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Animal digestive strategies versus anaerobic digestion bioprocesses for biogas production from lignocellulosic biomass

Ali Bayané · Serge R. Guiot

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Abstract Herbivorous mammals and wood-eating insects are fairly effective in the digestion of plant polymers, such as lignocellulosics. In order to improve methane production from the lignocellulosic biomass, several kinds of anaerobic digestion processes derived from animal models have been devised. However, the rates of biodegradation occurring in the anaerobic bioreactors still remain lower than in animal guts. The effectiveness of the digestive systems of those animals results from the concerted action of the various enzymes (e.g. cellulases, xylanases, esterases, ligninases) produced in their guts as well as their integration with mechanical and chemical actions. Powerful pretreatment (prefermentation) operations are integrated to and support efficiently the microbial fermentation system, e.g. the rumination (i.e. mechanical) in ruminants and the secretion of endogenous cellulases (i.e. enzymatic) or the alkaline treatment (chemical) at mid-way in xylophagous insects. The oxygen gradients along the gastrointestinal tract may also stimulate the hydrolytic activities of some microbial populations. In addition, the solid retention time, the digesta flow and the removal of the end-products are well ordered to enable animals to thrive on a complex polymer such

as lignocellulose. At the same time, technologies were developed to degrade lignocellulosic biomass, such as the rumen derived anaerobic digestion (RUDAD) process and the rumen simulating technique (RUSITEC), more elaborated and using rumen microbial consortia. Overall, they showed that the fermentation taking place in the rumen fermentation and even in the hindgut are biological processes that go beyond the limited environmental conditions generally found in anaerobic digesters. Hence, knowledge on herbivores' digestion mechanisms might be better exploited in the design and operation of anaerobic digesters. This literature review is a cross-analysis of the relevant information about the digestive strategies of herbivorous and wood-eating animals and the bioengineering techniques in lignocelluloses degradation. The aim is to highlight strategies of animals' digestion simulation for more effective anaerobic digestion of lignocellulosic compounds and other solid residues.

Keywords Anaerobic digestion · Rumen fermentation · Hindgut · Termite · Lignocellulose

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The main structural polysaccharides of plant tissues, cellulose and hemicelluloses, are linked with lignin and together called lignocellulose. Lignocellulosic biomass is more and more considered as a potential

energy resource for ethanol fermentation as well as for biogas (methane) production through *in vitro* fermentation bioprocesses (Bolzonella et al. 2003; Gijzen 2002; Pauss and Nyns 1993). The hydrolysis of both cellulose and hemicellulose are however rate-limiting steps in all fermentation processes designed to date, because of both the crystalline structure of cellulose and the lignin, which shields polysaccharides from enzymatic attacks (Sanchez 2009). Yet in nature, a number of invertebrate animals, and to some extent, their gut microbiota have evolved specialized enzymes that cooperate in lignocellulose processing (Scharf and Tartar 2008). Then again, some vertebrate animals such as ruminants have evolved through time the ability to feed on herbaceous plants such as soft grasses with the aid of their intestinal symbionts. The ruminants cannot digest woody plant, in contrast to xylophagous insects (longicorns, bark beetles, cockroaches, termites, etc.). Unlike mammalian herbivores that are able to degrade lignocellulosic materials only because they harbour cellulolytic symbionts, the wood-eating insects are capable to produce their own cellulases (endogenous cellulases or endocellulases). The most prominent and studied wood degrading invertebrates are termites, which can digest 74–99% of the cellulose in wood (Breznak and Brune 1994). In lower termites which harbour symbiotic flagellate protozoa, high digestibility of cellulose is due to both endogenous and protozoal enzymes acting independently. In higher termites, which do not possess symbiotic protozoa in the gut, lignocellulose is digested by endogenous and bacterial cellulases (Arakawa et al. 2009; Tokuda et al. 2005; Warnecke et al. 2007). Through various physiological mechanisms the gastrointestinal tracts of ruminants provide suitable environmental conditions to an extremely dense population of symbiotic microorganisms, capable to degrade plant polymers (Yokoyama and Johnson 1988). The symbiotic microorganisms provide their host with breaking down of complex polysaccharides, which could not be carried out by ruminants themselves (Fonty et al. 1995). The microbiota of the fermentation chamber of the ruminant gut, the so-called rumen, is constituted with prokaryotic (bacteria and archaea), and eukaryotic (protozoa and fungi) organisms (Bryant 1959; Chesson and Forsberg 1988; Dehority 2003; Stevens and Hume 1998). The digestive strategies of both herbivorous insects (primarily termites) and mammalian herbivores (primarily

ruminants) have been studied for years in order to highlight their key mechanisms that could benefit to the reduction of lignocellulosic wastes (pulp and paper waste, municipal solid waste, etc.) (Czerkawski and Breckenridge 1977; Muetzel et al. 2009; Owens and Goetsch 1988). It has not been clear, however, as to the lower performance of those industrial anaerobic digesters in lignocellulosics' bioconversion compared to that occurring in animal digestive tracts (Nair et al. 2005). What are the basic parameters of lignocellulosics digestion in herbivorous and xylophagous gastrointestinal tracts? What mechanisms from animals should be applied to biogas digesters? What is the mimicry level of the anaerobic bioreactors designed to date? We address those questions here, by analysing the functioning of herbivorous and xylophagous gastrointestinal tracts and the comparison of these systems with the current anaerobic digestion practices.

1 Lignocellulosic biomass digestion through animal guts

The digestion of lignocellulosic compounds by mammalian herbivores is entirely accomplished by symbiotic microorganisms, due to the fact that mammals do not produce endogenous cellulolytic enzymes (Bjornhag 1994). Relative to the gut portion where fiber digestion takes places, two groups of mammalian herbivores are distinguished i.e. the foregut fermenters and the hindgut fermenters (Hume 1989). The ruminants are the main foregut fermenters, with pregastric fermentation chambers (rumen) dedicated to fibers digestion. In the hindgut fermenters, fibrous material retention and microbial fermentation take place in the posterior part of the digestive tract, i.e. the large intestine (colon and caecum). Mammalian hindgut fermenters include rodents (rabbit, hare, pika), small monkeys (tamarins, marmosets) and large herbivores such as horse, zebra, rhinoceros, elephant, gorilla etc. (Hofmann 1988). In horse the caecum is the first fermentation site with a size of about 16% of the gut volume. The colon is the second fermentation site with a size of about 45% of the gut volume (Frape 2004). Like the ruminants, the horses finely crush plant materials before swallowing. The ingested materials pass relatively quickly through the stomach and the small intestine where digestive enzymes break down lipids, starch, and peptides.

Finally the digesta arrives in the large intestine where its passage takes days to allow the microbial digestion of cellulose and hemicellulose (Fransson et al. 2003). Regular contractions of caecum and colon mix their contents to promote the microbial digestion and to expose the content to the epithelial surface for volatile fatty acids (VFA) absorption. Environmental condition such as pH, temperature and redox potential are identical between caecum and colon and are suitable for the growth of a variety of bacteria as well as protozoa and fungi (although in lower concentration than in rumen) (Mountfort et al. 2002). The dry matter content of the hindgut is about 60% as compared to 13% in the rumen. The epithelial walls of the caecum and the colon play an essential role in microbial fermentation, by continued absorption of VFAs and also secreting bicarbonate ions that buffer the pH (Frape 2004). The biochemical reactions (i.e., hydrolysis, acidogenesis, acetogenesis and methanogenesis) involving in both foregut and hindgut fermentation are similar to those of the anaerobic digestions bioprocesses, even if the microorganisms involved in each case are different. However, only the rumen fermentation system has been intensively studied as model for the anaerobic digestion, as it is likely more effective in lignocellulosic fiber digestion than mammalian hindgut fermentation system. Nevertheless the digestive system of termites is more effective in lignocellulose degradation than that of ruminants. As an evidence the volumetric rate of lignocellulosic biomass breakdown in rumen, as measured from the VFA production rate is around 18 g COD-based VFA/L_{rumen}·d (chemical oxygen demand) (Colwell and Coddington 1994) against 6 g COD-based VFA/L·d in conventional anaerobic digesters (Nair et al. 2005). In comparison termites can consume organic matter at a rate varying between 0.65 and 0.84 mg/termite·d, and they can assimilate about 56% of this quantity (Okwakol 1980), which represents a potential rate of over 225 g VFA-COD·L_{tract}·d. Hence the review will focus on those two animal digestion systems, i.e. ruminants and wood-eating termites.

1.1 Fiber digestion by mammalian foregut fermenters

Ruminants possess a great stomach, specialized in the microbial fermentation of plant materials, of a size

varying from 3 to 15 L in sheep and from 35 to 100 L in cattle (Dehority 2002; Hofmann 1988). It is divided in four chambers i.e. reticulum, rumen, omasum and abomasum. The first three-chambers (reticulum-rumen-omasum), which are the main sites of microbial fermentation and VFA absorption are together the so-called forestomach. To accommodate the resident microbial population, the forestomach is separated from the acidic conditions of the abomasum also called true stomach. Environmental conditions such as pH, temperature and redox potential are quite similar between the rumen and typical anaerobic digestion processes (Table 1).

Unlike the anaerobic digesters, the rumen is not completely anaerobic environment. Oxygen enters the rumen regularly with food, saliva, by eructation and by diffusion from the blood. The rumen oxygen occupies about 1% of its gas content (Table 2). The dissolved oxygen is constantly removed by both chemical reduction and the facultative anaerobes microorganisms (mainly bacteria and protozoa) attached to rumen wall. The resistance of rumen fermentation to oxygen was confirmed in vivo and in vitro studies (Czerkawski and Breckenridge 1969; Scott et al. 1983).

The movement of digesta during rumen fermentation is also distinct from the mixing currently employed in the anaerobic digesters. The rumen fermentation combines both homogenization and stratification of the digesta, due to its compartmentalization. The digestion of ruminants begins with mechanical grinding and moistening of the ingested material before entering the rumen. Upon entering the rumen, the digesta sinks in the cranial sac, where it undergoes the contraction of the reticulorumen to be flooded with fermentation juice and inoculated with microorganisms. Then the digesta undergoes regular mixing provided by coordinated contractions of the reticulorumen about once a minute. During digestion, feed particle sizes are reduced and their densities are increased. Thus the small and dense particles can settle and reach the ventral sac (bottom of rumen) before being moved back to the cranial rumen where they are evacuated via the reticulo-omasal orifice into the omasum (Hofmann 1988). Therefore the residence time of lignocellulosic material in the rumen can be divided into two periods. The fermentation periods, where degradable materials circulate in the upper portion of reticulorumen (the top of reticulum

Table 1 Overall features of rumen and anaerobic digestion fermentations

Characteristics	Rumen (Dehority 2003)	Anaerobic digesters (Mata-Alvarez 2002; Vandevivere et al. 2002)
Size	3–100 L (according to animal sizes)	Variable
Dry matter content	10–13%	0.5–40%
Temperature	39°C	15–55°C
pH	5.5–7.0 (On average of 6.5)	6.5–7.5
Osmotic pressure	250–400 mOsm/kg (Hypotonic to plasma)	–
Redox potential	–350 to –150 mV	<–300 mV
Solid retention time (SRT)	20–30 h	–
Hydraulic retention time (HRT)	6–16 h	–
Solid turnover time	1.3–3.7 days	2–60 days
Volatile solids degradation	40–75%	–

The redox potential of rumen fermentation is higher than that occurring in anaerobic digesters. The osmotic pressure of rumen is also regulated. However, the most fascinating characteristics of rumen fermentation is the solids turnover time, which can be 20 times shorter than that of the anaerobic digestion processes

Table 2 Composition of biogas produced in rumen fermentation, in biogas digesters, and in landfill sites

Gas	Rumen fermentation (%)	Anaerobic digesters (%)	Landfill sites (%)
CO ₂	65–69	20–60	42
CH ₄	22–29	40–80	54
N ₂	3–10	1–5	3.1
O ₂	0.5–1	Traces	0.8
H ₂	0.18	0–3	–
H ₂ S	0.01–0.1	0.01–5	0.006

Karagiannidis et al. (2008); Kleiber et al. (1943)

The rumen is not completely an anaerobic environment. A part of the carbon dioxide produces in rumen fermentation is formed from saliva. The anaerobic digesters have better yield in methane compared to rumen, due to the fact that VFAs are largely absorbed through the mucus. Methanogenesis is a secondary activity far behind the hydrolytic and fermentative role of cellulolytic, xylanolytic and pectinolytic microorganisms

and the dorsal rumen), and the end period of fermentation where fine particles already digested, circulate in the lower portion of reticulorumen (the ventral and cranial sacs of the reticulorumen). It is during its stay in the upper reticulorumen that the digesta is likely to be regurgitated and chewed (rumination). Therefore, the digesta flow during rumen fermentation seems to be more complex than that occurring in conventional bioreactors. In addition to this complexity rumen fermentation couples both hydrolysis and absorption reactions. The removal of fermentation products such as VFAs is important for an efficient activity of cellulolytic microorganisms. The rumen epithelial provides an effective absorption of this product and also plays an important function

in the balance of water, nitrogen and mineral metabolism (Dehority 2002).

The most important feature of rumen digestion is rumination. This is the mechanism by which the semi-digested feed is regurgitated then chewed again finely during long periods (Bae et al. 1983; Weimer et al. 2009). Its purpose is to increase the digestible surfaces of feed materials in order to facilitate the access of microorganisms to degradable polymers. With some respect, the rumination could be compared to the upstream treatments used in certain anaerobic digestion processes in order to enhance the bioconversion of the lignocellulosic biomass. However the mechanism of the rumination (after the microbial attack) and its frequency (multiple grinding) are very specific and

clearly different from current physicochemical pretreatments applied in anaerobic digestion processes. An effective mastication is certainly instrumental in rumen digestion: it reduces plant material to smaller particulate size, then facilitates the depolymerisation of the lignocellulose by the microbial enzymes (Hendriks and Zeeman 2009; Palmowski and Muller 1999). In addition to the protective effect of lignin, plant leaves are also covered by cutin, which reduces even more their degradability. Forage tissues protected by cutin are more resistant to microbial adhesion (Akin 1989; Bauchop 1980). The mechanical grinding occurring with rumination helps to disrupt the protective layer of cutin and exposes more digestible portions of plant particles to hydration and fermentation (Miron et al. 2001). Thus, physical and chemical pretreatments of fibrous substrate before or during microbial fermentation can create more adhesion sites compared with untreated substrates (Miron et al. 2001). On the other hand, cellulolytic enzymes can get trapped in the pores of plant particles, so rumination contributes to their liberation and then facilitates their attachment to new digestible surfaces (Zhang and Lynd 2004).

In summary, rumen houses abundant microbial populations specialized in lignocellulosics digestion; solid retention time in the reticulorumen is relatively long, 1.3–3.7 days. The rumen is a micro-aerobic environment, which promotes the development of cellulase-producing bacteria, fungi and protozoa; this in turn improves their hydrolytic capacity. Effective digestive motility promotes the settling of fine particles, that undergo a second fermentation stage in the lower reticulorumen, and maintains in suspension in the upper portion of reticulorumen, the large particles that therefore remain in the rumination cycle. The rumination mechanism is the essence of the digestion effectiveness of the ruminants. This back and forth integration between grinding (mastication) and enzymatic and microbial attack namely allows for liberation of enzymes and/or microbes from digested particles to new digestible surfaces.

1.2 Fiber digestion by wood-eating termites

More than two thousand species of termite have been identified across the world. Termites are divided into seven families and two main phylogenetic groups i.e., the lower termites (hosting protozoa) and the higher

termites (Breznak and Brune 1994; Wood and Johnson 1986). The digestive system of termites comprises the mouth, the esophagus, the salivary gland, the foregut (crop and gizzard), the midgut and the hindgut (Kane 1997; Watanabe and Tokuda 2010). Digestion begins with the mechanical grinding of wood into particles smaller than 50 μm (Watanabe and Tokuda 2010). Drastic downsizing of wood particles (to 10–20 μm) has recently been reported in the foregut of lower termite *Coptotermes formosanus* (Fujita et al. 2010) in addition to the midgut of higher termite *Nasutitermes takasagoensis* (Tokuda et al. 2001). Cellulolytic enzymes synthesized by the salivary glands are secreted in the foregut, where they act on crystalline and amorphous cellulose (Watanabe et al. 1998). Lower termites produce endoglucanases and endogenous β -glucosidases in the salivary glands (Tokuda 2004; Tokuda et al. 2002; Watanabe et al. 1998). Higher termites secrete endoglucanases only in the midgut (Tokuda 2004; Tokuda et al. 1999), while they produce endogenous β -glucosidases both in the salivary glands and the midgut (Tokuda et al. 2009). The midgut (10–20 nL) is the primary site of cellulose digestion in the flagellate-free termites (Tokuda and Watanabe 2007). The extremely high cellulase concentration in the midgut is a particularity of the termite digestion system. The cellulase concentration inside the midgut of lower termite *Neotermes koshunensis* is about 3 mg/mL with an activity of 10^3 U/mL (Fujita et al. 2010). In comparison the cellulase activity measured from rumen fluid is about $4 \cdot 10^{-2}$ U/mL (Koppolu and Clements 2004). The resulting soluble sugars (mainly glucose and cellobiose) are absorbed through the midgut wall (Fujita et al. 2010). While lignin is degraded in the gut of termites within hours (Breznak and Brune 1994; Geib et al. 2008; Katsumata et al. 2007; Tartar et al. 2009), the mechanism by which termites digest lignin is not clear yet. No lignin digesting enzymes have yet been revealed in termites' symbiotic protozoa. However, endogenous laccases, phenoloxidases and esterases/carboxylesterases were presumed to be involved in lignin degradation, without direct evidence of degradation, on the basis of transcriptome analysis of termite *Reticulitermes flavipes* (Scharf and Tartar 2008; Tartar et al. 2009). These esterases and phenoloxidases enable the solubilisation of hemicellulose by cleaving ester bonds between hemicellulose and monolignols and/or phenolic acids.

The termite's hindgut (approximately 1 μL of volume) is divided into five segments: the ileum, the enteric valve, the paunch, the colon and the rectum (Watanabe and Tokuda 2010). The ileum is a simple tube occupying 20% of the total gut length, which has an internal pH ranging from 10 to 12. Therefore plant materials undergo alkaline treatment along this segment. The paunch, a microbe-packed enlarged hindgut compartment, is known as the fermentation chamber and the site of cellulolytic symbiosis (Hogan et al. 1988a; Noirot 1995; Noirot and Noirot-Timothee 1969). Although some microbes colonize the midgut and rectum, their contribution to wood digestion seems negligible compared to those of the paunch (Tokuda et al. 2001). The paunch region has the most favorable conditions for microbial fermentation with a redox potential ranging from -50 to -270 mV and pH from 6 to 7.5 (Brune and Friedrich 2000). In addition to this axial pH variation along their gut, termites show also radial variations of pH.

Furthermore, the termite's paunch is not completely anaerobic; it shows radial variations of O_2 . Oxygen penetrates into the peripheral hindgut content up to about 150–200 μm below the epithelial surface in both lower and higher termites. Thus only the central portion of the paunch region (40% of this environment) is completely anoxic and allows microbial fermentation (Brune et al. 1995; Veivers et al. 1980). This feature is likely related to lignin breakdown, which requires oxygen (Brune et al. 1995; Scharf and Tartar 2008).

In the hindgut of lower termites, although the presence of numerous bacteria (Brune and Friedrich 2000), protozoa are the main decomposers of cellulose, mainly into acetate (Brauman et al. 1992). Enzymes such as glycosyl hydrolases which are highly active in the hydrolysis of crystalline cellulose, have been revealed by metatranscriptomic analyses of symbiotic protozoal communities from four termites species and a wood-eating cockroach (Todaka et al. 2009). Enzymatic activities such as amylases and proteases have also been detected in crude extracts of hindgut's protozoa (Kane 1997). In the hindgut of higher termites which lack protozoal populations, polysaccharides are degraded by prokaryotic cellulases (Brune 1998; Brune et al. 1995; Watanabe et al. 1998); Warnecke et al. 2007). However, higher termites primarily degrade cellulose in the midgut, which shows a cellulolytic activity ca. 70% higher than the hindgut (Tokuda and Watanabe 2007).

In summary, the mechanisms involved in lignocellulosics digestion by wood-eating termites are not only more complex than those occurring in the biogas digesters, but also than those occurring in the gastrointestinal tracts of mammalian herbivores. Watanabe and Tokuda (2010) pictured the wood digestion system in termites as a three-unit chain: a mechanically grinding system, an enzymatic (cellulases and ligninases) reaction chamber (the midgut) combined with a permeation filter for product recovery (cellobiose, glucose), and a final fermentation chamber (the hindgut) which completes the digestion (74–99% for cellulose and 65–87% for hemicellulose) towards VFAs, mainly acetate. Therefore, contrary to the rumen fermentation, lignocellulose degradation by termites takes place in several stages through the gastrointestinal transit. Mechanical pretreatments take place in the foregut and the midgut. The salivary glands and the midgut epithelium are the main sites of endogenous cellulases production. The first procto-deal segment of the hindgut is a site of alkaline treatment with pH 10–12. The digestion of cellulose is carried out by both endogenous and microbial cellulases (either protozoal in lower termites, or fungal and bacterial in higher termites). The diversity of termites' cellulolytic enzymes as well as the synergism between the host and symbiont enzymes seem critical to release a maximum of glucose units trapped in the lignocellulosic matrix. Lignin, which limits the lignocellulose degradation, undergoes oxidative depolymerization catalyzed by both endogenous enzymes and microbial enzymes that are likely stimulated by oxygen gradients along the gastrointestinal tract. Those lignin depolymerizing phenoloxidases and esterases involved in termites' digestion but also found in several rumen's bacteria (*Lactobacillus*, *Bacillus*, *Clostridium*, etc.) and fungi, mediate the foremost lignocellulose treatment while cellulases or hemicellulases, the downstream polysaccharides hydrolysis that immediately precedes simple sugar fermentation, mainly in the paunch.

2 Microbial communities in herbivores' gut versus biogas digester

Prokaryotic (bacteria and archaea) and eukaryotic (protozoa and fungi) microorganisms play significant roles in the degradation of plant materials in both the

foregut fermentation and the hindgut fermentation (Breznak and Brune 1994; Flint and Bayer 2008; Hungate 1982; Hungate et al. 1964; Larue et al. 2005; Savage 1986). Although a large number of microorganisms living in these environments remains uncultivable to date, numerous microorganisms responsible for the main biochemical activities have been revealed by metagenomic and functional analyses (Flint and Bayer 2008; Warnecke et al. 2007). The rumen microflora is the best known due to the pioneering work of Hungate (1950). The rumen contains approximately 10^{10} bacteria and 10^6 ciliate protozoa per mL, as well as 10^3 – 10^6 cellulolytic fungi per mL. The most active cellulolytic bacteria isolated from the rumen are *Fibrobacter succinogenes*, which belong to a phylum of gram-negative bacteria, and the Firmicutes *Ruminococcus albus* and *Ruminococcus flavefaciens*, which are gram-positive (Table 3). In addition, a variety of hydrolytic and fermentative acidogenic bacteria belonging to the genus *Bacillus*, *Lactobacillus*, *Clostridium*, *Bifidobacterium*, etc. contribute to rumen digestion. Many ciliate and flagellate species of protozoa (*Dasytricha*, *Diplodinium*, *Eodinium*, *Trichomitopsis*) have been isolated from rumen, but the ciliate species were the most important in number (Akkada and Howard 1961; Dehority 2003). The ciliate protozoa are responsible for 30–40% of fiber degradation in the rumen (Demeyer and Van Nevel 1979) (Table 4). All rumen's fungi are capable to colonize plant materials and hence to degrade the major polysaccharides, such as cellulose, starch, pullulan (Williams and Coleman 1985; Williams and Orpin 1987). The main anaerobic fungi found in the rumen belong to the genera *Neocallimastix*, *Piromonas*, and *Sphaeromonas* (Table 5). The fungal rhizoids are able to penetrate plant tissues better than bacteria and protozoa (Lee et al. 2004). Thus, these fungi would be the most rapid to colonize feed surface and hence the first microorganisms to have access to some fraction of the forage (Heath 1988). The main function of rumen fermentation is to provide VFAs from plant polysaccharides. Methanogenesis appears as a secondary microbial activity. However, archaeal methanogens enhance the growth of fermentative bacteria by removing H_2 and CO_2 . That is why among the archaea, the hydrogenotrophic methanogens are the most abundant (*Methanobacterium*, *Methanobrevibacter*, *Methanomicrobium*), while in anaerobic

digesters the acetoclastic methanogens are dominant. Several non-cellulolytic microorganisms also play an essential role in rumen fermentation as they create favourable conditions for cellulolytic organisms by eliminating toxic substances and providing them with growth factors. Like the rumen, different organisms belonging to the *Eucarya*, *Bacteria* and *Archaea* domains have been isolated from the large intestine (caecum and colon) of the mammalian hindgut fermenters, such as horse (Daly et al. 2001; Yamano et al. 2008), elephant (Eloff and van Hoven 1980), hominoid primates (Lambert 1998), etc.

At least 61% of the hindgut content of the termite *Reticulitermes flavipes* consists of microbial cells (Odelson and Breznak 1983). The bulk of these microflora is housed in the paunch. Most of bacteria found in termite guts remain uncultivable to date, but metagenomic analyses have revealed the presence of numerous species of *Spirochete* and *Fibrobacter* (Table 3). Most of the termites' *Spirochete* and *Fibrobacter* have genes encoding for cellulases and xylanases (Schäfer et al. 1996; Tokuda and Watanabe 2007; Warnecke et al. 2007). However, no ligninase coding genes have yet been identified in termites' symbiotic bacteria (Scharf and Tartar 2008; Tartar et al. 2009). Several and unique genera and species of protozoa that are not found anywhere else were isolated in the hindgut of lower termites (Table 4), such as the species of the groups *Hypermastigotes* and *Oxymonads* (Dyer 2002; Todaka et al. 2007). Over 434 species of protozoa have been reported from the intestine of lower termites (Hogan et al. 1988b). In lower termites such as *Reticulitermes flavipes*, eukaryotic (protozoal) symbionts are considered to contribute significantly to cellulose digestion (Breznak and Brune 1994; Scharf and Tartar 2008). Higher termites in the subfamily *Macrotermittinae* have established an ectosymbiotic relationship with the basidiomycete fungi belonging to the genus *Termitomyces* (Table 5) (Breznak and Brune 1994; Tokuda 2004). Ectosymbiotic fungi provide termites with carbohydratases, including cellulases and xylanases, and lignin peroxidases (Hyodo et al. 2003; Johjima et al. 2006; Martin and Martin 1978; Taprab et al. 2005). Expressed sequence tag analyses applied to *Termitomyces* have also revealed genes encoding for several glycosyl hydrolase families and laccases under conditions of the termite digestion system (Johjima et al. 2006).

Table 3 Bacteria and archaea commonly isolated from rumen, termite guts and biogas digesters

Bacteria	Rumen (cells/mL)	Termites (cells/mL) ^a	Anaerobic digesters (cells/mL)	Function	Reference
<i>Acetoanaerobium</i>	10 ⁵ –10 ⁶		10 ⁵	Acetogenic	(Anderson et al. 2003; Morvan et al. 1996)
<i>Acetobacterium</i>	10 ⁵ –10 ⁶		10 ⁵	Acetogenic	(Anderson et al. 2003; Morvan et al. 1996)
<i>Acetogenium</i>	10 ⁵ –10 ⁶		10 ⁵	Acetogenic	(Anderson et al. 2003; Morvan et al. 1996)
<i>Acinetobacter</i>	10 ⁷ –10 ⁸	10 ¹⁰ –10 ¹¹		Oxidative bacterium	(Konig 2006; Stewart and Bryant 1988)
<i>Bacillus</i>	10 ⁶ –10 ⁷	10 ¹⁰ –10 ¹¹	10 ⁸ –10 ⁹	Hydrolytic, acidogenic	(Anderson et al. 2003; Konig 2006; Williams and Withers 1983)
<i>Bacteroides</i>	10 ⁸ –10 ⁹	10 ¹⁰ –10 ¹¹	10 ⁶ –10 ⁸	Acidogenic, acetogenic	(Anderson et al. 2003; Morvan et al. 1996)
<i>Bifidobacterium</i>	10 ⁸ –10 ⁹			Hydrolytic, acidogenic	(Stewart and Bryant 1988)
<i>Butribacterium</i>	10 ⁵ –10 ⁶		10 ⁵	Acetogenic	(Anderson et al. 2003; Morvan et al. 1996)
<i>Butyribacterium</i>			10 ⁶ –10 ⁸	Acidogenic	(Anderson et al. 2003)
<i>Butyrivibrio</i>	10 ⁸ –10 ⁹			Hydrolytic, acidogenic	(Rychlik and Russell 2002)
<i>Citrobacter</i>		10 ¹⁰ –10 ¹¹		Oxidative bacterium	(Konig 2006)
<i>Clostridium</i>	10 ⁶ –10 ⁷	10 ⁹ –10 ¹¹	10 ⁸ –10 ⁹	Hydrolytic, acidogenic	(Anderson et al. 2003; Reilly and Attwood 1998; Wenzel et al. 2002)
<i>Desulfobacter</i>			10 ⁶ –10 ⁸	Acidogenic	(Anderson et al. 2003)
<i>Desulfovibrio</i>		10 ¹⁰		Hydrolytic, acidogenic	(Wenzel et al. 2002)
<i>Enterobacter</i>	10 ⁸ –10 ⁹	10 ¹⁰		Hydrolytic, acidogenic	(Stewart and Bryant 1988; Wenzel et al. 2002)
<i>Escherichia</i>			10 ⁶ –10 ⁸	Acidogenic	(Anderson et al. 2003)
<i>Eubacterium</i>	10 ⁷ –10 ⁸		10 ⁶ –10 ⁸	Acidogenic	(Anderson et al. 2003; Stevenson and Weimer 2007)
<i>Fibrobacter</i>	10 ⁸ –10 ⁹	10 ¹⁰ –10 ¹¹		Hydrolytic bacteria	(Shinkai et al. 2009)
<i>Klebsiella</i>		10 ¹⁰ –10 ¹¹		Oxidative/fermentative bacterium	(Konig 2006)
<i>Lactobacillus</i>	10 ⁸ –10 ⁹		10 ⁶ –10 ⁸	Acidogenic	(Hungate et al. 1964)
<i>Methanobacterium</i>	10 ⁴ –10 ⁸	10 ⁸ –10 ⁹	10 ⁶ –10 ⁸	H ₂ /CO ₂ -methanogens	(Anderson et al. 2003; Hungate et al. 1964; Morvan et al. 1996; Tokura et al. 2000)
<i>Methanobolus</i>			10 ⁶ –10 ⁸	Ac-methanogens	(Anderson et al. 2003)
<i>Methanobrevibacter</i>	10 ⁴ –10 ⁸	10 ⁸ –10 ⁹	10 ⁶ –10 ⁸	H ₂ /CO ₂ -methanogens	(Anderson et al. 2003; Dehority 2002; Morvan et al. 1996; Tokura et al. 2000)
<i>Methanococcus</i>			10 ⁶ –10 ⁸	H ₂ /CO ₂ -methanogens	(Anderson et al. 2003)
<i>Methanogenium</i>			10 ⁶ –10 ⁸	H ₂ /CO ₂ -methanogens	(Anderson et al. 2003)

Table 3 continued

Bacteria	Rumen (cells/mL)	Termites (cells/mL) ^a	Anaerobic digesters (cells/mL)	Function	Reference
<i>Methanomicrobium</i>	10 ⁴ –10 ⁸	10 ⁸ –10 ⁹	10 ⁶ –10 ⁸	H ₂ /CO ₂ -methanogens	(Anderson et al. 2003; Hungate et al. 1964; Morvan et al. 1996; Tokura et al. 2000)
<i>Methanosaeta</i>			10 ⁶ –10 ⁸	Ac-methanogens	(Anderson et al. 2003)
<i>Methanosarcina</i>	10 ⁴ –10 ⁸	10 ⁸ –10 ⁹	10 ⁶ –10 ⁸	Ac-methanogens	(Anderson et al. 2003; Hungate et al. 1964; Morvan et al. 1996; Tokura et al. 2000)
<i>Methanospirillum</i>			10 ⁶ –10 ⁸	H ₂ /CO ₂ -methanogens	(Anderson et al. 2003)
<i>Methanotrix</i>			10 ⁶ –10 ⁸	Ac-methanogens	(Anderson et al. 2003)
<i>Micrococcus</i>			10 ⁸ –10 ⁹	Hydrolytic, acidogenic	(Anderson et al. 2003)
<i>Ochrobactrum</i>		10 ¹⁰		Hydrolytic, acidogenic	(Wenzel et al. 2002)
<i>Pelobacter</i>	10 ⁵ –10 ⁶			Acetogenic	(Anderson et al. 2003; Morvan et al. 1996)
<i>Peptococcus</i>			10 ⁸ –10 ⁹	Hydrolytic	(Anderson et al. 2003)
<i>Prevotella</i>	10 ⁸ –10 ⁹			Hydrolytic	(Stevenson and Weimer 2007)
<i>Propionibacterium</i>			10 ⁸ –10 ⁹	Acidogenic	(Anderson et al. 2003)
<i>Pseudomonas</i>			10 ⁶ –10 ⁸	Acidogenic	(Anderson et al. 2003)
<i>Ruminococcus</i>	10 ⁸ –10 ⁹		10 ⁶ –10 ⁸	Acidogenic	(Anderson et al. 2003)
<i>Spirochaeta</i>	10 ⁶ –10 ⁷	10 ¹⁰ –10 ¹¹		Hydrolytic, acidogenic, acetogenic, N ₂ fixation	(Paster and Canale-Parola 1982; Paster et al. 1996)
<i>Streptococcus</i>	10 ⁸ –10 ⁹		10 ⁶ –10 ⁸	Acidogenic	(Anderson et al. 2003; Stewart and Bryant 1988)
<i>Streptomyces</i>		10 ¹⁰		Hydrolytic	(Wenzel et al. 2002)
<i>Syntrophobacter</i>			10 ⁴ –10 ⁵	Acetogenic	(Anderson et al. 2003; Morvan et al. 1996)
<i>Syntrophomonas</i>			10 ⁴ –10 ⁵	Acetogenic	(Anderson et al. 2003; Morvan et al. 1996)
<i>Vibrio</i>			10 ⁸ –10 ⁹	Hydrolytic	(Anderson et al. 2003)

H₂/CO₂-methanogens, Hydrogenophilic methanogens; Ac-methanogens, Acetoclastic methanogens

Data shown in this table give an idea on the bacterial genera commonly isolated from rumen, termites, and anaerobic digestion facilities. However, the distribution and abundance of microbial populations are based on the substrates to be digested and the environmental conditions. Several bacterial species not included in this table have also been isolated from rumen, termite guts and anaerobic bioreactors in either large (more than 10⁶ cell/mL) or small quantities, but their direct involvement in the fermentation of organic matters was not demonstrated

^a Bacteria content in termites has been expressed in No. cells/mL assuming a termites' gut volume of 1 µl

Table 4 Protozoa commonly isolated from rumen, termite hindguts and biogas digesters

Protozoa	Rumen (cells/mL)	Termites (cells/mL) ^a	Anaerobic digesters (cells/mL)	Function	Reference
<i>Amoeboflagellates</i>			10 ² –10 ³	Unknown role in digestion	(Brad et al. 2008; Priya et al. 2008)
<i>Cyclidium</i>			10 ² –10 ³	Unknown role in digestion	(Brad et al. 2008; Priya et al. 2008)
<i>Dasytricha</i>	10 ⁵ –10 ⁶			Hydrolytic, acidogenic	(Dijkstra and Tamminga 1995; Williams and Coleman 1985)
<i>Diplodinium</i>	10 ⁵ –10 ⁶			Hydrolytic, acidogenic	(Williams and Coleman 1985)
<i>Dinenympha</i>		10 ⁷ –10 ⁹		Hydrolytic, acidogenic	(Hongoh et al. 2007; Lai et al. 1983; Moriya et al. 2003)
<i>Entodinium</i>	10 ⁵ –10 ⁶			Hydrolytic, acidogenic	(Dijkstra and Tamminga 1995; Williams and Coleman 1985)
<i>Eodinium</i>	10 ⁵ –10 ⁶			Hydrolytic, acidogenic	(Dijkstra and Tamminga 1995; Williams and Coleman 1985)
<i>Hexamastix</i>	10 ⁵ –10 ⁶			Hydrolytic, acidogenic	(Dijkstra and Tamminga 1995; Williams and Coleman 1985)
<i>Isotrichia</i>	10 ⁵ –10 ⁶			Hydrolytic, acidogenic	(Dijkstra and Tamminga 1995; Williams and Coleman 1985)
<i>Naegleria</i>			10 ² –10 ³	Unknown role in digestion	(Brad et al. 2008; Priya et al. 2008)
<i>Oxomonas</i>		10 ⁷ –10 ⁹		Hydrolytic, acidogenic	(Hongoh et al. 2007; Moriya et al. 2003)
<i>Pyrsonympha</i>		10 ⁷ –10 ⁹		Hydrolytic, acidogenic	(Hongoh et al. 2007; Moriya et al. 2003)
<i>Prorodon</i>			10 ² –10 ³	Unknown role in digestion	(Brad et al. 2008; Priya et al. 2008)
<i>Pseudotrichonympha</i>		10 ⁷ –10 ⁹		Hydrolytic, acidogenic	(Hongoh et al. 2007; Lai et al. 1983)
<i>Sporotrichonympha</i>		10 ⁷ –10 ⁹		Hydrolytic, acidogenic	(Hongoh et al. 2007)
<i>Rhynchomonas</i>			10 ² –10 ³	Unknown role in digestion	(Brad et al. 2008; Priya et al. 2008)
<i>Tetramitus</i>			10 ² –10 ³	Unknown role in digestion	(Brad et al. 2008; Priya et al. 2008)
<i>Tricercomitus</i>	10 ⁵ –10 ⁶			Hydrolytic, acidogenic	(Dijkstra and Tamminga 1995; Williams and Coleman 1985)
<i>Trichomitopsis</i>	10 ⁵ –10 ⁶	10 ⁷ –10 ⁹		Hydrolytic, acidogenic	(Hongoh et al. 2007; Lai et al. 1983)
<i>Trichonympha</i>		10 ⁷ –10 ⁹		Hydrolytic, acidogenic	(Hongoh et al. 2007; Lai et al. 1983)
<i>Vorticella</i>			10 ² –10 ³	Unknown role in digestion	(Brad et al. 2008; Priya et al. 2008)
<i>Trichomonas</i>			10 ² –10 ³	Unknown role in digestion	(Anderson et al. 2003; Fenchel and Finlay 1990)
<i>Metropus</i>			<2.10 ²	Unknown role in digestion	(Anderson et al. 2003; Fenchel and Finlay 1990)
<i>Saprodinium</i>			<2.10 ²	Unknown role in digestion	(Anderson et al. 2003; Fenchel and Finlay 1990)
<i>Hartmanella</i>			<2.10 ²	Unknown role in digestion	(Anderson et al. 2003; Fenchel and Finlay 1990)
<i>Trimyema</i>			<2.10 ²	Unknown role in digestion	(Anderson et al. 2003; Fenchel and Finlay 1990)

^a Protozoa content in termites has been expressed in No. cells/mL assuming a termites' gut volume of 1 µl. Like the bacterial populations, several species of protozoa can be found in anaerobic digestion facilities but their direct participation in the hydrolysis of organic matters remains to be determined

Table 5 Fungi commonly isolated from rumen, termite hindguts and biogas digesters

Fungi	Rumen (cells/mL)	Termites (Cells/mL) ^a	Anaerobic digesters (cells/mL)	Function	Reference
<i>Anaeromyces</i>	10 ³ –10 ⁶			Hydrolytic, acidogenic	(Heath 1988; Savage 1986)
<i>Ascomycetes</i>			10–10 ³	Unknown role in digestion	(Schnurer and Schnurer 2006)
<i>Aspergillus</i>		nd	10–10 ³	Hydrolytic	(Schnurer and Schnurer 2006; Varma et al. 1994)
<i>Alternaria</i>		nd		Hydrolytic, acidogenic	(Schnurer and Schnurer 2006; Varma et al. 1994)
<i>Caecomyces</i>	10 ³ –10 ⁶			Hydrolytic, acidogenic	(Heath 1988; Savage 1986)
<i>Cladosporium</i>		nd		Hydrolytic	(Schnurer and Schnurer 2006; Varma et al. 1994)
<i>Fusarium</i>		nd		Hydrolytic, acidogenic	(Schnurer and Schnurer 2006; Varma et al. 1994)
<i>Neocallimastix</i>	10 ³ –10 ⁶			Hydrolytic, acidogenic	(Heath 1988; Savage 1986)
<i>Orpinomyces</i>	10 ³ –10 ⁶			Hydrolytic, acidogenic	(Heath 1988; Savage 1986)
<i>Penicillium</i>		nd		Hydrolytic, acidogenic	(Schnurer and Schnurer 2006; Varma et al. 1994)
<i>Phycomycetes</i>			10–10 ³	Unknown role in digestion	(Schnurer and Schnurer 2006)
<i>Piromyces</i>	10 ³ –10 ⁶			Hydrolytic, acidogenic	(Heath 1988; Savage 1986)
<i>Termitomyces</i>		nd		Hydrolytic	(Schäffer et al. 1996)
<i>Thermomyces</i>			10–10 ³	Unknown role in digestion	(Schnurer and Schnurer 2006)

Only the higher termites in the subfamily *Macrotermitinae* have an ectosymbiotic relationship with the basidiomycete white-rot fungus *Termitomyces*. These play important role in fiber digestion in the termites *Macrotermitinae*. On the other hand, lower termites harbour various fungi such as *Aspergillus* and *Cladosporium* with genes encoding for cellulases and xylanases, but the abundance and the contribution of these fungi in the in vivo degradation of plant materials need to be clarified

^a The quantities of these fungi in the gut of termites is not available

Like mammalian herbivores and wood feeding insects, the microbial community of anaerobic digesters is composed of large number of different organisms belonging to the *Eucarya*, *Bacteria*, and *Archaea* domains. However, anaerobic bacteria and archaea represent the major phylogenetic group within the anaerobic digesters community. Anaerobic digestion populations include such physiological groups as hydrolytic and fermentative acidogenic *Bacteria* (e.g. *Clostridium*, *Bacillus*, *Bacteroides*, *Vibrio*, *Ruminococcus*), acetogenic syntrophic bacteria (e.g. *Acetobacterium*, *Butyribacterium*, *Syntrophomonas*), hydrogenotrophic and acetotrophic methanogens (e.g. *Methanobacterium*, *Methanosarcina*, *Methanococcus*), and sulfate-reducing *Bacteria* (e.g. *Desulfobacter*, *Syntrophobacter*, *Desulfovibrio*). The Eukaryotic microorganisms (fungi, yeast and protozoa) isolated from anaerobic digesters have no known role in the degradation of complex polymers such as lignocellulose (Priya et al. 2008).

The attachment of the cellulolytic microorganisms to plant materials is a prerequisite for the digestion of lignocellulosics in both the rumen and the anaerobic digesters (Confer and Logan 1997; McAllister et al. 1994; Pell and Schofield 1993). In rumen bacteria, protozoa and fungi rapidly colonize plant materials entering the rumen (Fonty et al. 1995). Thus, the rumen's microflora occupies three distinct ecological areas, i.e. the liquid phase, the food particles and the gut mucus (McAllister et al. 1994). More than seventy-five percent of rumen bacteria are tightly attached to both feed particles and mucus (Hungate et al. 1966; McCowan et al. 1978). The rumen fungi are primarily attached to food particles, while protozoa are attached to both food particles and gut mucus. The cellulolytic microorganisms preferentially attach to readily digestible inner tissues through stomata and mechanically damaged areas (Akin and Benner 1988; Fonty et al. 1988). Three structures seem to play essential role in microbial adhesion, i.e. the cellulosome (Adams et al. 2006; Doi 2008; Wilson 2008), the exopolysaccharides (Lamed and Bayer 1988) and the cellulase binding domaine (Brulc et al. 2009; Doi 2008). The attachment of microorganisms to both the gut mucus and plant particles improves cellulolytic activities by concentrating cellulase on target plant tissues. In addition, the attachment of microbial cells such as protozoa (whose doubling times are longer than the turnover of both solids and liquids) prevents

them to be washed-out. Hence microbial adhesion appears as the first and an essential step in the microbial digestion of plant materials through both the rumen and anaerobic digester.

3 From animal digestive tracts to anaerobic digestion processes

Analogies have been established between both hindgut and foregut fermentation systems and typical ideal chemical reactors, such as the batch reactor (BR), the continuous flow stirred-tank reactor (CSTR), and the plug-flow reactor (PFR, also called tubular reactor), either alone, or in serial combinations (Dehority 2002; Horn and Messer 1992; Hume 1997, 2002; Jumars 2000b; Logan et al. 2003; Penry and Jumars 1987; Sakaguchi 2003). Due to the continuous flow of digesta through a tubular vessel i.e. the caecum and colon, the hindgut fermentation was first compared to plug-flow reactor (PFR) (Hume 1997). In typical PFR the reactants are instantaneously and continuously mixed in the radial direction, but not axially with appreciable extent, while materials leave the reactor in the same order as they enter (Jumars 2000b). Plant materials entering the large intestine pass along a tubular reactor as a plug whose concentration decreases during passage from the inlet to the outlet. However the large intestine fermentation is not an ideal PFR because of: (1) an axial mixing due to the peristaltic and antiperistaltic contraction of the gut (Lai et al. 1983; Weems 1987); (2) the secretion from blood to lumen across the mucus (Engelhardt et al. 1984); (3) the absorption of water and solutes from lumen to blood (Jumars 2000a). Thus the hindgut fermentation could be evaluated as a modified-PFR, with several CSTR connected in series (Hume 2002). In the modified-PFR the feeding was instantaneously mixed in all directions in each CSTR vessel and along the reactor series. The modified-PFR also integrated hydrolysis and absorption reactions in series. The robustness of this model has been demonstrated experimentally by comparing the magnitude of both hydrolysis and absorption between one PRF and ten CSTR in series (Jumars 2000a). The foregut fermentation (rumen fermentation) could be compared to a modified-CSTR. Like the hindgut fermentation, the rumen fermentation also couples hydrolysis and absorption

reaction during microbial digestion. However the rumen fermentation is special in that it leads to a long settling of finely digested material in its ventral sac while undigested particles remain in the dorsal rumen. Numerous bioreactors were designed to degrade the lignocellulosic biomass with rumen microbial consortium. The processes developed in this field range from the very simple apparatus such as a batch reactor (Cerrato-Sanchez et al. 2007; Hu et al. 2004, 2007; Raizada et al. 2003; Seon et al. 2003) to a complicated apparatus designed to work on the continuous mode and even equipped with semi-permeable membranes to remove the VFAs produced (Czerkawski and Cheng 1988; Dalhoff et al. 2003; Gijzen et al. 1988; Nair et al. 2005). These anaerobic digestion processes were related to both animal physiology and environmental bioengineering in order to respectively improve the nutrition of livestock as in the rumen simulation technique (RUSITEC) and to improve the production of biogas from lignocellulosic wastes as in the rumen derived anaerobic digestion system (RUDAD).

The RUSITEC technique remains to date the most widely used continuous-flow type of rumen simulating system (Hillman et al. 1991). The RUSITEC technique was developed by Czerkawski and Breckenridge (1977) and was then the subject of many reproductions and adjustments. The RUSITEC technique could be briefly defined as an anaerobic digestion process using a rumen-based microbial inoculum, whose compartmentalization through the reactor provides three main configurations of microbial populations in the rumen. Firstly the free microorganisms markedly influenced by dilution rate, then the microorganisms loosely attached to feed particles, and finally the microorganisms firmly bound to feed particles (cellulolytic bacteria). To achieve this, solid rumen content was placed in a nylon bag and the material to digest, in a second bag, then both bags were placed in a perforated cage, slided in a reactor filled with artificial saliva. Despite its utilization in several works the RUSITEC technique remains a subject of critical discussion (Czerkawski 1990; Muetzel et al. 2009). The RUSITEC obviously lacks the microbial populations attached to the mucus. In addition, the solid turnover time is fixed by a manual replacement of the undigested solids in the nylon bags, which led to the removal of important microbial populations attached

to feed particles. Hoover et al. (1976) designed a continuous flow system of rumen fermentation in order to increase the maintenance of rumen protozoa in the fermentation vessel by filtering the overflow effluent. This delayed the solid retention time, without affecting the fermentation performance. However, the outflow filtration system was continuously clogged. Teather and Sauer (1988) proposed a partially stirred fermenter using rumen-based microbial inocula, with the overflow at the bottom third of the reactor and a spiral blade that gently mixed the material by slicing through it. This reactor allowed the stratification of the solid suspended particles according to their density, because materials thoroughly digested tended to sink in the reactor bottom. Thus materials that left the system were selected by gravity, in much the same manner as feed particles do through the rumen. This fermentation system mimics better the rumen functioning than the RUSITEC practice described previously. However, like previously, the outflow system was easily clogged, especially when fibre-rich substrates were used. A continuous apparatus similar to RUSITEC was proposed to circumvent the clogging problems (Muetzel et al. 2009). A modification of the overflow allowed maintaining of both the settling of digesta as in the previous model and the dispersion of suspended solid particles, which prevented the outflow system from clogging. This process also allowed the formation of a raft mat inside the fermentation vessel where protozoa could survive. Hence the apparatus represents quite well rumen fermentation in terms of both end products and microbial activity. However a change in the microbial balance was reported by authors. All those RUSITEC trials targeted the understanding of feedstuff degradation by rumen microorganisms, hence the improvement of the VFAs availability. The importance of eukaryotic organisms such as ciliate protozoa and fungi in the digestion of lignocellulosic material and the strategies to keep them alive in fermentation vessels have been demonstrated with RUSITEC reactors (Fuchigami et al. 1989; Hino et al. 1993; Weller and Pilgrim 1974). Those RUSITEC-based results have clearly shown that the microbial fermentation taking place in both the rumen and large intestine are biological processes that go beyond the environmental conditions generally found in the biogas digesters.

Researchers have long considered the application of the rumen fermentation principles into industrial anaerobic digestion systems as an attractive strategy to reduce and stabilize lignocellulosic wastes with simultaneous recovery of biogas as renewable energy. The most elaborated one-stage anaerobic digestion system using rumen-based microbial inoculums is the rumen anaerobic sequencing batch reactor (ASBR) (Barnes and Keller 2003). The Rumen-ASBR is based on the ASBR technique proposed by Dague et al. (1992). The operating principle of the ASBR consists of a four-step cycle (feeding, reaction, settling and decanting). The reaction stage functions as an ideal batch reactor with instantaneous and complete mixing of the reactant after entering the reactor. Barnes and Keller (2003), operated the Rumen-ASBR with 6-h cycles. The reactor was fed with rumens' inoculums, 2 L of artificial saliva and 20–30 g L⁻¹ of α -cellulose. It produced 210–230 mg COD/L·h of VFAs at cellulose loading rate of 10 g/L·h. However, the Rumen-ASBR remains quite different from the rumen functioning. The only link that can be done between this fermentation system and the rumen functioning regards to the microbial population. In addition, the authors do not provide information about the influence that may have fermentation cycles on the retention of eukaryotic organisms (protozoa and anaerobic fungi). On the other hand, the design of the Rumen-ASBR seems to be complicated and requires both continuous solids handling and filtration of the high-solids slurry that makes the process expensive and difficult to scale-up.

The two-stage RUDAD system, first developed by Gijzen et al. (1987), was more effective as compared to one-stage processes. The RUDAD concept assumed that a single reactor could not provide at the same time optimal conditions to both the hydrolytic and acidogenic microorganisms and the archaeal methanogens (Kivaisi and Mtila 1998). Thus, the RUDAD functions with two reactors in serial connection. Hydrolysis and acidogenesis are performed in the first reactor (acidogenic rumen reactor) and methane is produced from the liquid effluent of the acidogenic reactor in a high-rate methanogenic reactor. The acidogenic rumen reactor could be a continuous stirred reactor or a batch reactor. The high-rate methanogenic reactor could be an upflow anaerobic sludge blanket (UASB) reactor, an upflow anaerobic filter, a downflow anaerobic filter, or a fluidized bed reactor. The two-stage technology

was thought (1) to increase the steadiness of the overall process (Lai et al. 1983), (2) to provide archaea with protection against shock loads (Poirot et al. 1991), (3) to remove (at least in part) the compounds toxic to archaea (Kivaisi and Eliapenda 1995), (4) to enrich the methane content of the biogas, because most of diluent CO₂ would be produced during acidogenic stage (Kivaisi and Eliapenda 1995). The steadiness and the efficiency of the RUDAD process in biogas production have been demonstrated in numerous studies (Gijzen et al. 1987; Kivaisi and Eliapenda 1995; Nair et al. 2005). Certain RUDAD processes have been patented (Hack and Vellinga 1995; Kubler 1996) and at least four industrial plants using RUDAD technique were built in Europe for the treatment of municipal solid wastes (Deublein and Steinhauser 2008). Attempts were done to improve the hydrolysis stage of RUDAD, including mechanical pretreatment and enrichment of the rumen microbial consortia with ciliate protozoa (Hack and Vellinga 1995; Kubler 1996). However, the rate of solids breakdown in the RUDAD processes as measured from the production of VFA (around 6 g COD-based VFA/L·d) remained lower than that of the true rumen (around 18 g COD-based VFA/L_{rumen}·d) (Nair et al. 2005). Thus the acidogenic stage still remains the limiting step of the overall fermentation system and further the filtration of the liquid effluent from the acidogenic causes problems that keep RUDAD process difficult to scale-up. Hence, the effectiveness of the RUDAD fermenters based on the high rate of biogas recovered seems to be related to the high-rate methanogenic reactors (UASB) used in the second stage and not in the effectiveness of the acidogenic reactor. Therefore, the development of the RUDAD technique still has lessons to learn from the digestive strategy of ruminants.

4 Conclusion and prospects

Bioreactors are designed to control and amplify biochemical reactions taking place in nature. For years, several bioreactors were designed to reproduce rumen fermentation conditions for in vitro anaerobic digestion of lignocellulosic biomass. However, no system developed in this context has yet reached animal digestive performances. The rate of solids breakdown through rumen is almost three times as higher as anaerobic digesters, and much higher in

termites, which undoubtedly highlights the effectiveness of animal digestion. Both the mammalian herbivores and the wood-eating insects do not only provide environmental conditions (redox potential, pH, temperature, etc.) in their guts for microbial fermentation as observed in most biogas digesters. The microbial fermentation taking place in the foregut and the hindgut are only one aspect of the digestive strategy of animals. Hence digestion should not be reduced to only the microbial interactions taking place in these environments. Additional digestion mechanisms of ruminants and termites can be recapitulated as following.

- Along the gastrointestinal tracts, the microbial fermentation is integrated with other non-microbial treatments. For instance rumination provides for back and forth integration of mechanical, enzymatic and microbial treatments. Another instance is the mechanical/enzymatic or alkaline/enzymatic treatment in termites at mid-way between the mouth and the hindgut.
- The bolus motility in the rumen separates fine particles that settle in the ventral sac for final fermentation, from large particles (undigested) that are maintained in the rumination cycle.
- The concerted actions of a variety of enzymes including cellulases, xylanases, esterases and ligninases, some of them being produced by eukaryotic organisms, or by the xylophagous insects themselves improve the yield of the microbial digestion.
- The radial and axial oxygen gradients along the gastrointestinal tract may stimulate protozoal, fungal and facultative bacterial populations, and in turn their hydrolytic activities.
- Continuous removal of VFAs by absorption and of H_2 by methanogenesis, from both the rumen and the hindgut, eases the fermentation rate.
- Adhesion to the epithelium and trapping in the mucus dissociate microbial populations from the bolus transit and retain them in the digestive tract, while adhesion to plant particles or fibers allows the bacteria to reach the digestible surface and improves the hydrolytic attack.

Developments for betterment of anaerobic digestion of lignocellulosic biomass should go beyond the simple imitation of the environmental conditions (pH, redox, temperature, etc.) of both the rumen and the

hindgut. Attempts to biomimick the digestive strategies of ruminants and hindgut fermenters such as termites, as above outlined, may include the following adjustments: back and forth cycling of mechanical/chemical pretreatment and microbial fermentation; decoupling of the acidogenic and methanogenic stages so to focus on conditions optimization of the fermentative microbial group towards significant improvement of their hydrolytic functionalities; mixing patterns so to partition large undigested particles from the fine digestate; bioaugmentation with protozoa and fungi populations, namely in the hydrolytic compartment; controlled micro-aerobic conditions to stimulate protozoa and fungi populations as well as hydrolytic facultative bacteria; fixation of part of the microbial populations and dissociation between the retention times of microorganisms, solids and liquid.

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