Anaerobe xxx (2014) 1–12

Contents lists available at ScienceDirect

Anaerobe

journal homepage: www.elsevier.com/locate/anaerobe

Clinical microbiology

Flux analysis of the human proximal colon using anaerobic digestion model 1

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ARTICLE INFO

Article history:
Received 26 January 2014
Received in revised form
30 April 2014
Accepted 19 May 2014
Available online xxx

Keywords:
ADM1
Anaerobic digestion
Human colon
Intestinal microbiota
MCS
Modeling
SCFA production
TIM-2
In silico
In vitro

ABSTRACT

The colon can be regarded as an anaerobic digestive compartment within the gastro intestinal tract (GIT). An *in silico* model simulating the fluxes in the human proximal colon was developed on basis of the anaerobic digestion model 1 (ADM1), which is traditionally used to model waste conversion to biogas. Model calibration was conducted using data from *in vitro* fermentation of the proximal colon (TIM-2), and, amongst others, supplemented with the bio kinetics of prebiotic galactooligosaccharides (GOS) fermentation. The impact of water and solutes absorption by the host was also included. Hydrolysis constants of carbohydrates and proteins were estimated based on total short chain fatty acids (SCFA) and ammonia production *in vitro*. Model validation was established using an independent dataset of a different *in vitro* model: an *in vitro* three-stage continuous culture system.

The *in silico* model was shown to provide quantitative insight in the microbial community structure in terms of functional groups, and the substrate and product fluxes between these groups as well as the host, as a function of the substrate composition, pH and the solids residence time (SRT). The model confirms the experimental observation that methanogens are washed out at low pH or low SRT-values. The *in silico* model is proposed as useful tool in the design of experimental setups for *in vitro* experiments by giving insight in fermentation processes in the proximal human colon.

1. Introduction

The colon constitutes an anaerobic digestive compartment within the gastro intestinal tract (GIT) that is heavily colonized by bacteria, the so-called microbiota. Over 90% of the total microbial cells in the human body reside in the colon, and some 65% of the fecal mass consists of these prokaryotic cells [7,38]. The colonic microbiota ferments substrates that are not taken up in the small intestine. The main products of carbohydrate fermentation by colonic bacteria are short-chain fatty acids (SCFAs), mainly acetate, propionate and butyrate [6]. SCFAs are rapidly absorbed and provide about 5% of the total energy requirements of the host [25,27]. In addition, SCFAs have beneficial health effects; butyrate in particular is thought to be important to impact the regulation of the immune system [25]. As a result, the intestinal microbiota and the SCFAs produced play an important role in human health and wellbeing. Unlike the fermentation products of carbohydrates, that are considered healthy, some fermentation products derived from proteins (like ammonia) can be harmful for the host [34]. Of major interest to the food industry are the effects of food and functional ingredients on the composition of the microbiota and the metabolites produced in the gut.

A particular group of functional ingredients constitutes the prebiotics. The prebiotic concept is defined as 'The selective stimulation of growth and/or activity(ies) of one or a limited number of microbial genus(era)/species in the gut microbiota that confer(s) health benefits to the host' [34]. Different types of prebiotics have been described, though most constitute non-digestible oligosaccharides (NDOs), like fructans (fructo-oligosaccharides and inulin) and galacto-oligosaccharides (GOS) [31,38]. These prebiotics reach

Abbreviations: ADM1, anaerobic digestion model 1; TIM-2, *in vitro* fermentation of the proximal colon; GOS, galactooligosaccharides; SCFA, short chain fatty acids; SRT, solids residence time; MCS, multichamber continuous culture system.

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http://dx.doi.org/10.1016/j.anaerobe.2014.05.008
1075-9964/

the colon, where they are metabolized by beneficial members of the microbiota, amongst others, bifidobacteria and lactobacilli [38].

Most studies on the effects of prebiotics on the microbiota composition and metabolites produced in the colon make use of *in vitro* and *in vivo* (animal) models [22]. Examples of *in vitro* models are the three stage continuous culture system, here referred to as multichamber continuous culture system (MCS), the simulator of the human intestinal microbial ecosystem (SHIME), and the TNO Intestinal Model of the large intestine (TIM-2) [12,27,39]. The MCS consists of three vessels representing the proximal, transverse and distal colon. SHIME consists of five vessels representing the stomach, small intestine and the three colonic regions. In the mucosal-SHIME (M-SHIME), a mucosal environment is incorporated in the vessel representing the proximal colon [39]. No peristaltic movements, water and nutrient diffusion are implemented in the MCS, SHIME or M-SHIME [12,39]. The TIM-2 system simulates the proximal colon, including peristaltic movement and water and nutrient absorption [27].

Even though *in silico* modelling has been applied extensively in waste digestion, only limited attempts have been made to model the human colon [30,45]. Interestingly, in the large intestine as well as in anaerobic digesters of waste streams rich in organic matter, complex substrates are degraded step-wise by a mixed microbial community. The anaerobic environments are comparable and similar conversion processes take place: hydrolysis of carbohydrates and proteins, formation of SCFAs, increase and decay in biomass [2,6]. A widely used *in silico* model of anaerobic digestion is the Anaerobic Digestion Model 1 (ADM1) developed by the International Water Association (IWA) [2]. ADM1 has been developed to describe the anaerobic digestion of wastes for the production of methane containing biogas or bio-hydrogen [8,18,30]. The model describes and predicts the conversion processes in anaerobic digestion and covers about 30 components (soluble compounds, particulates and gases). The particulates include lumped groups of micro-organisms that catalyse a reaction in the anaerobic degradation. SCFAs are included as soluble compounds. The catabolic end-products of the process are methane, carbon dioxide, ammonia and inert material.

Here, we present an *in silico* model of the component fluxes in the lumen of the proximal colon of the human colon based on ADM1. To our knowledge, the *in silico* approach to model the fermentation processes in the human proximal colon presented is the first that combines the two fields of anaerobic digestion modelling of waste and *in vitro* modelling of the human proximal colon by TIM-2 and MCS.

It enables the description of substrates and product fluxes between groups of microorganisms and between microorganisms and the host, and provides a quantitative description of the microbial functional groups in the microbial community established in the proximal colon. After calibration of the model using *in vitro* data, the model will be used to predict the impact of operational changes (as potentially imposed by dietary changes) on the fluxes in the proximal colon.

2. Materials and methods

The *in silico* ADM1 model was used as a basis to develop a computational model of proximal colon fermentations. The model was used to describe the results of two *in vitro* bioreactor model experiments in TIM-2 and MCS, which are schematically shown below (Figs. 1 and 2).

Both model experiments were conducted under anaerobic conditions, inoculated with feces and medium and continuously fed [13,21]. In both systems, the bioreactors were continuously stirred and the temperature was maintained at 37 °C. The pH was controlled at 5.8 in TIM-2 and at 5.5 in MCS [13,21].

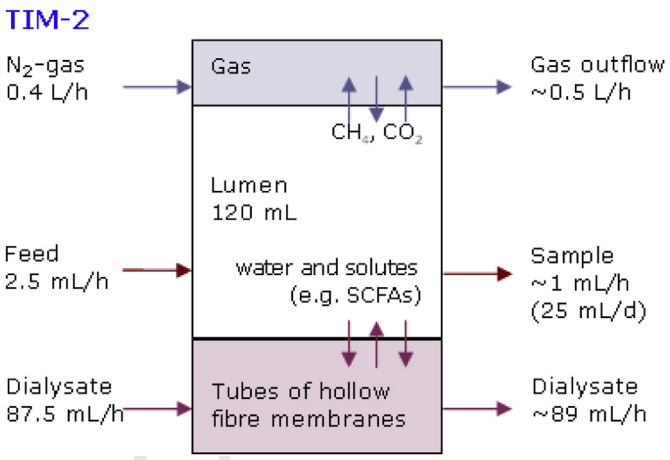

Fig. 1. Scheme of TIM-2 [21], an *in vitro* model of the proximal colon.

In the TIM-2 system, the hollow-fibre membranes in the bioreactor absorb water and nutrients mimicking the uptake of small solutes (<50 kD) by the host [27]. Peristaltic movement was accomplished by a flexible wall. The TIM-2 system was used to investigate standard carbohydrate fermentation and GOS fermentation [21]. The TIM-2 system was run for three days and had a solid retention time of about 5 days, indicating that no steady state had been reached.

The MCS *in vitro* model was run with standard medium until a stable conversion was established (steady state after about 13 days, which is defined as a stable SCFA concentration over 3 days) [13]. After a steady state had been established, the feeding medium was changed to a GOS-containing medium until a stable conversion was reached.

A detailed description of the setup of the *in vitro* experiments as applied in both experimental systems can be found in the Appendix.

3. Model development

3.1. Colon model based on ADM1

The general *in silico* fermentation process model of the human colon developed in this work is based on ADM1 [2]. In ADM1 the conversion reactions in the anaerobic digestion process are modeled as the resultant from the activity of a range of functional groups of microorganisms. Substrate conversion, bacterial growth, and product formation are described according to Monod-kinetics for the limiting carbon substrate and ammonia as nitrogen source. Several specific processes are assumed to be inhibited at low pH-values, or a high hydrogen partial pressure. The pH of the system is estimated by solving the charge balance taking into account all cations and anions in the system including all bases and acids. The acid/base equilibrium equations are expressed as algebraic equations and all differential equations are numerically solved as a set of ODEs.

The characteristics of anaerobic digestion of waste and anaerobic digestion in the colon are compared in Table 1. One major difference is the significantly shorter solids retention time in the colon compared to an anaerobic waste digester. As a consequence, acetate consuming methanogens are washed out and the SCFA concentration (which is an intermediate product of carbohydrate, protein and long chain fatty acid digestion) is relatively high in the colon compared to anaerobic digesters aiming for production methane containing biogas. Due to the higher SCFA concentration,

Please cite this article in press as: Motelica-Wagenaar AM, et al., Flux analysis of the human proximal colon using anaerobic digestion model 1, Anaerobe (2014), http://dx.doi.org/10.1016/j.anaerobe.2014.05.008