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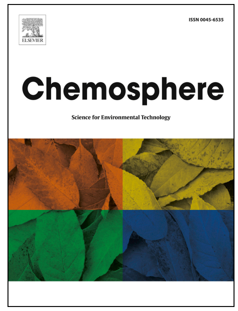

Application of rumen and anaerobic sludge microbes for bio harvesting from lignocellulosic biomass

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Application of rumen and anaerobic sludge microbes for bio harvesting from lignocellulosic biomass

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Abstract

This study investigated the production of biogas, volatile fatty acids (VFAs), and other soluble organic from lignocellulosic biomass by two microbial communities (i.e. rumen fluid and anaerobic sludge). Four types of abundant lignocellulosic biomass (i.e. wheat straw, oaten hay, lurence hay and corn silage) found in Australia were used. The results show that rumen microbes produced four-time higher VFAs level than that of anaerobic sludge reactors, indicating the possible application of rumen microorganism for VFAs generation from lignocellulosic biomass. VFA production in the rumen fluid reactors was probably due to the presence of specific hydrolytic and acidogenic bacteria (e.g. *Fibrobacter* and *Prevotella*). VFA production corroborated from the observation of pH drop in the rumen fluid reactors indicated hydrolytic and acidogenic inhibition, suggesting the continuous extraction of VFAs from the reactor. Anaerobic sludge reactors on the other hand, produced more biogas than that of rumen fluid reactors. This observation was consistent with the abundance of methanogens in anaerobic sludge inoculum (3.98% of total microbes) compared to rumen fluid (0.11%). VFA production from lignocellulosic biomass is the building block chemical for bioplastic, biohydrogen and biofuel. The results from this study provide important foundation for the development of engineered systems to generate VFAs from lignocellulosic biomass.

Key words: Lignocellulosic biomass; Rumen fluid; Anaerobic sludge; Volatile fatty acids, Biogas; Bio harvesting.

1. Introduction

Lignocellulosic biomass are residues from agricultural and forestry industries with an estimation of 10 billion tons annually. The conventional view of the residues is that they need to be disposed of to prevent the spread of disease in the next cropping season. An alternative view is that the residues, as lignocellulosic biomass, are a great reserve of carbon, the keystone of energy and raw chemical production (Nanda et al., 2015; Sawatdeenarunat et al., 2015). Lignocellulosic biomass has a net calorific value of up to 20 MJ/kg. However, the economic value of alternate uses such as electricity generation through incineration is relatively small due to high moisture content in lignocellulosic biomass. An alternative use of lignocellulosic biomass will probably pave the way for the production of raw chemicals and energy that currently depends on fossil resources. Harvesting processes from lignocellulosic biomass have gained an upward trajectory in the last two decades; however, the recalcitrant structure of lignocellulosic biomass is the main bottleneck that still requires substantial research to overcome (Rouches et al., 2016; Sawatdeenarunat et al., 2015).

Current methods to extract raw chemicals and energy from lignocellulosic biomass have low productivity (Nanda et al., 2015; Sawatdeenarunat et al., 2015). This is because the chemical compositions and structure of lignocellulosic biomass (which includes cellulose, hemicellulose and lignin) requires high energy or corrosive chemicals to break it down (Sawatdeenarunat et al., 2015; Zabed et al., 2016). Processes that have been investigated include a physical process (e.g. steam explosion and grinding); chemical process (e.g. sulphuric, nitric acids, sodium hydroxide and urea soaking); and protein engineering to improve the performance of existing lignocellulose-degrading enzymes (Sawatdeenarunat et al., 2015; Wen et al., 2009). The physical process methods and chemical process methods are limited in their effectiveness, create environmental hazards, and are energy intensive. The protein engineering methods have achieved only modest results in improving lignocellulosic biomass hydroxylation (Wen et al., 2009). This is mostly due to our limited understanding of the mechanisms of biomass hydroxylation and the relatively low activity of currently available hydrolytic enzymes.

Specific microbial communities from a termite gut, from the digestive tract of ruminant animals and from anaerobic digester have shown the capability of degrading lignocellulosic biomass. The rumen microbial community has evolved in the rumen environment for million years to digest lignocellulosic biomass to produce volatile fatty acids (VFAs) and biogas. The symbiotic relationship between the rumen and its microbial community has led to the evolution of lignocellulosic-degrading bacteria that have not been found to proliferate elsewhere. Likewise, the microbial community in 1 μL termite gut is also specific for lignocellulosic degradation. Recently, Lazuka et al. (2018) has reported that a consortium of lignocellulosic-degrading bacteria can be achieved in an engineered anaerobic reactor under sterile conditions. Anaerobic microbial community from the anaerobic digester has demonstrated the efficiency of converting organic waste to energy (i.e. biogas) (Nghiem et al., 2017; Yue et al., 2013). Research in the application of these microbial communities for lignocellulosic biomass degradation has gained promising results (Sawatdeenarunat et al., 2015; Takizawa et al., 2018; Wang et al., 2018; Zhang et al., 2017). Takizawa et al. (2018) reported that rumen fluid pretreatment of paper sludge increased 3.4 times methane production. Zhang et al. (2017) observed an enhancement of cellulose degradation due to rumen microbes addition in anaerobic digestion. Therefore, microbial community sources (e.g. rumen fluid and anaerobic sludge) could be used to produce VFAs and energy from lignocellulosic biomass.

The study aims to investigate the production of VFAs and biogas as well as soluble chemical oxygen demand from lignocellulosic biomass by rumen fluid and anaerobic sludge microbial communities. The production rate was investigated in a biomethane potential assay that provided conditions simulating anaerobic digestion process. 16S rRNA gene-based community was employed to reveal the microbial community composition in rumen fluid and anaerobic sludge. The results of microbial community analysis provided support evidence to the different observation in production rate between two communities. Results from this study provided preliminary background for the development of an engineered system to generate VFAs from lignocellulosic biomass.

2. Materials and methods

2.1 Lignocellulosic biomass and inoculum sources

Four lignocellulosic materials namely wheat straw (WS), lurence hay (LH), oaten hay (OH) and corn silage (CS) were obtained from a local pet store. These are some of the most abundant lignocellulosic biomass in Australia. They were washed with Milli-Q water and dried at 60 °C for 24 h. Then, they were milled and sieved through a 600-µm pore size sieve (Fig 1a). The resultant was characterized for moisture, volatile solid (VS) and ash content and stored in a zip bag at room temperature until use. The VS contents of all four lignocellulosic materials were above 90% (Table 1). The lignocellulosic biomasses have substantial levels of COD (500-1000 kg COD/kg biomass). Therefore, these materials have high potential as feedstocks for anaerobic digestion.

Table 1: Characteristics of lignocellulosic biomass (mean ± standard deviation from 3 samples).

Materials	Moisture (%)	VS (%)	Ash (%)	COD (kg/kg)
Wheat straw (WS)	2.8 ± 0.5	92.5 ± 0.0	4.7 ± 0.5	846.5 ± 168.9
Lurence hay (LH)	4.9 ± 0.5	91.3 ± 0.2	3.8 ± 0.6	1014 ± 33.6
Oaten hay (OH)	4.2 ± 1.1	94.6 ± 0.2	1.7 ± 0.9	531 ± 8.5
Corn silage (CS)	4.4 ± 0.7	95.3 ± 0.5	0.3 ± 0.2	738.5 ± 112.4

Rumen fluid and anaerobic sludge were two inoculum sources (Table 2). The former was collected from a 12-year old fistulated cow after 2 hours feeding. Rumen fluid was strained through two layers of cheesecloth to remove any coarse materials, and then stored in insulated thermos bottles that had been pre-heated with warm water to maintain a temperature of approximately 39 ºC during transportation to the laboratory. Anaerobic sludge was obtained from a full-scale anaerobic digester at the wastewater treatment plant in NSW, Australia. Anaerobic sludge was stored in pre-heated insulated thermos bottles during transportation and used within four hours of collection.

Table 2: Key properties of inoculum (mean ± standard deviation of 3 measurements).

	Rumen fluid	Digested sludge
TS (%)	2.2 ± 0.2	1.6 ± 0.2
VS (%)	1.8 ± 0.1	1.1 ± 0.2
pH	7.0 ± 0.0	7.3 ± 0.0
Total COD (g/L)	14.8 ± 1.7	1.8 ± 0.5

2.2 Biochemical methane potential assay

Biochemical methane potential assay was conducted using a set of test rigs similar to that used by Nghiem et al., (2014). The test rigs contained fermentation bottles, a water bath, and a biogas collection gallery. The fermentation bottles were made of glass with 100 mL active volume. Each bottle was equipped with a rubber stoper and aluminium cap. The water bath was Model TWB-20D Thermoline Scientific Pty Ltd and the biogas collection gallery included a 50-mL syringe connected with the needle via an inter lock. Biogas production was recorded daily following the change of syringe piston position on the graduated syringe.

Rumen fluid and anaerobic sludge (50 mL) were inoculated with 1.5 g lignocellulosic biomass equivalent to 3% w/v into a 100-mL fermentation glass bottle that was pre-flushed with N_2 gas. The bottles were flushed again with N_2 gas and immediately sealed with a rubber stopper to maintain anaerobic condition. The fermentation bottles were submerged in a water bath to maintain a constant temperature of 39 ± 1 °C and 35 ± 1 °C for rumen fluid and anaerobic sludge fermentation, respectively (Fig 1b).

The fermentation process was conducted for 7 days with rumen fluid and anaerobic sludge inocula, respectively. For each lignocellulosic material, six fermentation bottles were prepared. Two bottles were taken for soluble COD and total organic acids (as acetate) analysis every two days. Another set of bottles was prepared with only either inoculum or lignocellulosic materials as the controls. Fermentation bottles were mixed manually three times each day.

(a)

(b)

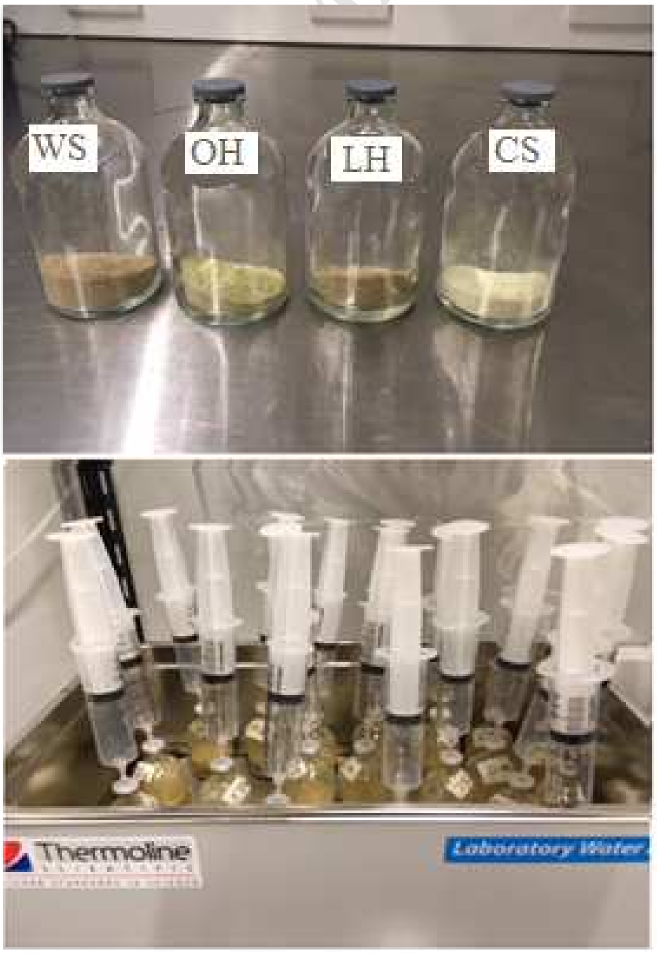

Figure 1: Four selected lignocellulosic biomass: WH = wheat straw; OH = oaten hay; CS = corn silage; LH = lurence hay (a) and a photograph of biomethane potential setup (b).

2.3 Analytical methods

Moisture, volatile solid (VS) and ash content of lignocellulosic biomass were determined according to Standard Methods 1684. Briefly, five gram of lignocellulosic biomass was transferred into a ceramic bowl and dried at 100 °C for 24 h. The ceramic bowl was then allowed to cool to room temperature in a desiccating glass chamber. The weight of ceramic bowl and material was recorded. Then the ceramic bowl was heated to 550 °C in a furnace for 15 min. The residual weight was recorded and used to calculate moisture, VS and ash content.

Total COD and soluble COD concentration were measured by using digestion vials (Hach, Australia) and Hach DR3900 spectrophotometer program number 435 COD HR, following the US-EPA Standard Method 5220 D.

Total organic acids (TOA) as acetate (mg/L) were measured following US-EPA Standard Method 5560C, including acidification, distillation and titration. Fermented broth (3 mL) from each fermentation bottle was diluted into 200 mL with Milli-Q water. Then 5 mL of 98% H_2SO_4 was mixed into samples. The sample was distilled using the Vapodest 300 (Gerhardt Germany) with set up program of heating power 80% and distillate time of 8 min. The final sample was titrated using an Auto Titrator 885 (Metrohm Australia). TOA concentration was calculated using the following equation:

$$\text{Total organic acid}\left(\frac{\text{mg}}{\text{L}}\right) = \frac{(\text{mL NaOH sample} - \text{mL NaOH blank}) \times \text{N} \times 60000}{\text{mL sample} \times 0.6}$$

Where: N= normality of NaOH and 0.6 is the recovery factor (60%).

2.4 Microbial community analysis

Rumen microbial community results were obtained from Duarte et al. (2017), who sampled rumen fluid from the same fistulated cow. Anaerobic sludge microbial community samples were collected before the inoculation process. Anaerobic sludge was mixed with 100% ethanol (1:1 v/v) to preserve the cells. Detail sample preparation procedure is available elsewhere (Nguyen et al., 2019a). Briefly, samples were stored in an ice bag during transport and immediately transferred to - 20 °C freezer upon arrival to the laboratory. Genomic DNA was extracted using DNeasy PowerSoil Pro Kit (QIAGEN Pty Ltd, Australia) following the manufacturer's instruction. The integrity, purity and concentration of the extracted DNA were determined by a spectrophotometer (Nanodrop ND2300). The mass of DNA in each sample was always more than 10 µg and the concentration was normalized to 50 ng/µL using DNA/RNA free water. Samples were stored at - 20 °C until DNA sequencing.

The variable regions (V3-V4) on the 16S rRNA gene of extracted DNA were amplified using the universal primers Pro341F (5'-CCTACGGGNBGCASCAG-3') and Pro805R (5'-GACTACNVGGGTATCTAATCC-3') (Takahashi et al., 2014). The amplified fragments were sequenced on the Illumina MiSeq sequencing platform at the Australian Genome Research Facility, Australia. Raw paired-end (2×300 bp) 16S rRNA gene sequence data were analyzed according to the Quantitative Insights into Microbial Ecology (QIIME2) pipeline (Caporaso et al., 2010). In brief, raw sequences were denoised using DADA2 with the following parameters: trim left-f = 17, trim left-r = 20, trunc-len-f = 280, trucn-len-r = 220, and all other parameters at their default setting. The sequences were clustered into representative OTUs based on a 97% nucleotide identity cut-off. The 16S rRNA gene sequencing generated 120,000 to 450,000 sequences per sample after pre-processing. The taxonomical assignment was performed against MiDAS database version 2.1 (McIlroy et al., 2017). The 16S rRNA gene sequences were deposited in GenBank with the accession numbers PRJNA507317.

3. Results and discussion

3.1 Volatile fatty acid production

Rumen fluid is a potential source of microorganisms for bio harvesting of volatile fatty acids (VFAs) from lignocellulosic biomass. The rumen fluid reactors generated significantly higher total volatile fatty acid (VFA) levels than that of the anaerobic sludge reactor (Fig 2). An average 100 mg VFA per g of lignocellulosic biomass was produced after two days of inoculation with rumen fluid, whereas this value was 23 in the reactors with anaerobic sludge (an estimated of four times higher). VFAs (i.e. acetic, propionic and butyric acid) are the products of hydrolytic and acidogenic steps during the fermentation process. The level of VFAs indicate the efficiency of hydrolytic and acidogenic process. Results suggest that rumen fluid microorganisms can hydrolyses lignocellulosic biomass for production of VFAs. VFAs are building blocks for biodegradable plastics and biofuel. The market for VFAs is growing with an annual demand growth rate of 7.4% (Atasoy et al., 2018). The global demand for VFAs (i.e. acetic, butyric, and propionic) is predicted to be about 18 million tons by 2023 (Atasoy et al., 2018; Reddy et al., 2018). The VFAs generation during the incubation of rumen microorganism with lignocellulosic biomass suggest an alternative source to offset the future VFA demand that currently relies on fossil resources.

Anaerobic hydrolysis and acidogenesis of lignocellulosic biomass by rumen microbes caused a decline in pH (Table S1). The pH of the reactor dropped from 7.0 to 5.6 after four days incubation. This observation is in consistent with the high level of VFAs production. Extending the incubation period to 6 days resulted in no further pH drop. Therefore, it is inferred that hydrolytic and acidogenic processes were inhibited by high level of VFAs accumulation. Likewise, the VFA concentration profiles along incubation times showed no significantly different after two days incubation with rumen microbes (Fig S1). This study suggests that pH is a detrimental factor to hydrolytic and acidogenic processes. This result is in consistent with the observation that rumen microbes are inhibited at pH below 5.5 (Zhang et al., 2017). On the other hand, hydrolysis and acidogenesis are possible the rate limiting steps in the anaerobic sludge reactor. In consistent with the low level of VFAs production, pH of the reactor was relatively stable (Table S1). In conclusion, VFAs produced from rumen microbe fermentation should be collected from the reactor or on a regular basis.

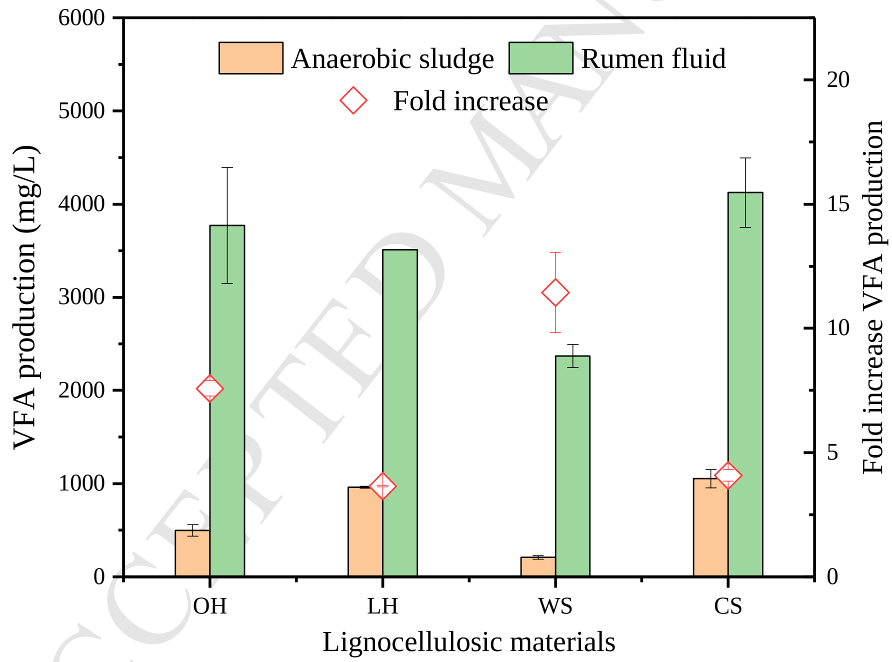

Figure 2: Volatile fatty acid production from anaerobic digestion of lignocellulosic biomass by rumen fluid and digested sludge inocula. Data was recorded after four days incubation. Value and error bars are mean and standard deviation (*n* = *4*).

3.2 Biogas production

BMP results indicated a higher biogas production from the anaerobic sludge than from rumen fluid reactor (Fig. 3). At the end of the incubation period (i.e. 7 days), the BMP bottles with anaerobic sludge produced an average 2.5 times higher biogas than the rumen fluid reactors. Biogas production is a direct indicator of methanogenesis in the anaerobic digestion process. Many studies have demonstrated the positive correlation between biogas production and the abundance of methanogens (Hao et al., 2016; Nguyen et al., 2019a; Tale et al., 2011). Results from this study suggest that methanogenesis is a limiting step in the rumen fluid reactor. That is because of the low abundance of methanogens in the rumen fluid (Patra et al., 2017). Methanogens are often outcompeted by hydrolytic and acidogenic microbes in ruminant microbiota. VFAs compounds, which are substrate for methanogens, are continuously adsorbed in the rumen of host animals (Patra et al., 2017). Another notable observation is the accumulation of VFAs and drop in pH in rumen fluid reactor (Section 3.1). Methanogens are slow-growing microbes and sensitive to pH environment. These conditions indicate an onset of the inhibition for the methanogenesis process (Nguyen et al., 2019b).

Anaerobic sludge reactors produced 120 to 170 mL biogas per g VS added of lignocellulosic biomass (Fig. 3). These values are lower than that typically obtained from the anaerobic digestion of municipal solid waste, waste activated sludge and organic wastes (Nghiem et al., 2014; Nguyen et al., 2019a). This result is likely due to the limitation in hydrolysis and acidogenesis of lignocellulosic biomass by anaerobic sludge microbes. Overall, rumen fluid microbes can be used for the production of VFAs, whereas anaerobic sludge can be used for biogas production. The complementary effect of these two inocula presents a potential solution for bio harvesting from lignocellulosic biomass.

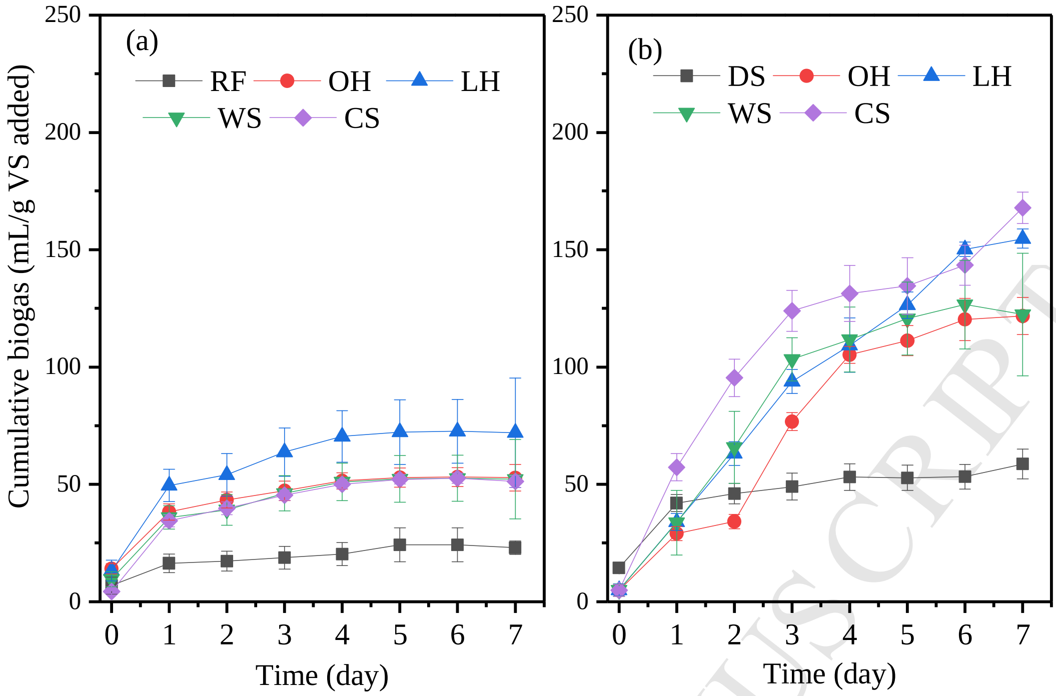

Figure 3: Cumulative biogas production (mL/g VS added) plotted against time from anaerobic digestion of lignocellulosic biomass by rumen fluid (a) and digested sludge (b) inocula. Value and error bars are mean and standard deviation (*n = 4*).

3.3 Soluble chemical oxygen demand

Rumen fluid and anaerobic sludge inoculum have an impact on soluble COD production from lignocellulosic biomass fermentation (Fig. 4). Lignocellulosic biomass is insoluble. The control reactor (i.e. contain lignocellulosic biomass only) has negligible amount of sCOD. Therefore, any increase in sCOD is mainly due to the biological conversion of lignocellulosic biomass. The rumen fluid reactors produced 227 (OH), 251 (LH), 187 (WS) and 340 (CS) mg sCOD/g VS added, whereas the anaerobic sludge reactors produced 135 (OH), 32 (LH), 56 (WS) and 256 (CS) mg sCOD/g VS added.

The levels of sCOD depend on the methanogenic microbes. According to the COD balance calculation, Xie et al., (2017) estimated about 50% conversion of input COD to biogas. Therefore, the activity of methanogens could negatively correlate with sCOD concentration. In the rumen fluid reactors, the sCOD concentration was high after two days of inoculation and remained stable towards the end of incubation period (Fig. 4). On the other hand, in the anaerobic sludge reactors, the sCOD concentration gradually decreased from day 2 to day 6 (Fig. 4). Furthermore, the ratio of sCOD and VFA from rumen fluid reactors (ca. 1.88 [OH], 2.14 [LH], 2.36 [WS], and 2.48 [CS]) was much lower than those of the anaerobic sludge reactors (ca. 7.5 [OH], 10.37 [LH], 8.0 [WS], and 7.11 [CS]). This observation indicated two scenarios (i) sCOD was converted to VFAs in the rumen fluid reactors and (ii) sCOD was converted to VFAs and biogas in the anaerobic sludge.

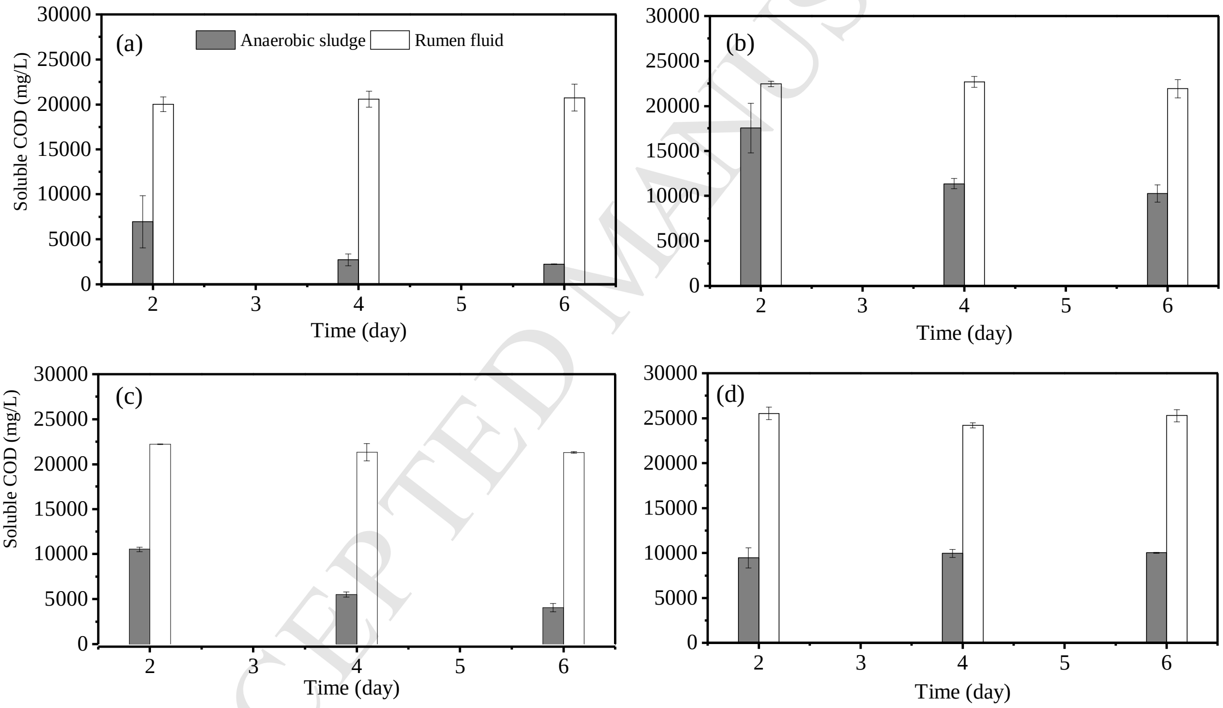

Figure 4: Soluble COD production from anaerobic digestion of lignocellulosic biomass by rumen fluid and digested sludge inocula: (a) control sets with inocula only or lignocellulosic biomass only (a) and tested sets inoculated with WS (b), LH (c), OH (d) and CS (e). Value and error bars are mean and standard deviation (*n = 4*).

3.4 Microbial community in rumen fluid and anaerobic sludge inocula

The first difference between rumen fluid and anaerobic sludge microbial community is the presence of bacteria in the phylum of *Fibrobacteres* (Table 2). The abundance of the phylum *Fibrobacteres* in the rumen fluid (i.e. 8.8%) was significantly higher than that in the anaerobic sludge inoculum (i.e. 0.06%). Bacteria in the phylum of *Fibrobacteres* are the major rumen microbes, allowing for the degradation of plant-based cellulose in ruminant animals. For example, the genus of *Fibrobacter* is specific hydrolytic bacteria that have genes encoding for enzymes cellulases and xylanases. *Fibrobacter succinogenes*, which is one of two cultivated species in the phylum of *Fibrobacter*, degrades effectively crystalline cellulose. Its genome contains high number of genes that were classified into 31 identified cellulases (Suen et al., 2011). This species also encodes hemicellulose-degrading enzymes to remove hemicelluloses for other enzymes to attach on cellulose. These enzymes are highly specific for hydrolysis (i.e. cellulolysis) of lignocellulosic biomass (i.e. 30-60% cellulose, 10-25% lignin and 8-40% hemicellulose).

The second difference is the presence of bacteria in the *Prevotellaceae* family in the rumen fluid inoculum (Table 2). In this family, *Prevotella* was dominant in rumen microbiota (Duarte et al., 2017). Baba et al. (2017) observed that species in the *Prevotella* family presented at 50.5% of total microbial abundance in the rumen fluid of cattle. Member of the *Prevotella* family such as *P. brevis*, *P. ruminicola* and *P. bryantii* produce cellulolytic enzymes such as CMCase and xylanases. The *Prevotella* species function synergistically with other cellulolytic organisms to contribute to the ruminal fibrolytic activity. In contrast, *Prevotellaceae* were present at very low abundance in anaerobic sludge (Table 2). The presence of *Fibrobacter* and *Prevotella* at high abundance and their cellulolytic functions probably explain for the generation of soluble COD and VFAs in the reactor inoculated with rumen fluid and lignocellulosic biomass.

Another possible difference between rumen fluid and anaerobic sludge inoculum is the presence of flagellate protozoa and fungi in the rumen fluid. The number of protozoa in the rumen fluid inoculum was $6x10^4$ cells/mL (Figure S2). Endogenous and protozoal enzymes could act independently or synergistically with bacterial enzymes to breakdown lignocellulosic biomass in the rumen. For example, ruminal protozoan *Polyplastron multivesiculatum* comprise a family of 22 carbohydrate-binding module (CBM) that binds strongly to various crystallinities cellulose (Devillard et al., 2003). Fungi are unique among rumen microorganism in which they penetrate the cuticle of plant cells. With high levels of cellulases and hemicellulases, rumen fungi hydrolyse or solubilize the entire plant cell wall. However, the potential of rumen protozoa and fungi to degrade more recalcitrant plant walls is not always achieved in the rumen. Future study is recommended to investigate the proliferation of rumen protozoa and fungi in anaerobic digestion of lignocellulosic biomass.

The compositions and relative abundance of methanogenic communities in the rumen fluid were different from the anaerobic sludge inoculum (Table 2). Three genera including *Methanobacterium, Methanobrevibacter* and *Methanomicrobium* were present at the relative abundance of less than 0.1%. These genera have been described as hydrogenotrophic rumen methanogens. This is consistent with the physiology of the rumen. Volatile fatty acid, CO_2 and H_2 are formed during hydrolysis and fermentation of plant polymers in the rumen. While the ruminant consumes VFAs, CO_2 and H_2 are used by rumen methanogens to produce methane. These methanogens via hydrogenotrophic pathway function as hydrogen sink and thus support the activity of hydrolytic and fermentative bacteria. Consistently, hydrogenotrophic methanogens have been observed in many rumen microbial community studies (Agematu et al., 2017; Bayané & Guiot, 2011; Patra et al., 2017). On the other hand, aceticlastic methanogens dominated the methanogenic community in the anaerobic sludge (Table 2). The genus of *Methanosaeta* is strictly aceticlastic methanogens, presented at

3.16% of total microorganism population. This is consistent with the high abundance of the genera *Methanosaeta* in most of the anaerobic digestion process (Nguyen et al., 2019b). The genus of *Methanosaeta* is strictly aceticlastic methanogens. Chen et al. (2015) reported the robustness of *Methanosaeta* genus at high levels of acetate in anaerobic digestion (44 mM). Overall, the relative abundance of methanogens in anaerobic sludge was significantly higher than that of rumen fluid, explaining for the high biogas production and no accumulation of VFAs in reactor inoculated with anaerobic sludge.

Results from the analysis of rumen fluid and anaerobic sludge microbial community compositions revealed the possible complementary between two inocula. The co-inoculation of specific lignocellulolytic consortium (i.e. rumen fluid) with the high methanogenic consortium (i.e. anaerobic sludge) can increase the digestion of lignocellulosic biomass for biogas production. Recent studies have achieved some progress in improving anaerobic digestion of cow manure by co-inoculation of cow rumen fluid and anaerobic sludge (Ozbayram et al., 2018). However, knowledge into the interactions between rumen microbes and anaerobic sludge microbes as well as their associations with the environmental conditions (i.e. may be different from the rumen conditions) is required to fully realise the co-inoculation approach. This study preliminary suggests maintaining the abundance of lignocelulolytic bacteria (e.g. *Fibrobacter* and *Prevotella*) in anaerobic digestion is necessary for the degradation of lignocellulosic biomass.

Table 2: Relative abundance (%) of specific genera in rumen fluid and anaerobic sludge inocula

Genera	Relative abundance (%)		Ecological function
	Rumen fluid (n = 2)*	Anaerobic sludge (n = 4)	
Bacteria			
Fibrobacter	8.8	0.06	Hydrolytic
Prevotellaceae	35.8	0.08	Hydrolytic
Firmicutes	25.9	11.4	Hydrolytic, acidogenic
Methanogens			
Methanobacterium	0.01	0.003	Hydrogenotrophic
Methanobrevibacter	0.09	0.04	Hydrogenotrophic
Methanomicrobium	0.01	nd	Hydrogenotrophic
Methanolinea	nd	0.62	Aceticlastic
Methanospirillum	nd	0.10	Aceticlastic
Methanosaeta	nd	3.16	Aceticlastic
Methanoculleus	nd	0.05	Aceticlastic
Methanosphaera	nd	0.01	Aceticlastic
Total abundance (%)	0.11	3.98	

[*] Data were retrieved from Duarte et al. (2017); nd = not detected.

4. Conclusions

Lignocellulosic biomass (i.e. wheat straw, oaten hay, lurence hay and corn silage) can be used for VFAs and biogas production depending on the inoculum sources. Rumen fluid microbes demonstrated the efficiency to digest lignocellulosic biomass into VFAs (at four-time higher than anaerobic sludge). This was likely due to the presence at the high abundance of lignocellulolytic bacteria in the genus of *Fibrobacter* (8.8% of total microbes) and *Prevotella* (35.8%). On the other hand, anaerobic sludge produced higher biogas than rumen fluid reactors. Consistently, the methanogenic abundance in anaerobic sludge was at 3.98% of total microbes, significantly higher than in the rumen fluid inoculum (0.11%). The results of this study suggest the use of rumen fluid microbes together with a continuous extraction of produced VFAs can be an alternative solution to enhance the environmental and economic benefits of lignocellulosic biomass.

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Table 2: Key properties of inoculum (mean ± standard deviation of 3 measurements).

	Rumen fluid	Digested sludge
TS (%)	2.2 ± 0.2	1.6 ± 0.2
VS (%)	1.8 ± 0.1	1.1 ± 0.2
pH	7.0 ± 0.0	7.3 ± 0.0
Total COD (g/L)	14.8 ± 1.7	1.8 ± 0.5

Table 3: Relative abundance (%) of specific genera in rumen fluid and anaerobic sludge inocula

Genera	Relative abundance (%)		Ecological function
	Rumen fluid (n = 2)*	Anaerobic sludge (n = 4)	
Bacteria			
Fibrobacter	8.8	0.06	Hydrolytic
Prevotellaceae	35.8	0.08	Hydrolytic
Firmicutes	25.9	11.4	Hydrolytic, acidogenic
Methanogens			
Methanobacterium	0.01	0.003	Hydrogenotrophic
Methanobrevibacter	0.09	0.04	Hydrogenotrophic
Methanomicrobium	0.01	nd	Hydrogenotrophic
Methanolinea	nd	0.62	Aceticlastic
Methanospirillum	nd	0.10	Aceticlastic
Methanosaeta	nd	3.16	Aceticlastic
Methanoculleus	nd	0.05	Aceticlastic
Methanosphaera	nd	0.01	Aceticlastic
Total abundance (%)	0.11	3.98	

[*] Data were retrieved from Duarte et al. (2017); nd = not detected.

Highlight

- Rumen fluid produced 4 times more VFAs from biomass than anaerobic sludge microbes
- Lignocellulolytic bacteria (*Fibrobacter*, *Prevotella*) were abundant in rumen fluid
- Methanogenic abundance was high in anaerobic sludge inoculum
- Continuous extraction of VFAs from rumen fluid reactor is required for efficiency