Effect of inoculum-substrate ratio on the start-up of solid waste anaerobic digesters

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Abstract: The anaerobic systems start-up for solid waste treatment is a fundamental step, especially for those with two phases. It is necessary to know both the waste characteristics and the inoculum conditions.

The objective of this work was to study the inoculum-substrate ratio (ISR) influence as a previous step of the start-up of an anaerobic system for the solid waste digestion. During this research spent grain was chosen as residue, working at three different concentrations (7; 13 and 20% w/v), studying the ISR effect in terms of anaerobic degradability (AD) and specific methane productivity (SMP). The initial acetoclastic activities ($A_0$) were calculated based on the equation which describes the methane accumulation during each test. The model constants were also calculated and were adjusted to the experimental data. The results showed that in general the ISR variation has less impact on AD than on SMP. While maximum AD were reached in those tests with high ISR, the greatest values of SMP were with the lowest values of ISR ratio. A low ISR caused a slow hydrolysis, although the methane production was fast. So, during the start-up of a two-phase anaerobic system an elevated ISR would not be necessary in order to reach a good AD and a good intermediate products production, because the hydrolysis and the VFA production must be optimised in the first phase of these systems. While in conventional systems, where phases are together, it is much better to optimise the methane production. The ISR and the SMP indicated which inoculum percentage would be interesting based on the objective of the whole system: methane or intermediate compounds (VFA) production. All this information is important during the conventional anaerobic reactors operation because these tests would show which ISRs avoid inhibition.

Keywords: Anaerobic degradability; industrial solid waste; inoculum substrate ratio; start-up

Introduction

The treatment of organic solid waste is developing as a huge investigation area, in order to find new options that substitute the conventional treatment systems, inside the environmental politics and the economic development. Instead of incineration process or the usual landfill disposal, there is a biotechnology option: anaerobic digestion. Adequate equipment selection depends on the limiting step of the anaerobic process and on the substrate characteristics.

Anaerobic digestion is a common process for the treatment of organic effluents, where the methanogenic phase is normally considered as limiting step of the process. On the other hand, the hydrolysis is the lowest and controlling step if substrates are particulate, as for example, with organic solid waste (Eastman and Ferguson, 1981). The substrate solubilisation is also important because the low biodegradabilities obtained for some residues are due to some refractory and/or inhibitory polymers generated during this solubilisation (Delgenès et al., 2000). It is always essential to know the characteristics of both the residue and the inoculum. The inoculum-substrate ratio (ISR) shows the effect of substrate concentration during the anaerobic digestion like any other biodegradability test, but in this case the inoculum concentration is varied to find how anaerobic degradability (AD) and specific methane productivity (SMP) change.
So the ISR study is a previous step because the start-up process of the solid waste anaerobic treatment systems is always a delicate and fundamental stage if we want to reach a successful operation. The objective of this work was to study the influence of the inoculum-substrate ratio (ISR) in order to select and start-up an anaerobic system for industrial solid waste. For that, a residue produced in great amounts in the brewery industry was chosen as model of industrial waste: the spent grain (20 kg waste/hLbeer).

Methods
Spent grain and anaerobic sludge
Brewery spent grains were supplied by Cerveceria Santiago, Chile. The moisture content is 75.1% and the main components are shown on Table 1. The elevated protein percentage predicts an important ammonium production during the hydrolysis step of the anaerobic digestion.

The anaerobic sludge was also supplied by Cerveceria Santiago, Chile, and was activated in a lab-scale UASB of the Biochemical Engineering School of the Catholic University of Valparaiso. Its methanogenic activity varied between 0.22 and 0.53 gCODCH4/gVSS·d.

Anaerobic degradability test
The degradability tests for solid wastes were based on a modification of Field methodology (Field et al., 1988) developed previously (Poirrier et al., 1997). These assays were done in duplicate plus blank, with three different substrate concentrations (7, 13 and 20% w/v) and with eight specific sludge activities (AE, from 0.005 to 0.980 gCODCH4/gTswaste·d). The tests were 150 mL reactors at 37°C and without mixing. The regular measurement of methane was made by displacement of a NaOH 5M solution inside Mariotte Tubes. The initial and final content of total solids (TS) and volatile solids (VS) of each test were determined by Standard Methods (1985).

The AD was defined as the relation between degraded and initial TS (g/L) and it was expressed in %. The SMP was calculated by using a biomass base (SMPVS in LCH4/kgVS·d) and substrate base (SMPWASTE in LCH4/kgWASTE·d). The ISR was defined as the relation between specific sludge activity AE divided by substrate mass (g).

![Figure 1](https://iwaponline.com/wst/article-pdf/44/4/103/430167/103.pdf)

**Figure 1.** Variation of methane accumulation through time at three ISR. ((a) ISR = 0.0016; (b) ISR = 0.0052; (c) ISR = 0.0307)

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Spent grain (percentage on dry base)</th>
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<tbody>
<tr>
<td>VS</td>
<td>Fibre</td>
</tr>
<tr>
<td>---------</td>
<td>-------</td>
</tr>
<tr>
<td>80.0</td>
<td>5.0</td>
</tr>
</tbody>
</table>

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Results and discussion

Figure 1 shows the accumulative methane through time for 7, 13 and 20% w/v tests with different specific activities $A_E$. In general for all tests, a quick initial methane production was observed due to hydrolysis of the most soluble compounds of the spent grain. Afterwards in a second step, the methane production became slow because of the metabolism of complex material, more difficult to hydrolyse, was taking place. The velocity during this second stage was approximately constant at the end for these assays (120 days). However, 20% w/v tests did not present a second stage at 120 days of performing and all ISR behaved in a similar way, without any effect of the ISR on methane production.

In Figure 1, the effect of inoculum amount can be observed and how solubilization affects the substrate disposability (the presence of intermediate degradation products). In general, for all assays the accumulative methane increased when the inoculum concentration was high. This could be observed especially for all 7% and 13% w/v tests. The degradation steps were clear when substrate concentration was 7% for all ISRs tested and 13% when ISR was small. An increase in substrate and inoculum did not clarify the steps, as is shown in Figure 1c.

The accumulative methane for three substrate concentrations is presented in Figure 2. With the same ISR, the substrate concentration generated different steps of methane production. The compounds originated during the spent grain solubilisation and/or their degradation products could inhibit anaerobic bacteria. This could be an explanation for those periods of no methane accumulation. For example, in Figure 2a the assays with less inoculum presented no accumulation periods: so much ammonium and VFA have been accumulated in such a way that methanogenic bacteria stopped their methane production. Although, with the increase of ISR, these detentions disappeared, except in 7% tests.

Different stages were observed during the assays but mainly two were clear: the initial degradation during the first five days and a second degradation when enough intermediate compounds were accumulated. So the acetoclastic activities were calculated by an equation which describes the methane production (Veeken et al., 2000):

$$\text{CH}_4(t) = \left[ -\frac{A_0}{\mu_{\text{max}}} \right] \exp(\mu_{\text{max}} \cdot t)$$

Where $\text{CH}_4(t)$ is the accumulative methane at time $t$ (L); $A_0$ is the acetoclastic activity (mg COD/g VS·d), $\mu_{\text{max}}$ is the maximum growth rate (d$^{-1}$) and $t$ time (d).

$$\ln(\text{CH}_4(t)) = \ln\left[ -\frac{A_0}{\mu_{\text{max}}} \right] + \mu_{\text{max}} \cdot t$$

![Figure 2](https://iwaponline.com/wst/article-pdf/44/4/103/430167/103.pdf)

Figure 2. Variation of the methane accumulation through time at three spent grain concentrations. (△) 7% w/v, (□) 13% w/v, (○) 20% w/v
As an example with ISR = 0.0307, the initial acetoclastic activities $A_0$ and the maximum growth rate $\mu_{max}$ are presented in Table 2. This fitting was made in two visually separated intervals: (a) day 0 to 5, (b) day 15 to 40. The values of the (a) interval are higher than in the literature (Veeken et al., 2000), but those values obtained into (b) interval were confirmed by the literature.

With these results, real accumulative methane curves were modelled for 7, 13 and 20% w/v tests. Figure 3 shows this model when ISR = 0.0307. For all concentrations, the adjustment of experimental data was quite good, although more time intervals would be necessary for those tests with an elevated substrate content.

Anaerobic degradability AD and specific methane production SMP were calculated at the end of all tests. The maximum values of AD, $\text{SMP}_{VS}$ and $\text{SMP}_{WASTE}$ were:

- 80%, 58.5 L$_{\text{CH}_4}$/kg$_{VS}$·d and 0.14 L$_{\text{CH}_4}$/kg$_{WASTE}$·d for 7% w/v tests,
- 81%, 48.7 L$_{\text{CH}_4}$/kg$_{VS}$·d and 0.16 L$_{\text{CH}_4}$/kg$_{WASTE}$·d for 13% w/v tests, and
- 78%, 6.7 L$_{\text{CH}_4}$/kg$_{VS}$·d and 0.03 L$_{\text{CH}_4}$/kg$_{WASTE}$·d for 20% w/v tests.

The effect of ISR variation is less on AD than on SMP. In Figure 4, AD and SMPs versus ISR are presented for all tests. During the development of this study, it was observed that the curve AD versus ISR tended to a saturation value (around 80%), reached at different ISR values depending on substrate concentration. The maximum AD for 7% tests was reached when ISR was approximately equal to 0.1, but for 13 and 20% ISR was 0.02 and 0.01 respectively. All calculated AD were better than literature estimated values for brewery spent grain (Kang, 1993).

While AD maximums depend on high ISR values, SMP maximums were reached at small ISRs. These results show that small ISR ratios caused slow or limiting hydrolysis, although the methane production was very quick. The SMP$_{WASTE}$ curve for 7 and 13% w/v tests is very interesting, because its maximum varies with ISR value. The SMP$_{WASTE}$ dropped from 1.4 to 0.3 L$_{\text{CH}_4}$/kg$_{WASTE}$·d in 7% w/v tests, while it was under 1 L$_{\text{CH}_4}$/kg$_{WASTE}$·d for all 13% w/v tests. This difference was due to biomass content in

![Figure 3](https://iwaponline.com/wst/article-pdf/44/4/103/430167/103.pdf)  
**Figure 3** Model of experimental data based on maximum growth rate and acetoclastic activities from equation (1) for 7, 13 and 20% w/v tests when ISR = 0.0307. (△) 7% w/v; (□) 13% w/v; (○) 20% w/v; (— model)

<table>
<thead>
<tr>
<th>% w/v</th>
<th>$\mu_{max}$ (d$^{-1}$)</th>
<th>$A_0$ (mgDQO/gSV·d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Interval (a)</td>
<td>Interval (b)</td>
</tr>
<tr>
<td>7</td>
<td>0.453</td>
<td>0.022</td>
</tr>
<tr>
<td>13</td>
<td>1.728</td>
<td>0.010</td>
</tr>
<tr>
<td>20</td>
<td>3.560</td>
<td>0.015</td>
</tr>
</tbody>
</table>

Table 2 Maximum growth rate ($\mu_{max}$) and acetoclastic activities ($A_0$) for degradability tests when ISR = 0.0307.
7% w/v assays: biomass hydrolyses and produces volatile fatty acids quicker than 13% biomass.

Conclusions
Brewery spent grain is an organic industrial waste that can be anaerobically degraded because the AD result was 80% for almost all tests and the SMP values were, for example 70LCH4/kg VS·d for 13% w/v tests.

In general, the best values were related to 7% w/v tests, where highest ADs were reached, but SMPVS were similar for all three series. The AD and SMP curves have the same behaviour for 7, 13 and 20% w/v tests; so this behaviour confirms the nature of the substrate with the anaerobic sludge. However, the maximum values of SMPWASTE depend on the ISR ratio for each substrate concentration.

Related to the start-up of an anaerobic system with brewery spent grain, the ISR ratio and AD relation showed that it was not necessary to start-up with an elevated inoculum concentration in order to reach good degradation results. It means that the inoculum for the start-up of an anaerobic system for organic solid waste, as spent grain, does not need an excessive biomass concentration. For example, during the start-up of a two-phase anaerobic system would be enough a low ISR ratio for getting a good AD and intermediate compounds production as first-phase effluent. But in conventional anaerobic systems with one reactor, SMP should be optimised in order to find a good biogas production.

The ISR and AMP relation showed something similar to AD, but in this case the results lead to what inoculum concentration would be interesting based on the final objective: methane or intermediate compounds production, as VFA. If methane production is more important, then methanogenic inhibition could appear if the start-up begins with high ISR (low SMPVS). In particular for brewery spent grain, the ammonium concentration was so high that adaptation periods were observed. So for conventional anaerobic systems this results would be especially important in order to avoid such an inhibition. So the brewery spent grain could be degraded in a two-phase anaerobic system with 7% w/v substrate concentration and a ISR around 0.032.

As final conclusion, the ISR tests for each residue are an important tool to establish the start-up protocol for anaerobic systems in order to optimise this stage, which is fundamental to reaching good operation. The correct ISR might avoid problems such as inhibition or incorrect inoculum concentration.

Acknowledgements
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References


