Cellulosic waste degradation by rumen-enhanced anaerobic digestion

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Abstract Anaerobic digestion of lignocellulosic material is carried out effectively in many natural microbial ecosystems including the rumen. A rumen-enhanced anaerobic sequencing batch reactor was used to investigate cellulose degradation to give analysis of overall process stoichiometry and rates of hydrolysis. The reactor achieved VFA production rates of 207–236 mg COD/L/h at a loading rate of 10 g/L/d. Overloading of the reactor resulted in elevated production of propionic acid, and on occasion, the presence of succinic acid. With improvements in mixing and solids wasting, the anaerobic sequencing batch reactor system could enable full-scale application of the process for treatment of cellulosic waste material.

Keywords Anaerobic digestion; anaerobic sequencing batch reactor; lignocellulosic waste; rumen

Introduction
Lignocellulosic waste is generated in significant amounts from both domestic and rural activities. It includes material such as paper products, yard waste, verge grass from highways, processed crops and animal wastes. Anaerobic digestion of cellulosic waste is becoming favourable as a sustainable technology which promotes energy recovery from waste and provides an alternative to publicly unpopular solid waste processing methods such as incineration and landfilling. However, the kinetics of anaerobic digestion are still limited by the first step of the process; hydrolysis or depolymerisation of structural material into constituent molecules (Verstraete et al., 1996, Pavlostathis and Giraldo-Gomez, 1991).

Anaerobic digestion is carried out effectively in many natural anaerobic microbial ecosystems including the rumen of ruminant animals such as sheep, cows, deer and kangaroos. The rumen is a highly cellulolytic ecosystem with a complex microbial population of Bacteria, Archaea, Protozoa and Fungi. This consortium produces volatile fatty acids (VFAs) as the animal’s energy source, biomass as the protein source for the animal and biogas which is eructated mainly via the mouth. Using the rumen as a model for the development of an anaerobic digestion system could significantly improve conventional systems by providing increased depolymerisation rates and possibly greater extents of degradation of lignocellulosic material.

As rumen research proliferated over the last few decades, a need for greater experimental control and reproducibility developed while also seeking to reduce the use of live surgically-altered animals. This led to the development of rumen simulation by growing rumen microbes in a reactor (in vitro). Numerous “artificial rumen” systems have provided new insights into the biological processes and microbial ecology of the rumen (see: Rufener et al., 1963; Hoover et al., 1976; Teather and Sauer, 1988; Fuchigami et al., 1989).

A summary of the performance of systems found in the literature is outlined in Figure 1. The figure shows VFA production rate versus volatile solids loading rate. The rate of VFA production ($r_{\text{vfa}}$) provides a good estimate of the hydrolysis rate and has been used to evaluate prefermenter performance in wastewater treatment (v. Muench, 1997). This approach is
validated as studies have shown that the carbohydrate sub-units such as glucose do not nor-
mally accumulate in anaerobic digestion systems.

The literature data shows a high degree of variability in the VFA generation rate in com-
parison to the loading rate indicated by the low correlation factor (R²). This is predominant-
ly due to variations in substrate type and degradability. Generally, the results are similar
and show a clear trend to increasing volumetric VFA generation rates with higher loading,
even beyond the typical level of loading in actual rumen systems (around 60 g/L/d, Rufener et
al., 1963). No information has been reported for in-vivo volatile fatty acid production
rates. All reactors were continuous systems either using a simple continuously stirred tank
reactor (CSTR) arrangement with an effluent overflow or more recently, a filter to decouple
solids and liquid residence times.

Lignocellulosic material is extremely heterogenous and structural material is defined by
methods of analysis such as neutral detergent fibre (Goering and Van Soest, 1970). For the
experiments described here, fibrous cellulose (detailed below) was chosen as a pure
substrate to simplify analysis of data. The use of fibrous cellulose was regarded as being
similar to possible substrates for a full-scale system, such as paper products.

An investigation of rumen-enhanced anaerobic digestion of cellulosic waste using an
anaerobic sequencing batch reactor (ASBR) has been undertaken to investigate limits of
system performance and give analysis of overall process stoichiometry and rates of hydrol-
ysis. The effects of loading rate on rates of hydrolysis are highlighted including reactor per-
formance during overloading.

**Materials and methods**

An ASBR system utilising rumen as the inoculum was operated to investigate the degrada-
tion of cellulose to VFAs and biogas. Rumen was sourced from a fistulated cow, filtered to
remove coarse solids (mesh; 1 mm by 1 mm) and diluted 1:1 with buffer solution for reactor
start-up.

Fibrous alpha-cellulose (Sigma Chemicals, C8002) was fed to the reactor at loading
rates of 5–15 g/L/d along with a modified artificial saliva buffer solution (Slyter et al.,
1966; Buffer A). Two systems were trialled, one using manual feeding once daily and one
feeding cellulose suspended in buffer solution automatically at the beginning of each 4 hr
cycle. In order to prevent degradation occurring in the feed vessel for the automated sys-
tem, the buffer was fed equally as two solutions: Buffer 1 contained the nitrogen source as
ammonium chloride (1.5 g/L) and trace metal solution (4 mL/L, Haris, 2001) while Buffer
2 contained the feed cellulose. The operating phases of the ASBR were filling (12 min),
unmixed react (48 min), mixed react (120 min), settle (53 min) and decant (7 min). Solids
wasting from the reactor was achieved by mixing for 4 minutes during one decant cycle
daily giving a solids residence time of approximately 7 days. The reactor working volume
was 2 L and the cycle exchange volume of 500 mL resulted in a dilution rate of 1.5/d or a hydraulic retention time of 16 h for the system.

Decanted effluent collected from all cycles was analysed for volatile fatty acids (HPLC, Biorad HPX-87H column, UV detection 210 nm), ammonia-nitrogen (FIA, Lachat QuikChem8000, 630 nm), pH, and suspended solids (APHA/AWWA, 1995). Buffer and effluent bicarbonate concentration was estimated by titration (Anderson and Yang, 1992). The pH data was logged by computer at all times and was controlled one-way to a set-point of 6.25 by the addition of 1.5 M NaOH during periods of mixing. The reactor was contained in a waterbath at a constant temperature of 39°C. Biogas production was measured by a rotary gas meter and analysed daily for hydrogen, methane and carbon dioxide composition by gas chromatograph (Alltech Haysep Q column, TCD detector).

Results and discussion
A graph of volatile fatty acid production rate ($r_{VFA}$) over time for the rumen-based ASBR is shown in Figure 2. The COD generation rates for the individual acids (acetic, propionic and butyric acid) and total COD are shown.

During this experiment, cellulose was fed daily manually and the loading rate was increased from 5 g/L/d to 10 g/L/d on day 8. A drop in COD production rate from the inoculum can be seen initially but after the loading increase, the acid production rises as expected. The experiments showed major shifts in fermentation products over time and concurrent shifts in the microbial community have been observed using fluorescent in-situ hybridisation targeting key ruminant bacterial groups (results not shown). On day 19, a significant rise in total COD production is caused by a higher propionic acid generation. This is a key indicator of overloading and will be discussed further below.

Fermentation products
Studies have been carried out previously to investigate the rumen for degradation of cellulotic materials such as office paper, newspaper and pure cellulose of differing properties. Comparisons of the performance of the ASBR system and other systems are detailed below.

Blasig et al. (1992) undertook rumen fermentation using a dual-flow continuous culture which utilised a filtration step (30 µm pore size) to decouple solids and liquid residence times. The substrate used in these studies was newspaper that was pre-treated using ammonia fibre explosion (AFEX). Another study using a dual-flow system was carried out by
Gijzen et al. (1987, 1988) using pure filter-paper cellulose as the substrate. The system was almost identical to the one described above also using a filter (30 µm pore size). The operating parameters of the systems and the rumen ASBR are shown in Table 1. Data from steady-state operation was shown here for two different rumen ASBR trials at a loading rate of 10 g/L/d.

The ASBR was operated at a significantly longer solids residence time and a lower loading rate than the literature studies. Typical HRT and SRT for the rumen are 6–16 h and 20–30 h respectively (Ishler et al., 1996).

Table 2 gives a performance comparison between the ASBR and literature data. It can be seen that VFA production rates (both COD and molar) are comparable with those reported by Gijzen et al. when taking the different loading rate into account. The VFA production rate of 207–236 mg COD/L/h of the ASBR compares favourably with that predicted by the linear regression of literature data above (115 mgCOD/L/h). VFA yields are also similar but the ASBR gave a higher yield than studies conducted by Blasig et al. The difference could be attributed to newspaper being a more difficult material to degrade than the pure celluloses. Filter paper cellulose is a readily degradable solid substrate which is used for studies of cellulase enzyme activity (Dijkerman et al., 1997). Alpha cellulose used in this study has been shown to have lower rates of reaction than microcrystalline cellulose (Weimer et al., 1990).

Variability between the two ASBR trials is considered to be comparable with that shown by the two separate studies conducted by Gijzen et al. The composition of VFAs was similar although both ASBR trials had a lower butyric acid fraction than the others documented from the literature. This variation in fermentation could be due to different environmental conditions or differences in the rumen inoculum. This might also indicate that there are different micro-organisms present in this system compared to the ones reported in the literature since those used shorter solids retention times compared to the study in the ASBR.

A major difference between the trials lies in the reactor set-up. The use of a filter in dual-flow rumen fermentation can be problematic especially with regard to filter blocking. Blasig et al. stated that filters needed to be changed every 12 to 24 hours due to blocking which decreased liquid flowrates. No such problems were found with filter paper cellulose

<table>
<thead>
<tr>
<th>Reference</th>
<th>LR g/L/d</th>
<th>HRT H</th>
<th>SRT h</th>
<th>pH (average)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blasig et al. (1992)</td>
<td>14.1</td>
<td>11</td>
<td>44</td>
<td>6.7</td>
</tr>
<tr>
<td>Gijzen et al. (1988)</td>
<td>16.6</td>
<td>12</td>
<td>60</td>
<td>6.6</td>
</tr>
<tr>
<td>Gijzen et al. (1987)</td>
<td>15</td>
<td>12</td>
<td>60</td>
<td>6.5</td>
</tr>
<tr>
<td>Rumen ASBR 9</td>
<td>10</td>
<td>16</td>
<td>16B</td>
<td>6.3</td>
</tr>
<tr>
<td>Rumen ASBR 8</td>
<td>10</td>
<td>16</td>
<td>16B</td>
<td>6.4</td>
</tr>
</tbody>
</table>

LR – loading rate; HRT – hydraulic retention time; SRT – solids retention time

<table>
<thead>
<tr>
<th>Reference</th>
<th>Ac mol%</th>
<th>Pr mol%</th>
<th>Bu mol%</th>
<th>VFA Prod mmol/L/d</th>
<th>VFA Yield g VFA/gVS</th>
<th>$t_{vfa}$, mg COD/L/h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blasig et al.</td>
<td>72.7</td>
<td>21.4</td>
<td>5.9</td>
<td>81</td>
<td>0.31</td>
<td>248</td>
</tr>
<tr>
<td>Gijzen et al. (1988)</td>
<td>70</td>
<td>24</td>
<td>6</td>
<td>130</td>
<td>0.51</td>
<td>437</td>
</tr>
<tr>
<td>Gijzen et al. (1987)</td>
<td>68</td>
<td>25</td>
<td>7</td>
<td>95</td>
<td>0.41</td>
<td>328</td>
</tr>
<tr>
<td>Rumen ASBR 9</td>
<td>71</td>
<td>25</td>
<td>3</td>
<td>70</td>
<td>0.46</td>
<td>236</td>
</tr>
<tr>
<td>Rumen ASBR 8</td>
<td>69</td>
<td>28</td>
<td>3</td>
<td>60</td>
<td>0.39</td>
<td>207</td>
</tr>
</tbody>
</table>

$t_{vfa}$ – volatile fatty acid production rate; Ac – acetic, Pr – propionic, Bu – butyric acids
in the trials conducted by Gijzen et al. The influence of filter pore size on rumen fermentation performance is outlined by Kiwaisi and Barugahare (1996). They found that the VFA production rate using a grass/grain feed dropped by 53% as filter pore size increased from 30 to 300 µm. The ASBR reactor used only a settling cycle which might not settle out biomass flocs smaller than 50–100 µm. However, such a system would be considered significantly easier to scale-up to a larger process.

Methane production from the ASBR system was found to vary between the two trials (0.41 L/L/d and 0.46 L/L/d) and was half of the 0.91 L/L/d reported by Gijzen et al. (1987). Reasons for this difference are unknown.

Process stoichiometry and biomass yield

Analysis of process stoichiometry was carried out using mass balances over the ASBR (Table 3). Key assumptions for calculations were that consumption of ammonia-nitrogen was solely converted to biomass which had the empirical formula C₅H₇O₂N and the sole fermentation products were acetic, propionic, butyric and valeric acids, methane and CO₂ gas, and biomass.

The stoichiometry presented by Keshtkar et al. (2002) is for the acidogenic process only and does not incorporate methane production or butyrate or propionate-utilising bacteria. It was developed to study conventional anaerobic digestion of cattle manure. It was assumed that 75% of insoluble substrate was solubilised in the hydrolysis step.

By analysing bicarbonate (represented as aqueous carbon dioxide), it is possible to estimate the amount of carbon dioxide yielded from the reactor by stripping as carbon dioxide gas. It was also assumed that methane was derived solely from the consumption of carbon dioxide and hydrogen by hydrogen-utilising Archaea due to the low operating pH of the reactor (Grady and Lim, 1980).

For ASBR8 and ASBR9, it can be seen that inlet liquid-phase carbon dioxide accounts for 46% and 62% of carbon removed as biogas, respectively. Very few studies incorporate bicarbonate into calculations and it can be concluded that a significant proportion of biogas is in fact resulting from the physical bicarbonate stripping process which will occur with acid formation. Gas and liquid phase carbon dioxide was assumed to be at equilibrium by Keshtkar et al. due to the long residence time of the modelled reactor.

There were differences in acid production between the two ASBR trials with SBR 9 yielding higher amounts of acetate and propionate. In comparison with the acidogenic process, it can be seen that the distribution of acids (shown as mol% in brackets) was significantly different especially with regard to butyrate (3% c.f. 25%). The actions of butyrate (and propionate) utilising acetogenic bacteria likely account for most of the difference.

<table>
<thead>
<tr>
<th>Species</th>
<th>Species</th>
<th>ASBR 8 (10 g/L/d)</th>
<th>ASBR 9 (10 g/L/d)</th>
<th>Keshtkar et al. (1990)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellulose</td>
<td>+C₆H₁₀O₅</td>
<td>1 (basis)</td>
<td>1 (basis)</td>
<td>1 (basis)</td>
</tr>
<tr>
<td>Ammonia</td>
<td>+NH₃</td>
<td>0.35</td>
<td>0.26</td>
<td>0.1115</td>
</tr>
<tr>
<td>Carbon dioxide (Aq)</td>
<td>+CO₂(aq)</td>
<td>0.84</td>
<td>0.85</td>
<td></td>
</tr>
<tr>
<td>Acetic acid</td>
<td>−C₃H₆O₂</td>
<td>0.96 (69%)</td>
<td>1.29 (71%)</td>
<td>0.744 (44%)</td>
</tr>
<tr>
<td>Propionic acid</td>
<td>−C₄H₇O₂</td>
<td>0.39 (28%)</td>
<td>0.44 (25%)</td>
<td>0.5 (30%)</td>
</tr>
<tr>
<td>Butyric acid</td>
<td>−C₅H₁₀O₂</td>
<td>0.05 (3%)</td>
<td>0.06 (3%)</td>
<td>0.4409 (26%)</td>
</tr>
<tr>
<td>Valeric acid</td>
<td>−C₆H₁₂O₂</td>
<td>0.00</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Total acids</td>
<td></td>
<td>1.40</td>
<td>1.8</td>
<td>1.68</td>
</tr>
<tr>
<td>Methane gas</td>
<td>−CH₄(g)</td>
<td>0.41</td>
<td>0.45</td>
<td></td>
</tr>
<tr>
<td>Carbon dioxide gas</td>
<td>−CO₂(g)</td>
<td>1.41</td>
<td>0.92</td>
<td>0.6909</td>
</tr>
<tr>
<td>Biomass</td>
<td>−C₅H₇O₂N</td>
<td>0.35</td>
<td>0.26</td>
<td>0.1115</td>
</tr>
<tr>
<td>Water</td>
<td>−H₂O</td>
<td>0.37</td>
<td>0.73</td>
<td>0.0254</td>
</tr>
</tbody>
</table>
The presence of these might be related to the longer SRT in the ASBR, thereby giving both a higher biomass concentration and also a different microbial community structure.

Ammonia was assumed to be consumed solely to form biomass whose empirical formula was estimated at C₅H₇O₂N to allow comparison between the studies.

The microbial biomass yield \( Y_{X/S} \) was estimated at 0.29 and 0.22 for ASBR8 and ASBR9, respectively, on a carbon-mole basis. This was significantly higher than reported values for acidogenic carbohydrate utilisation in anaerobic digestion systems of between 0.14–0.17 gCOD/gCOD (Pavlostathis and Giraldo-Gomez, 1991). A higher yield is consistent with the function of the rumen to generate biomass as the protein source for the animal.

### Overloading

At the long solids retention time used in the ASBR, a significant build-up of cellulose was found in the reactor at higher loading rates. This was caused by settling and compaction of cellulose which was then not completely mixed during the wasting cycle, therefore effectively lengthening the SRT. This build-up of cellulose was found to lower VFA yields, lower biogas production, and alter fermentation stoichiometry significantly.

Figure 3 shows the characteristic changes which occur with overloading of the ASBR system. A loading rate of 15 g/L/d was used and the effective SRT was estimated at up to 15 days. It can be seen that propionate production becomes dominant from day 17 to 21. Propionic acid accumulation is an indication of process instability. For this period, maximal hydrogen biogas concentrations reached 0.4 mol%. This was caused by a reduction in the activity of hydrogen-utilising methanogens as evidenced by the concurrent reduction in methane production during this period (data not shown). In some trials, hydrogen represented up to 15% of the gas phase. Hydrogen is normally undetectable in the gas phase during stable fermentation. Succinic acid appears as the dominant fermentation product for a limited period of time between day 11 and day 18 reaching liquid concentrations of around 550 mg/L. Succinic acid is formed as an intermediate in the pathway that ultimately leads to propionic acid in anaerobic fermentation (Brock et al., 2000).

On days 16–17, the pH control system was turned off causing the reactor pH to drop to levels of 5.90. This may be responsible for the shift in microbial community structure or fermentation leading to the subsidence of succinate and rise of propionate in the time following this fault.

After a continued period of elevated propionate concentration (greater than 25 mmol/L),
VFA production decreased and the system collapsed. The effects of prolonged elevated propionate formation appear irreversible as the hydrogen utilising methanogenic community is not re-established.

**Conclusions**

A rumen-enhanced ASBR was studied to analyse rates of hydrolysis and process stoichiometry and yields. The use of rumen-based anaerobic digestion has been shown to give high rates of degradation of cellulosic material with a VFA production rate of 207 to 236 mg COD/L/h (around 5,300 g COD/L/d) at a loading rate of 10 g cellulose/L/d. This compares favourably to the rate predicted by linear regression of literature data relating to rumen simulation systems (115 mgCOD/L/h). Microbial yields are higher in the rumen than for other systems for anaerobic digestion of carbohydrates.

Overloading of the reactor was characterised by high propionate production and the concurrent presence of hydrogen in the gas phase. Succinate, which is an intermediate to propionate formation, was found to accumulate in the reactor for a period of time. This overloading was likely to have been a consequence of the accumulation of substrate in the reactor due to inefficient mixing.

With improvements in mixing and solids wasting, the ASBR system could provide a practical alternative for filter-based rumen simulation systems and enable full-scale application of the process for treatment of lignocellulosic waste material.

**References**


