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Human colon: a complex bioreactor. Conceptual modelling for the anaerobic digestion of the functional trophic chain.

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Abstract This paper presents a description of the model structure for the anaerobic digestion of carbohydrates in the human colon. Since anaerobic digestion occurs similarly in both digesters and animal gastrointestinal tracts, a parallel between these systems is presented. Hydraulic behaviour, reaction mechanisms and transport phenomena of the process are identified as key preliminary step for the construction of a mathematical model. On this basis, the obstacles to validate the model are presented and a preliminary approach is proposed to evaluate the adequacy of the structure obtained, in terms of the construction of a minimal microbiota model. The mathematical model will be further validated on animal model, consisting of axenic rodents inoculated with a model microbial consortium from human large bowel. This approach would be very useful for a better understanding of the human digestive tract, as a complex system, and the role of the microbiota on human health.

Keywords Anaerobic digestion modelling; carbon trophic chain; human colon; microbiota

INTRODUCTION

The human colon (also called large intestine) is an anaerobic compartment within the intestine. Its main function is to get energy for the human body through the degradation of alimentary fibers, (*i.e.* complex carbohydrates) that are not hydrolysed and absorbed in the upper digestive tract (MacFarlane and Cummings, 1991). In addition to the metabolic aspects of fibre degradation, the interactions between the host and the bacterial community are just starting to be understood. The commensal micro-organisms play an important role on human health. Their involvement in inflammatory bowel disease is for instance now well documented (Gill *et al.*, 2006).

Despite this important role in human health, the gut microbiota composition and function still remain to be elucidated. The main limits of the study of gastrointestinal tract (GIT) microbiota are its microbial diversity exceeding several hundred species (Eckburg *et al.*, 2005) and the very few number of cultured bacteria, representing only 20% of total microbial cells (Suau *et al.*, 1999). However, molecular methods, first targeting 16SrDNA genes and now functional genes through metagenomics approaches, allow a better characterization of bacterial diversity and functions of uncultured species (Manichanh *et al.*, 2006, Gloux *et al.*, 2007, Gill *et al.*, 2006). In the literature, artificial systems (chemostats) or *in silico* approaches have been used to study specific aspects of the system such as microbial competition (Ballyk *et al.*, 2001), VFA absorption (Tyagi *et al.*, 2002; Minekus *et al.*, 1999) and interactions between the host and the microbiota (Wilkinson, 2002). However, none of these models integrate the physiological parameters of the large intestine, the biochemical processes and flux together with functional microbial diversity. Because of its biological complexity, modelling of the human intestinal ecosystem is indeed a challenging subject. It is also a key step towards a better understanding of carbohydrate fermentation. In addition, model development can play a major role in understanding the impact of microbial community in the stability of the digestive system in healthy individual, and in the development of inflammatory diseases. Such model will be used to the study the impact of dietary regimes on human gastro intestinal microbiota. This model will also be a very useful tool for *in vivo* and *in vitro* experiments design, as both the medical field and private companies (pharmaceuticals, nutrition, probiotics) require biological tools representing a “healthy gut microbiota”.

The objective of this paper is to propose a model structure of the carbohydrates degradation in the large intestine, as a preliminary stage of a mathematical model. For this, the applicability of the IWA anaerobic digestion model No1 (ADM1) (Batstone *et al.*, 2002) dealing with the human colon will be addressed. By integrating knowledge from both digester process and medical fields, it is indeed believed that the ADM1 model could help broadening the understanding of anaerobic digestion impact and perspectives.

DESCRIPTION OF THE SYSTEM

The human colon is an anaerobic compartment of 150 cm long receiving food material from the ileum. The contents are first retained and mixed in the right colon and, after this digesta goes to the left colon, are finally excreted or absorbed (Leclerc, 1997). The principal carbon sources for the microbiota in the human colon are polymers that have not been digested in the stomach and small intestine, also called dietary fibres. They are fermented in the colon where they quantitatively represent 85% of the substrates available. The main carbohydrates consist of resistant starch, cellulose and hemi-cellulose.

In addition to the dietary source, the secretion of mucus by the human epithelium is an endogenous carbon source available for the micro-organisms. Indeed, in the mucus, mucins support complex communities of intestinal bacteria *in vivo* in the absence of any dietary input (MacFarlane and Cummings, 1991) and bacteria able to degrade mucins have been isolated (Derrien *et al.*, 2004).

While the dietary carbon input is sequential, mucus secretion occurs continuously and throughout the whole large bowel. The mucus layer, of 105-155 μm thickness, forms a protective gel for the host cells. Mucus gel has the characteristics of a polymer-based matrix and allows the attachment or embedment of micro-organisms. As a consequence, within the large intestine, the microbiota is located in the lumen and associated with the mucus layer. In terms of bacterial species present, the mucosal population is distinct from the luminal one (Zoetendal *et al.*, 2002).

THE COLON AND OTHER ANAEROBIC SYSTEMS

One of the main differences between anaerobic processes used for wastewater treatment or bio-energy recovery and the natural ecosystems is system operation (Table 1). From a reactors design perspective, process characteristics such as degradation efficiency, organic load and biogas production can be associated with factors such as geometry, surface and hydraulic regimes. Among the natural habitats, the digestive tracts of animals show a specialized behaviour with excellent performance, in such a way that these systems can be used as references to improve the AD processes operated by human beings (*e.g.*, UASB reactors).

But other important differences exist. For example, end products of anaerobic digestion and energy harvest differ between the systems: all ruminants are methanogenic, whereas only some termites species (Brune, 1998), and in Western countries, 30-50 % of healthy human beings are (MacFarlane and Cummings, 1991). The VFA are absorbed and converted into energy by the animal host cells. It is to be noticed that the ratio (energy produced by VFA in AD/total energy required) dramatically differs between anaerobic digestion systems; as shown in Table 1.

Table 1. Comparison between anaerobic systems.

	Human colon	Termit gut	Bovine rumen	Full scale industrial AD bioreactor
Volume	1.84 l	1 μl	100 l	10-10 ³ m ³
A/V (m ² /m ³)	2500	5000	10	60- 200 fixed bed reactors 100- 200 moving bed reactors 1000- 3000 expanded bed reactors 1000-2500 fluidised bed reactor 2000- 5000 recycled bed reactors (Henze and Harremoes, 1983)

Solids residence time	60 h	24 h	38 h	1-30 d
pH	5.4-6.9	5-12	5.8-6.4	6.8-7.5
Temperature	39°C	30°C	39-41°C	PR: 0-20°C; MR: 20-45°C TR: 50-65°C*
COD load (kgCOD/ m ³ d)	30	400	225	35-100
Number of bacterial species	800	500	385	140
Molar ratio HAc: Pr:But	57:22:21	Hac:94	70:20:10	
Biogas production	0.5-4 l/d	2.48 µg /g of termite h	600-1000 l/d	0.6 l/g COD removed
Energy production yield				285 kWh/100 kg COD
Energy yield of AD	6 to 10%	30%	> 80%	NA

PR: psychrophilic range; MR: mesophilic range; TR: termophilic range.

CONCEPTUAL STRUCTURE OF THE MODEL

The premise in this work is that the colon can be mathematically modelled like as an anaerobic reactor and that the microbiota can be represented functionally since it has been demonstrated that complex communities of quite different composition can have similar functional characteristics (Fernandez *et al.*, 2000).

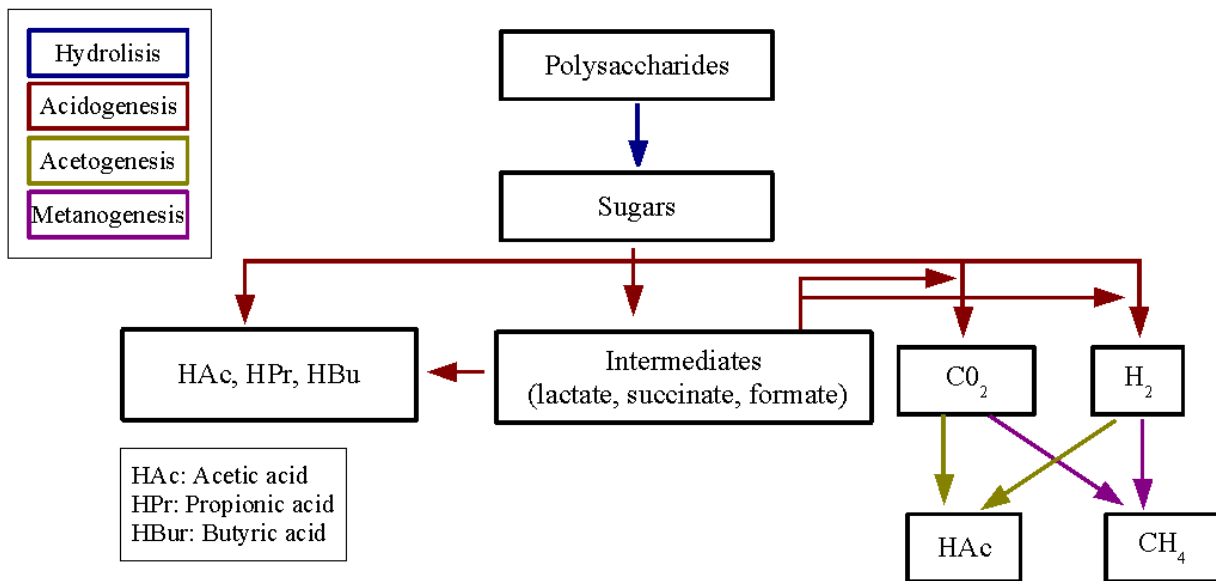


Figure 1. Carbohydrates degradation pathway in the human colon.

The reaction scheme (Figure 1) to be considered, takes into account the following differences with the ADM1 model:

1. ADM1 includes carbohydrates, proteins and lipids, as substrates. For the colon, the substrate is mainly carbohydrates.
2. In the colon, the methanogenesis is predominantly hydrogenotrophic, while for ADM1 the main reaction is the acetoclastic pathway.
3. For ADM1, the acetogenesis is carried out by the oxidation of butyric, propionic and valeric acids, whereas in the colon, acetogenesis is mainly hydrogenotrophic.
4. In ADM1, intermediate compounds such as lactate are omitted; however in the colon, the lactate production appears as a step to be considered.

These differences can be included in the model through changes of the Peterson matrix in the biochemical reactions proposed in ADM1. These catabolic reactions occur in both the mucus and the lumen. Each subsystem must have different kinetics parameters. Moreover, from a physical point of view, the lumen and the mucus are two different systems that interact with each other and with the host epithelium (Figure 2).

The colon can be defined as a high-rate system, with a high biomass concentration thanks to the formation of aggregates that resist the hydrodynamics forces (shed of epithelial cells, peristalsis movement and the flow of chyme) (Sonnenburg *et al.*, 2004). In addition, similarly to anaerobic digesters, the residence time affects the biomass concentration in the system and the biomass excreted (Child *et al.*, 2006).

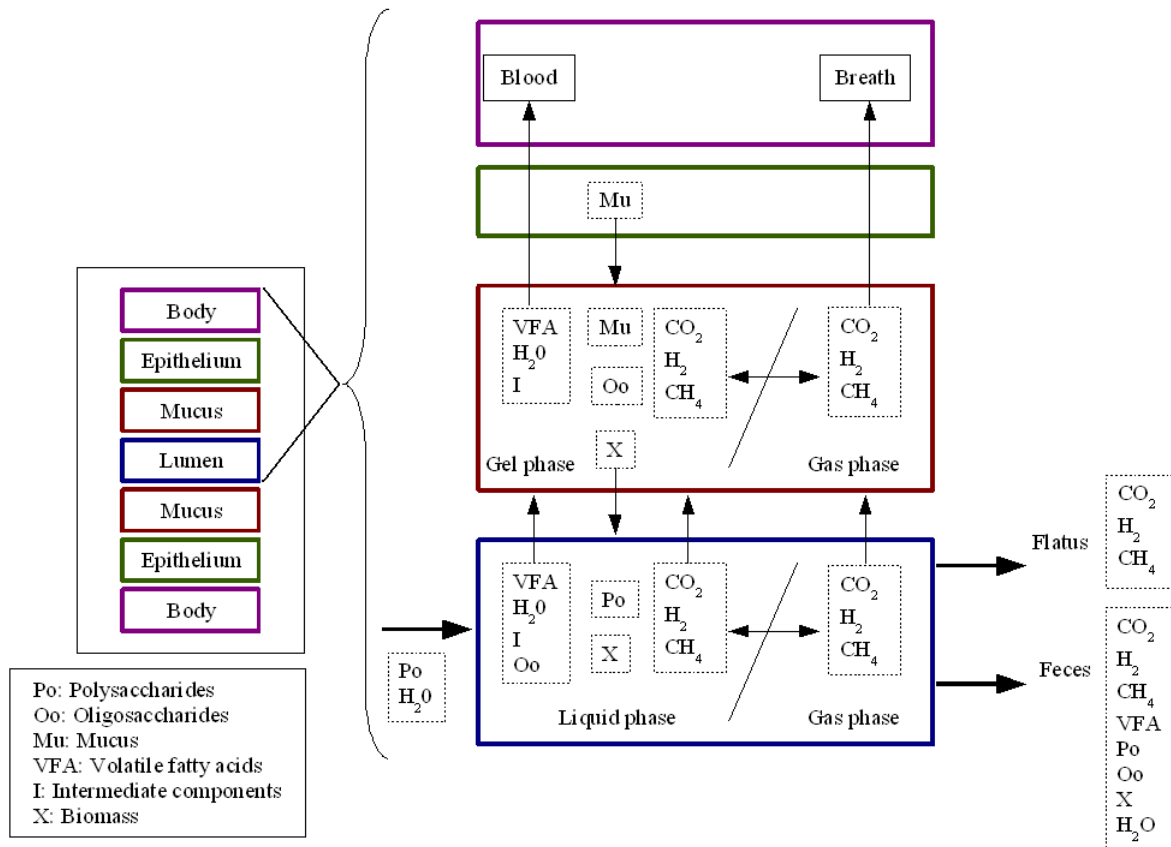


Figure 2. Interaction diagram in the colon.

The hypothesis underlying the diagram are the following:

1. Oligosaccharides, VFA, intermediates products, water and gases are able to diffuse from the lumen to the mucus.
2. In each subsystem (mucus, lumen), there is a liquid-gas transfer
3. Part of the VFA and intermediate products (lactate, succinate) are absorbed by the host.
4. Hydrogen, methane and carbon dioxide solubilities in the liquid phase must be taken into account.
5. Gas produced in the lumen is expelled in flatus and breath (crossing the mucus) and gas produced in the mucus is expelled in breath.
6. Part of the mucosal biomass goes to the lumen by means of a shear-loss phenomenon.

In spite of its geometry (a cylinder shape) and size, several factors can produce mixing (maybe not up to a completely stirred behaviour) along the colon, such as peristaltic movement, shed of epithelial cells and gas production. As a consequence, the colon has been represented as a series of chemostats (Child *et al.*, 2006). In each chemostat, a distinction between the lumen and the mucus

phase is proposed. As a first approach, following the physiological foundations (right colon, transverse colon and left colon), the colon will be represented as three systems in series with different transit time and viscosity because of the VFA and water absorption. This phenomenon does not occur in anaerobic reactors, where a constant flow rate is normally used for analysis. Figure 3 shows the hydraulic scheme for each partition, where the lumen and mucus are represented as CSTRs and a higher retention time is applied to the bacteria in the mucus.

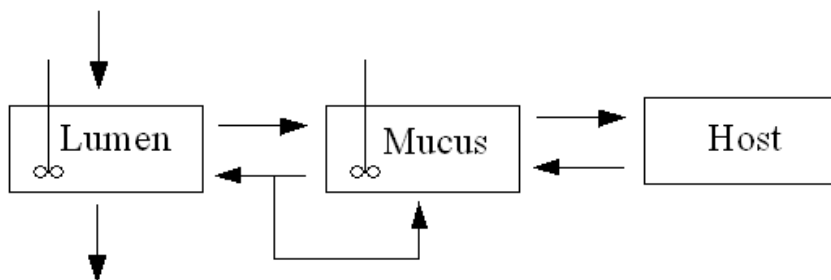


Figure 3. Hydraulic representation of each partition of the system.

This approach will allow comparisons of the resulting mathematical model with previous physical models where, in order to emulate the large intestine, continuous vessels with biofilm and planktonic phase were connected (see Child *et al.*, 2006).

PERSPECTIVES AND FUTURE WORK

The construction of the mathematical model and its validation are hindered by the lack of knowledge about some phenomena and the difficulty in measurements. Knowledge gained from *in vitro* experiments appears limited because most of the bacteria in colon are uncultured (Suau *et al.*, 1999). Moreover, many studies have been based on the microbiota in fecal matter; however important differences have recently been found between feces microbiota and colon microbiota. *In vivo* models using inoculated axenic rodents will therefore be very useful, although in such models many variables can be collected only once: this means that the availability of dynamic data is limited, except for the gases. That will be a very interesting topic to be studied in the state estimation of the system.

The ongoing modelling work consists in developing a simulation tool based on the structure here proposed and on the available information, and assessing through simulation the relative importance of the different phenomena involved.

In the future, information gained from 16S rRNA analysis will be used to first define a minimal functional microbiota and metagenomics approaches will allow a better functional characterization of GIT bacteria. Micro-sensor technology together with FISH (Fluorescent in situ hybridization) should enable us to study spatial distribution of substrates, products and biomass composition in the mucus. Such assays *in vitro* and *in vivo* will, for instance, help clarifying the diffusion and liquid-gas transfer phenomena and their importance in the colonic ecosystem dynamics. This will also help improving the mathematical model from which we expect, on the long term, a better insight of the phenomena involved and a significant feedback for reactor design.

CONCLUSIONS

A framework of the model structure for carbohydrates degradation in the human colon has been presented, making the analogy with conventional anaerobic digesters with respect to biological processes, kinetics, hydraulic and mass transfer. Despite the outlined differences between the pathways occurring in the colon and the mechanism proposed in ADM1, the ADM1 structure can be used to model the biochemical reactions in the large intestine, considering some changes in the Peterson matrix. The colon is structurally represented as a set of three compartments in series. In

each compartment, the lumen and the mucus are reactors that interact between them. A model following this first approach is under construction and the interaction structure will be validated through simulation.

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