number found in the fecal inoculum. Table 5 presents the mean values (± SEM) of the different microbial populations enumerated in the three fecal inocula and in the three incubation experiments in the system. The total anaerobic microflora was present in high numbers in the system (10^{10} to 10^{11} microorganisms/ml) at the different incubation periods as they were in fecal inocula. However, this population tended to decrease slightly after 6 days incubation in the system. The level of the facultative anaerobic population was similar in the system and in fecal samples (Table 5). The Enterobacteriacaea were enumerated in higher numbers in the fecal inoculum than in the system, regardless of the incubation period considered. The *Bacteroides* species were predominant in fecal samples as well as in the system, throughout the incubation. Lactic acid bacteria, i.e. *Bifidobacterium* and *Lactobacillus*, were enumerated in same number in feces and in the system, whereas the methanogenic population appeared to increase in the system compared to in the fecal inoculum.

The molar ratio of acetic acid, propionic acid and butyric acid was 55 ± 3.9%, 22 ± 1.8% and

Table 2 Average numbers of some groups of bacteria from duplicate experiments (± SEM) in the fermenter inoculum and during incubation in the system. The data presented are expressed as $\log_{10}$ colony-forming units/ml

	Fermenter inoculum	Day				
		1	2	3	4	5
Total anaerobes	10 ± 0.2	10.1 ± 0.1	9.8 ± 0.5	10.5 ± 0.1	9.9 ± 0.6	10.3 ± 0.3
Facultative anaerobes	ND	8.5 ± 0.2	8.0 ± 0.5	8.0 ± 0.6	ND	ND
Clostridia	7.1 ± 0.0	7.7 ± 0.3	6.1 ± 1.3	5.8 ± 1.5	7.9 ± 0.6	7.4 ± 0.2
Enterobacteriaceae	6.0 ± 1.2	6.2 ± 0.3	6.9 ± 0.2	6.8 ± 0.3	6.7 ± 0.1	6.0 ± 0.6
Bacteroides	ND	9.0 ± 0.1	8.6 ± 0.1	8.6 ± 0.1	ND	ND
Bifidobacterium	9.6 ± 0.1	9.7 ± 0.1	9.8 ± 0.2	9.5 ± 0.5	9.4 ± 0.1	9.6 ± 0.4
Lactobacillus	4.6 ± 0.1	4.5 ± 1.1	5.4 ± 0.7	5.9 ± 0.1	4.7 ± 0.0	6.2 ± 0.2

ND = not determined

Table 3 Enzyme activity pattern determined with the API-zym test over a period of 5 d in the system. The figures refer to a colour scale from 1–5, presenting the enzyme activity

Enzyme activity	Day				
	1	2	3	4	5
Alkaline phosphatase	5	5	5	4	5
Esterase (C4)	3	2	2	1	2
Esterase lipase (C8)	1	2	2	1	2
Lipase (C14)	0	0	0	0	0
Leucine arylamidase	4	3	3	3	5
Valine arylamidase	1	0	0	0	1
Cystine arylamidase	5	0	0	1	1
Trypsin	2	1	0	1	1
α-Chymotrypsin	2	0	0	0	1
Acid phosphatase	4	5	5	5	5
Naphthol-AS-BI-phosphohydrolase	5	5	5	5	5
α-Galactosidase	2	3	3	3	3
β-Galactosidase	4	5	5	5	5
β-Glucuronidase	5	5	5	5	5
α-Glucosidase	5	5	5	5	5
β-Glucosidase	3	4	3	2	4
n-Acetyl-β-glucosaminidase	4	5	5	5	5
α-Mannosidase	0	0	0	0	0
α-Fucosidase	3	1	1	1	1

23 ± 1.8% (±SEM), respectively. The mean concentration of total SCFA produced in the three experiments by the different fecal inocula, was 144 ± 40 mmol/l.

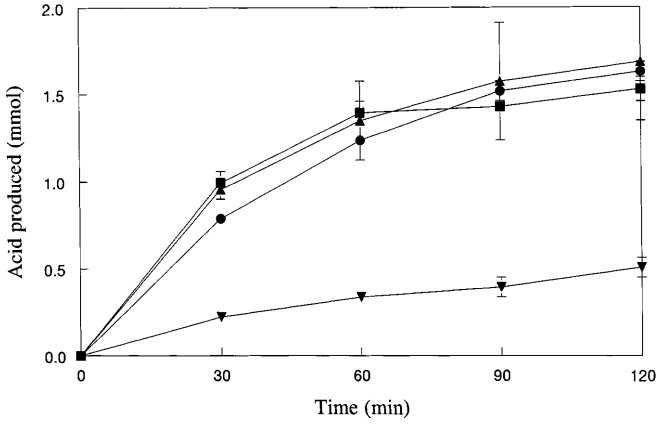

Fig. 4 Average acid production (±SEM, $n = 2$) from the fermentation of lactulose (●), pectin (■), fructo-oligosaccharides (FOS) (▲) and lactitol (▼) during 2 h in the system

Table 4 Molar ratio between acetic acid, propionic acid and butyric acid after 2 h fermentation of several types of dietary fibre in the system. Mean of duplicate experiments (±SEM). FOS, fructo-oligosaccharides

Dietary fibre	Acetic acid (%)	Propionic acid (%)	Butyric acid (%)
FOS	82 ± 6	12 ± 5	6 ± 0
Lactulose	89 ± 0	3 ± 0	8 ± 1
Pectin	74 ± 1	19 ± 0	8 ± 0
Lactitol	66 ± 3	13 ± 1	21 ± 2

The ratio of produced H_2, CO_2 and CH_4 was 2.8 ± 0.7, 55 ± 4.3, and 42 ± 4.5 (±SEM), respectively.

Discussion

There is a growing interest in systems to study the efficacy of functional foods and to evaluate the safety of novel foods. Since experiments in animals or human volunteers have drawbacks with respect to costs, ethics and complexity, studies are often performed in laboratory systems. Laboratory *s*ystems to simulate the large intestine have been used successfully (Rumney and Rowland 1992), but they generally do not combine physiological concentrations of metabolites with physiological numbers of microorganisms. If possible, this combination would increase the predictive value towards the in vivo situation, especially with respect to fermentation rates of substrates and when the interaction between microorganisms and cell-to-cell contacts are of importance (Gasson and Davies 1980). Simple static systems accumulate metabolites which eventually might influence the metabolic activity of the microflora. A drawback of more complex continuous culture systems is that they operate under steady-state conditions which means that the concentrations of metabolites are kept within the physiological range by the dilution rate and the limited amount of substrates in the influent (Edwards and Rowland 1992). Dilution, substrate limitation and product inhibition are the main factors that limit the number of microorganisms in these systems. Therefore, removal of the metabolites from the chyme and the absorption of water separately from the microorganisms, and concentrated feeding, are prerequisites to maintain the number of microorganisms as well as

Table 5 Average number (±SEM) of some groups of bacteria from three experiments and during incubation in the system after inoculation with a fecal inoculum. The data presented are expressed as $\log_{10}$ colony-forming units/g for the feces and $\log_{10}$ colony-forming units/ml for the samples from the system

	Faecal inoculum	Day		
		1	3	9
Total anaerobes	11.0 ± 0.4	10.5 ± 0.4	9.9 ± 0.0	10.2 ± 0.3
Facultative anaerobes	8.7 ± 0.7	8.6 ± 0.2	8.9 ± 0.8	8.7 ± 0.2
Enterobacteriaceae	8.6 ± 0.8	7.8 ± 0.7	7.8 ± 0.4	7.6 ± 0.5
Bacteroides	8.5 ± 0.4	8.5 ± 0.2	8.7 ± 0.1	8.6 ± 0.2
Bifidobacterium	8.1 ± 0.9	8.1 ± 0.5	8.3 ± 0.2	8.3 ± 0.1
Lactobacillus	7.9 ± 1.3	7.7 ± 1.0	7.6 ± 0.3	8.0 ± 0.7
Methanogens	6.9 ± 1.9	8.0 ± 1.8	8.5 ± 1.2	8.5 ± 0.7

their metabolites at physiological levels. However, the feeding and mixing of dense fibrous and viscous materials is a common problem in large intestinal systems (Edwards and Rowland 1992). In our system, mixing with nitrogen offered an adequate method to maintain the influent homogeneous and anaerobic. The peristaltic valve pumps proved adequate in pumping viscous and fibrous materials into the system and pumping chyme out of the system. Water and metabolites were absorbed adequately through hollow-fibre membranes inside the compartments. By feeding concentrated medium, water absorption could be minimized in order to decrease fouling of the membranes. During the experiments, up to 750 ml water could be absorbed with one set of membranes. Experiments were performed to demonstrate that the SCFA could be dialysed efficiently and that their concentration could be maintained within physiological limits (Fig. 3). The applicability of the system to study the fermentation of carbohydrates has been demonstrated with several substrates (Table 4). The kinetics of fermentation could easily be monitored by the dose of alkali used to neutralize the production of acid. A yield of approximately 40 g SCFA per 100 g FOS, pectin and lactulose was calculated, which is in agreement with data presented by Cummings (1994). The yield of 15 g SCFA/100 g lactitol in 2 h fermentation is low and in agreement with the slow fermentation pattern based on total acid production (Fig. 4). The molar ratios of SCFA after fermentation of FOS and pectin were similar to those obtained by Wang and Gibson (1993). For lactulose, the relative amount of acetic acid was higher than those reported by others (Vince et al. 1990; Wang and Gibson 1993), whereas lactitol resulted in a relatively low production of acetic acid. The molar ratio of SCFA after inoculation with feces was similar to values obtained in vivo (Cummings et al. 1987), while the fermenter flora showed higher amounts of acetic acid, similar to the results of Miller and Wolin (1981) and Manning et al. (1987).

It is not clear if these different molar ratios are due to differences between the faecal flora and the fermentor flora or to the different conditions such as the setpoint pH and the use of mucin with the feacal inoculum. For an adequate comparison between fecal inoculum, fermenter inoculum and the in vivo situation, all experiments should be performed under identical conditions, with material that is obtained from volunteers on a similar diet and in a statistically sound experimental design. This was not the intention of the presented study. The number of bacteria in the human colon usually ranges between 10^{10} and 10^{11} colony-forming units/ml, while the dry matter content increases from approximately 14% in the caecal material to 23% in the sigmoid rectum. It has been shown that about 50% of the dry matter originates from microorganisms (Cummings and Macfarlane 1991), the restbeing composed of bulking components such as plant cell material. The dry matter in the reactor increased from approximately 5% to approximately 10% due to the concentrating of microorganisms and unfermentable substances by removing water through the hollow fibres. The 10% dry mass was reached with a low level of bulking components in the medium and hence consisted mainly of microorganisms. With the addition of extra cellulose to the feeding medium, a dry matter content of up to 20% was obtained (data not shown). The circular shape of the system, resembling a loop reactor, proved adequate to circulate this high-density chyme with peristaltic movements. The enzyme activities measured in the system were close to the activities found in feces (Molly et al. 1993). The stability and activity of the microflora were tested during experiments with a fecal inoculum and with an inoculum from a fermenter, both of which have specific advantages. A fecal inoculum is easily to obtain, is considered representative of the colonic microflora and is therefore widely used as inoculum (Rumney and Rowland 1992). However, standardization of fecal inocula is problematic. The fermenter microflora offered the possibility to obtain a standardized culture that was adapted to the feeding medium and was directly available. The counts of the different microbial groups in the system for both studies were stable and within physiological ranges (Macfarlane et al. 1995). The higher number of methanogens in the system as compared to the feacal inoculum was probably due to the relatively longer residence time in the system. The presence of physiological quantities of methanogens indicated a redox potential sufficiently low to maintain strictly anaerobic microorganisms.

As in all laboratory systems that simulate the gastrointestinal tract, the use of this system is also limited by the fact that it does not include interactions with the host. In addition, the mechanism of absorption used in the system is not the same as that in vivo. However, taking these limitations into account, this system is a useful tool to study the fate of undigested components and their effect on microbial metabolism and ecology in the lumen of the large intestine. Such studies may include safety evaluation of foods that contain genetically modified material or antibiotic residues (Van der Vossen et al. 1997), bio-transformation of food components into toxic compounds, and the study of prebiotics and probiotics.

References

Balch WE, Fox GE, Magrum LJ, Woese CR, Wolfe RS (1979) Methanogens: reevaluation of a unique biological group. Microbiol Rev 43: 260–296

Beerens H (1990) A selective and elective isolation medium for Bifidobacterium spp. Lett Appl Microbiol 70: 155–157

Boisen S, Eggum BO (1991) Critical evaluation of in vitro methods for estimation digestibility in simple-stomach animals. Nutr Res Rev 4: 141–162

Clarke KR, Owens NJP (1983) A simple and versatile microcomputer program for the determination of Most Probable Number. J Microbiol Methods 1: 133–137

Corthier G, Muller MC, Lharidon R (1996) Selective enumeration of *Bacteroides vulgatus* and *B. distasonis* organisms in the pre-

dominant human fecal flora using monoclonal antibodies. Appl Environ Microbiol 62: 735–738
Cummings JH (1994) Anatomy and physiology of the human colon. ILSI Europe workshop on Colonic Microflora Barcelona
Cummings JH, Macfarlane GT (1991) The control and consequences of bacterial fermentation in the human colon. J Appl Bacteriol 70: 443–459
Cummings JH, Pomare EW, Branch WJ, Naylor CPE, Macfarlane GT (1987) Short chain fatty acids in human large intestine, portal, hepatic and venous blood. Gut 28: 1221–1227
Edwards CA, Rowland IR (1992) Bacterial fermentation in the colon and its measurement. In: Schweizer TF, Edwards CA (eds) Dietary fibre- a component of food. Springer, Berlin Heidelberg New York
Freter R (1992) In: Fuller R (ed) Probiotics, Chapman and Hall, NY, pp 111–144
Gasson MJ, Davies FL (1980) High frequency conjugation associated with *Streptococcus lactis* donor cell aggregation. J Bacteriol 143: 1200–1264
Gibson GR, Cummings JH, Macfarlane GT (1988) Use of a three-stage continuous culture system to study the effect of mucin on dissimilatory sulfate reduction and methanogenesis by mixed populations of human gut bacteria. Appl Environ Microbiol 54: 2750–2755
Jouany JP (1982) Volatile fatty acids and alcohols determination in digestive contents, sillage juice, bacterial culture and anaerobic fermenter contents. Sci Aliments 2: 131–144
Jouany JP, Senaud J (1978) Utilisation du monensine dans la ration des ruminants. II Effets sur les fermentations et la population microbienne du rumen. Ann Zootech (Paris) 27: 61–74
Leedle JAZ, Hespell RB (1980) Differential carbohydrate media and anaerobic replica plating techniques in delineating carbohydrate utilizing subgroups in rumen bacterial populations. Appl Environ Microbiol 39: 709–719
Macfarlane GT, Gibson GR, Drasar BS, Cummings JH (1995) Metabolic significance of the gut flora. In: Whitehead R (ed) Gastrointestinal and oesophageal pathology. 2nd edn. Churchill Livingstone, Edinburgh, pp 249–274
Manning BW, Federle TW, Cerniglia CE (1987) Use of a semi-continuous culture system as a model for determining the role of human intestinal microflora in the metabolism of xenobiotics. J Microbiol Methods 6: 81–94
Miller TL, Wolin MJ (1981) Fermentation by the human large intestine microbial community in an in vitro semi continuous culture system. Appl Environ Microbiol 42: 400–407
Minekus M, Havenaar R (1996) In vitro model of an in vivo digestive tract. United States Patent; nr. 5,525,305, dated June 11, 1996
Minekus M, Havenaar R (1998) Reactor system. European Patent; nr. 0642382 dated February 11, 1998
Minekus M, Marteau P, Havenaar R, Huis in't Veld JHJ (1995) A multicompartmental dynamic computer-controlled model simulating the stomach and small intestine. ATLA 23: 197–209
Molly K, Vande Woestyne M, Verstraete W (1993) Development of a 5-step multi-chamber reactor as a simulation of the human intestinal microbial ecosystem. Appl Microb Biotechnol 39: 254–258
Raibaud P, Dickinson A, Sacquet E, Charlier H, Mocquot G (1966) La microflore du tube digestif du rat. IV Implantation controlée chez le rat gnotobiologique de différents genres microbiens isolés du tube digestif du rat conventionnel. Ann Inst Pasteur 111: 193–213
Rumney CJ, Rowland IA (1992) In vivo and in vitro models of the human colonic flora. Crit Rev Food Sci Nutr 31: 299–331
Savoie L (1994) Digestion and absorption of food: usefulness and limitations of in vitro models. Can J Physiol Pharmacol 72: 407–414
Vince AJ, McNiel NI, Wager JD, Wrong OM (1990) The effect of lactulose, pectin, arabinogalactan and cellulose on the production of organic acids and metabolism of ammonia by intestinal bacteria in a fecal incubation system. Br J Nutr 63: 17–26
Van der Vossen JMBM, Havekes WALM, Koster DS, ten Brink B, Minekus M, Havenaar R, Huis in't Veld JHJ, Overeem J, Hendriks N, Hofstra H (1997) Development and validation of an in vitro gastro intestinal tract model for safety evaluation of genetically modified foods. Technical annex, EZ-Project "Markt introductie Genetisch Gemodificeerde Voedingsmiddelen": 9.D.1
Wang X, Gibson GR (1993) Effects if the in vitro fermentation of oligofructose and inulin by bacteria growing in the human large intestine. J Appl Bact 75: 373–380