Short Communication

Effect of microaerobic conditions on the degradation kinetics of cellulose

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Abstract

Limited oxygen supply to sludge digesters has shown to be an effective method to eliminate hydrogen sulfide from the biogas produced during anaerobic digestion but uneven results have been found in terms of the effect on the degradation of complex organic matter. In this study, the effect that the limited oxygen supply provoked on the “anaerobic” degradation of cellulose was evaluated in batch-tests. The microaerobic assays showed to reach a similar maximum production of methane than the anaerobic ones after 19 d and a similar hydrolytic activity (considering a first order rate constant); however, the microaerobic assays presented a shorter lag-phase time than the anaerobic test resulting in faster production of methane during the first steps of the degradation; specifically, the maximum methane production found in the anaerobic test in 19 d was found in the microaerobic test before the day 15.

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1. Introduction

Anaerobic digestion (AD) process is widely employed to reduce the organic content of wastes and wastewaters while producing biogas, mainly composed of methane that can be energetically recovered. However, when the organic matter contains sulfur compounds, hydrogen sulfide is also found in the gaseous stream, and a further step for removing hydrogen sulfide is required before the methane can be transformed to energy.

In this sense, the microaerobic removal of hydrogen sulfide (limited oxygen supply to the “anaerobic digester” to convert the hydrogen sulfide to elemental sulfur) has proved capable to reach a very low concentration of the pollutant (Díaz et al., 2010a, 2011; Fdz-Polanco et al., 2009); nevertheless, uneven results in terms of methane yield, hydrolytic activity and aerobic consumption of volatile fatty acids (VFA) have been reported.

Some authors found that microaerobic conditions did not substantially alter the methane production and organic matter removal (Díaz et al., 2011; Fdz-Polanco et al., 2009; Tang et al., 2004), whereas Nguyen et al. (2007) found an increase in methane yield, without any evidence of improving the hydrolytic potential. On the other hand, it has been reported that the limited oxygen supply caused an increase of the hydrolysis rates for the case of complex organic matter in batch-tests (Jenicek et al., 2008, 2010; Johansen and Bakke, 2006) and an improvement in digester performance and VFA concentration reduction in the study of Botheju et al. (2010a), Zhu et al. (2009), who reported that sufficient microaeration improved the hydrolysis of carbohydrates and proteins treating organic wastes in digestion in two stages, attributes these differences to the lack of comparison between the microaerobic gradient. Some other important factors apart from the amount of oxygen (or air) supplied are the mixing and the gas–liquid transfer, the presence of hydrogen sulfide, the kind of waste and the characteristics of the sludge.

The aim of this study was to evaluate the effect of the limited oxygen supply on the degradation of cellulose by different type of biomass coming from reactors operating at different mixing conditions. To do this, several batch assays were performed to complement previous research studies.

2. Methods

2.1. Anaerobic biomass and substrate

Original inoculum was obtained from a full-scale anaerobic digester from Villalonquejar WWTP (Burgos, Spain). Afterward, it was seeded and acclimated in three anaerobic pilot reactors which were operated in three different conditions: anaerobic conditions, microaerobic conditions with sludge recirculation and microaerobic conditions with biogas recirculation. All these pilot-plant digesters (200 L) were fed with the same sewage sludge (mixed sludge) and at the same hydraulic retention time (HRT) of 20 d. After this period of 39 d, biomass samples (which would be the inoculum for the anaerobic test) were withdrawn from each digester and degassed during 3 d at 35 ± 1 °C previous to the addition of buffer and sub-
strate. With regards to the substrate, amorphous cellulose (as suggested by Angelidaki et al. (2009)) was used as carbon source in order to estimate the hydrolytic activity of the anaerobic biomass.

2.2. Biodegradability tests

Glass batch-reactors with an effective volume of 2.1 L equipped with a deflector bar to facilitate mixing in rotatory beds were employed. The volume of seeded anaerobic biomass was around ~500 mL for every batch, sodium bicarbonate was added as buffer to achieve a concentration of 1 g/L, and finally, amorphous cellulose to reach a relation VSubstrate/VSinoculum of 0.5. The batch tests were maintained at 35 ± 1 °C and methane production was evaluated by pressure increase with a transducer and with the corresponding methane composition of the biogas.

The batch assays were performed in anaerobic and microaerobic conditions. Assay AN was performed in anaerobic conditions with inoculum acclimated in anaerobic conditions, assay MA1 was performed in microaerobic conditions with inoculum acclimated in microaerobic conditions with sludge recirculation and assay MA2 was carried out in microaerobic conditions with inoculum from the digester in microaerobic conditions with biogas recirculation.

Assays were performed in triplicate, employing three extra control-test for every batch to discount the endogenous methane production of the sludge. 10 mL of oxygen at atmospheric pressure were daily supplied to the assays MA1 and MA2, this rate was chosen to maintain a similar relation of oxygen per fed VS as that in continuous pilot-plant reactors from previous research (Fdz-Polanco et al., 2009).

2.3. Data processing

Experimental data was fitted with two kinetic equations: (1) the modified Gompertz equation (Eq. (1)) to ease the comparison between the data (Hu et al., 2004), and (2) the first order equation to estimate the hydrolysis constant. Eq. (2) was modified by including a term to adjust data with “lag-phase”.

\[
P(t) = P_{\infty} \exp \left[ -\exp \left( \frac{R_{\infty} e}{P_{\infty}} (\lambda - t) + 1 \right) \right]
\]

where \(P(t)\) is the cumulative total methane production (mL/gVS), \(P_{\infty}\) is the total methane production potential (mL/gVS), \(R_{\infty}\) the maximum methane production rate (mL/d), \(\lambda\) the lag-phase time (d) and \(t\) the elapsed time (d).

\[
B(t) = B_{\infty} \left[ 1 - \exp \left( -k_{H}(t - L_p) \right) \right]
\]

where \(B(t)\) is the cumulative total methane production (mL/gVS), \(B_{\infty}\) is the total methane production potential (mL/gVS), \(k_{H}\) the hydrolysis constant (d⁻¹), \(L_p\) the lag-phase time (d) and \(t\) the elapsed time (d).

In order to obtain information about the accuracy of the estimated kinetic parameter, the covariance matrix associated with each set of optimal parameters was calculated as in Lukasse et al. (1997).

An analysis of the variance (ANOVA) was performed to evaluate the effect of microaeration on the estimated parameters of the non-linear regressions (Eqs. 1 and 2). The goodness of models with constant parameters (indicating lack of significance of the different adjustments) that fit all the experiments was evaluated and compared to the application of different parameters for every experiment by utilizing the Eq. (3) (Montgomery, 2005):

\[
F_0 = \frac{RSS_1 - RSS_2}{RSS_2} \frac{DF_1}{DF_2}
\]

where RSS1 is the sum of the squares of residuals of the considered hypothesis, RSS2 is the sum of the squares of the fitted models independently, DF1 the degrees of freedom in the hypothesis and DF2, the degrees of freedom of the independent adjustment. Eq. (3) follows a distribution F-Snedecor, with DF1 – DF2, degrees of freedom in the numerator and DF2, degrees of freedom in the denominator, allowing to calculate the probability (p) of the hypothesis.

2.4. Analytical methods

Biogas composition was measured by sampling (100 µL) and subsequent injection in a VARIAN CP 3800 chromatograph (GC) equipped with TCD as described in Díaz et al. (2010). Biogas production was measured by pressure increase with a transducer. VFA concentration at the end of batch assays was measured by GC in a VARIAN 3900 gas chromatograph equipped with FID. Samples were centrifuged at 5000 rpm during 10 min, supernatant was filtered by 0.45 µm. Next, 5 mL of filtered sample were added 100 µL of sulfuric acid for carbon dioxide removal and stabilization. Finally 1.5 mL were placed in a vial and 10 µL were injected in the chromatograph.

TS and VS were evaluated according to Standard Methods (Clesceri et al., 1998).

3. Results and discussion

The performance of the digestion under microaerobic and anaerobic conditions was evaluated in batch assays (Fig. 1). The anaerobic test (AN) showed a final methane production after 19 d of 316 ± 11 mL CH4/gVS fed, while for the microaerobic test MA1 was 319 ± 11 and for the microaerobic MA2 was 327 ± 6. The relative difference between the highest production (MA2) and the lowest in AN was lower than 4%, these differences are not significant. These results match to those obtained by Díaz et al. (2010) who reported similar methane yields in digesters of sewage sludge with HRT of 20 d in anaerobic and microaerobic conditions. In this sense, the possible effect of limited oxygen supply on the hydrolysis to the bioreactor did not alter the expected methane production in anaerobic conditions for the common HRT employed in the anaerobic digestion of sludge.

The data fit to the Gompertz equation showed that the microaerobic assays, MA1 and MA2, presented a lower lag-phase time (\(\lambda\)) than the anaerobic assay AN (Table 1). This implies that limited oxygen supply accelerated the transformation of the cellulose to more simple organic compounds in the very early stages of the digestion leading to a higher methane production during these first days, which agree with the results presented by Jagadabbi et al. (2010) in the anaerobic degradation of grass-silage, a rich-cellulose substrate. The maximum methane production potential (\(P_{\infty}\)) was similar in MA1 and AN and slightly higher in MA2 than in AN.

The comparison between MA1 and MA2 presents overlapped intervals of confidence for all the parameters in the model; however the standards deviations in parameters from MA1 adjustment are considerably larger than those observed for MA2. This can be the result of a better acclimation to microaerobic conditions for MA2, whose inoculum belongs to a reactor mixed by biogas recirculation, where the contact with oxygen is expected to be higher as a consequence of a larger gas/liquid interface.

The analysis of the variance (Table 2) showed that there was a probability lower than 1% of that a unique model can describe properly all the experiments and a similar situation was found when only one \(\lambda\) was considered to describe all the experimental set; thus, indicating that the lag-phase time was significantly different between the experiments. On the other side, the hypothesis of using constant \(P_{\infty}\), \(R_{\infty}\), or both constant was not rejected as the probability was higher than 1%.

A similar behavior was found when fitting the data to the first order equation. \(L_p\) followed the same trend as abovementioned,
different in the ANOVA under microaerobic conditions suggest the possible utilization of other models, where the whole performance of hydrolysis can be described more specifically. A first approach in this sense was carried out by Botheju et al. (2010b) by modifying the anaerobic digestion model 1 (ADM1) in order that the amount of oxygen supplied can be specifically taken in account.

To explore the effect on the early stages of the hydrolysis step the specific methanogenic activity was analyzed (Fig. 2). The specific methanogenic activity can be related with the hydrolytic activity if there is no accumulation of intermediates as hydrolysis is the limiting step in the degradation of cellulose (Angelidaki et al., 2009). All the batch experiments showed a concentration of acetic acid at the end of the experiments lower than 20 mg/L with no presence of longer chain VFA. Then, it can be assumed no accumulation of intermediates.

Fig. 2 shows that MA1 and MA2 presented a higher methane production rate than AN before day 3, then indicating a more elevated hydrolysis of cellulose during this period. Conversely, the maximum specific methanogenic activity was presented in AN in day 3.3 with a value similar than MA2. Another important observation is the activity during the last days of the assay, from day 10 to 19, A showed a specific methanogenic activity above MA1 and MA2, what involves that a higher amount of cellulose was still hydrolyzed and transformed to methane lately.

Therefore, our findings indicate that the effect of limited oxygen supply on the anaerobic digestion of cellulose was found on the early hydrolysis steps resulting in higher production of methane during the first days; besides, oxygen did not inhibit methanogenesis, or compete for the consumption of volatile fatty acids, as the methane yield was not reduced in the microaerobic assays. The most important consequence of this is that a similar methane production rate than AN before day 3, then indicating a more elevated hydrolysis of cellulose during this period. Conversely, the maximum specific methanogenic activity was presented in AN in day 3.3 with a value similar than MA2. Another important observation is the activity during the last days of the assay, from day 10 to 19, A showed a specific methanogenic activity above MA1 and MA2, what involves that a higher amount of cellulose was still hydrolyzed and transformed to methane lately.

Table 1 contains the parameters of Gompertz and first order equations fitting experimental data. The ANOVA for the first order equation presented a similar performance provoked some effect on the lag-phase time. The rest of the hypothesis could not be rejected. The fact of that both considered models presented a lower HRT in comparison to the anaerobic.

Then, the most plausible effect of the introduction of oxygen in the batch assays was found on the lag-phase time. This parameter is interpreted as the time elapsed until a significant production of methane is found in batch assays. As a result, the impact of microaerobic conditions on the anaerobic degradation can be expected on the first steps of solubilisation of complex organic matter.

The ANOVA for the first order equation presented a similar behavior than for the Gompertz model. The probability of that a unique model can describe the behavior of all the experiments is lower than 1%. \( L_{F} \) was found to be statistically different between the experiments according to the F-test, indicating that microaerobation provoked some effect on the lag-phase time. The rest of the hypothesis could not be rejected.

The fact that both considered models presented a lower lag phase time while the maximum methane production rate (or hydrolysis rate constant) could not be considered statistically significant lower in the microaerobic assays in comparison to the anaerobic.

Table 2 contains the analysis of variance for a confidence level of 99. The probability of the Gompertz model (RSS2) is 24,176 with 99 degrees of freedom and 23,069 with 90 degrees of freedom for the first order equation.

Table 3 contains the parameters of the Gompertz model for each assay and the adjustment models. The Batch assay analysis was found on the lag-phase time. This parameter is interpreted as the time elapsed until a significant production of methane is found in batch assays.

(a) Assay MA1; (b) Assay MA2; (c) Assay AN.
4. Conclusions

The effect of limited oxygen supply in the degradation of cellulose was studied in batch-test. The supply did not substantially affect the maximum methane production after 19 d test and the hydrolysis constant (in a first order rate model) was not significantly different between the anaerobic and microaerobic assays. However, a shorter lag-phase was found in the microaerobic assays, during the first 2 d; the microaerobic assays presented a higher specific methanogenic activity compared to the anaerobic ones and the maximum methane production in the anaerobic test in 19 d was reached in the microaerobic tests before the day 15.

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