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Influence of lipid concentration on the hydrolysis and biomethanation of lipid rich wastes

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Abstract The influence of lipid concentration on hydrolysis and biomethanation of an artificial lipid rich (triolein) waste was evaluated. No inhibition on methane production was observed for tests with 5, 10 and 18 % (w/w, based on COD) of lipid. For higher amounts of lipid (31, 40 and 47 %) inhibition was observed. However, the process was able to recover from the inhibition. When the effect of lipase addition on enzymatic hydrolysis of lipids was studied, results showed that the higher the enzyme concentration, the more accentuated was the inhibition of the methane production. The enzyme seems to enhance the hydrolysis and produced intermediates are causing inhibition of the later steps of the degradation process. Since the VFA profiles presented similar trends for the different lipid amounts tested, the major obstacle to methane production is believed to be the LCFA formed.

Keywords Anaerobic digestion**;** biogas**;** methane; lipid; LCFA; hydrolysis;

Introduction

Lipid rich waste from food processing industry, slaughterhouse, edible oil processing, dairy products industry, are attractive substrates for anaerobic digestion due to the higher methane yield obtained when compared to proteins or carbohydrates (Hansen et al., 1999). Besides causing operational problems in the anaerobic digesters due to clogging, these lipids may as well lead to mass transfer problems for soluble substrates since they adsorb on the microbial biomass surface (Pereira *et al.*, 2004). The flotation of biomass due to fat adhesion may as well cause loss of active biomass through the outlet of the digester (Cammarota *et al.*, 2001).

Few studies have been conducted to investigate the influence of lipid concentration on hydrolysis dynamics. Nevertheless, to study the hydrolysis process for a wide range of concentrations of lipids would allow better understanding of the process. Some studies have been reported on the area but the, lipid amount is in most cases lower than 5% (w/v) (Masse *et al.*, 2002, 2003, Cammarota *et al.*, 2001). Furthermore, a process configuration allowing higher lipid concentration would improve the process economics. The aim of this study was to investigate the influence of the lipid concentration on the hydrolysis step and examine if the biomethanation of lipid rich wastes under inhibitory conditions could be improved by the addition of enzyme.

Methods

The influence of different concentrations of lipid (ranging from 5 to 47%) on the hydrolysis and biomethanation of an artificial waste was studied. The waste was composed of soluble starch, whey protein, α-cellulose and triolein as carbohydrate, protein, cellulose and lipid sources respectively. The amount of lipid was varied while it was kept constant for the other components (Table 1). The composition of substrate was based on chemical oxygen demand (COD).

Sludge from an anaerobic digester treating municipal and potato processing wastewater (total solids (TS) 8.3 % and volatile solids (VS) 4.6 %) (Ellinge, Sweden) was used as inoculum at VS ratio of 1.35 (waste:inoculum). Nutrients (Jantsch et al., 2002) were added to ensure that no nutrient deficiency would occur. Bicarbonate at a concentration of 14 g/l was added to provide buffering capacity. The final TS content in the experiments varied between 6 and 8 %. Assays were run using 11 replicates and each time one vial was eliminated for analysis. Three were used for gas phase studies during the experiment and liquid content content was analyzed at the end. Liquid phase sampling was performed on days 0, 1, 2, 3, 5, 9, 15, 21 and final day.

To test if the enzymatic hydrolysis of lipids was rate limiting for anaerobic digestion, the waste was treated with a commercial lipase in a separate series of experiments. The fat concentrations were selected based on the results from the previous experiment so that inhibitory and non inhibitory conditions were present. The three triolein concentrations tested were, 10, 18 and 31 %. The commercial enzyme used was lipase 80 000, from *Rhizopus oryzae* (Gist-Brocades SA, now owned by DSM). Each waste mixture was supplemented with three different enzyme concentrations 3.6 (E1), 61.0 (E2) and 120.8 (E3) kIU/kgVS. Autoclaved enzyme assays (inactive enzyme) were used as controls (40 min at 120 ºC). Inoculum and inoculum plus enzyme (active and inactive) were used as blanks. All tests were run in triplicate.

Biogas production was measured using a pressure transducer technique (Neves et al. 2004). Volatile fatty acids (VFA) concentrations were measured using HPLC according to Svensson *et al.* (2001). COD was measured according to APHA (1995).

Table 1 Substrate compositions tested.

Test lipid % (COD based)	Composition COD % (w/w)				Total COD (g)	TS % (w/v)
	triolein	Starch	Whey protein	α- cellulose		
5	5	32	47	16	0.76	6.1
10	10	30	45	15	0.80	6.2
18	18	27	41	14	0.88	6.5
31	31	23	35	11	1.04	7.1
40	40	20	30	10	1.20	7.7
47	47	18	26	9	1.36	8.3

Results and discussion

The methane production rate observed was similar for tests with 5, 10 and 18 % of lipid (Fig 1) indicating that no inhibition occurred at these lipid concentrations. Inhibition was observed for the other tests. For the test with 47% lipid the lag phase extended for 60d, but the process recovered. The methane recovery was 100 % for all the tests except for the one with 31 % lipid for which was 93%. pH decreased to 5.5 on day 5 in the test with 47 % lipid even though bicarbonate was added. Up to day 21 the profiles of VFA concentrations for acetic, propionic and n-butyric acids presented similar trends for all the tests except the one with 47% lipid which always contained higher amounts (Fig 2). Around day 15 acetic and n-butyric acids concentrations decreased considerably, while an increase in propionic acid could be observed. At the end of the experiment the VFA concentrations were very low for all the tests. The fact that the VFA concentrations profiles were similar (exception for 47%) and that inhibition was observed for tests with 31 and 40 % lipid indicates that VFAs are not the major cause of inhibition of gas production. If it was VFAs concentration causing the inhibition, the profiles observed should have been correlated to the concentration of lipid present in each test.

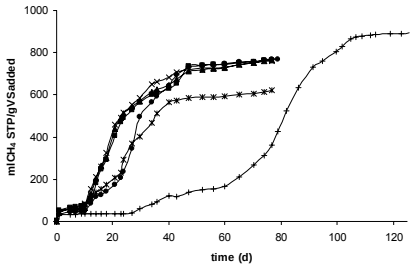

Figure 1 Cumulative methane production of the artificial waste containing different amounts of lipid (triolein); ■ -5%, ▲- 10%, × - 18%, * - 31%, ● – 40% and + - 47%.

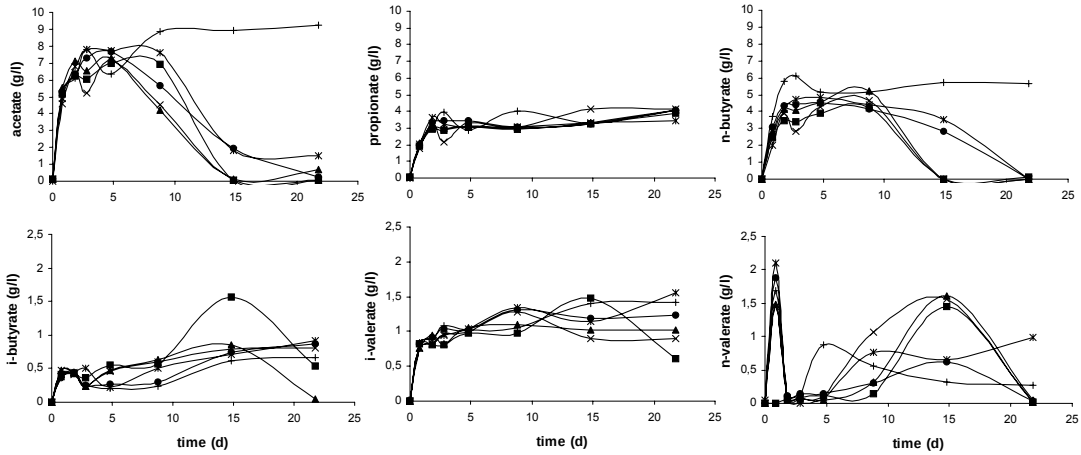

Figure 2 VFA profiles up to day 21; -■ -5%, -▲- 10%, -× - 18%, -* - 31%, -● – 40% and -+ - 47% lipid.

In the experiment in which the effect of lipase addition was studied, the results showed that the higher the enzyme concentration, the more accentuated was the inhibition on methane production (Fig 3). In the blanks, the methane rate was faster with increasing enzyme concentration. The maximum methane yield was higher for increasing enzyme concentrations added as well. This was an indication of that the enzyme was used as substrate. Rintala and Ahring (1994) also reported that on addition of enzymes during termophilic anaerobic treatment of household waste the added enzymes were used as substrate.

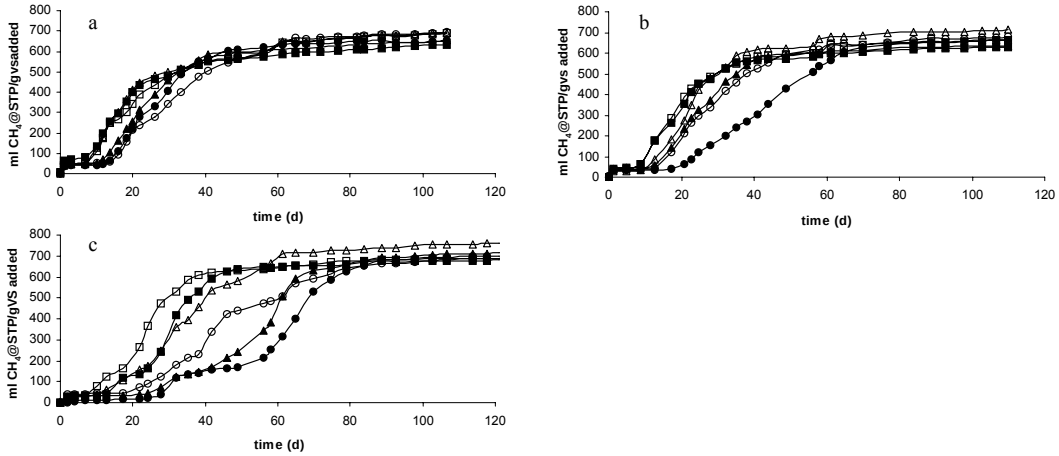

Figure 3 Cumulative methane production of the artificial waste containing different amounts of lipid (triolein) treated with three lipolytic enzyme concentrations. a – 10%, b – 18% and c – 40% lipid; -■- E1 active, -□- E1 inactive, -▲- E2 active, -Δ- E2 inactive, -●- E3 active and -○- E3 inactive.

A significant difference in methane production between the tests compared to controls with inactive enzyme was observed with increasing lipid content. This is an indication that the addition of enzyme was beneficial for lipid hydrolysis. In case the enzyme was not enhancing the hydrolysis process, similar curves should have been observed for tests and controls for all enzymes concentrations. Furthermore, in the first experiment, the tests with 10 and 18% lipid did not show any inhibition on methane production (Fig 1) contrarily to what was observed in the second one. Moreover, since the VFA profiles were similar in the first experiment for the different lipid amounts tested in this experiment, the major inhibitor of methane production is therefore believed to be the LCFA formed. This is according to other researchers' observations however those tested lower concentrations of lipid (Masse *et al*., 2003). The next step of the study is to analyze the LCFA and compare with the results presented here.

Conclusions

The results from this study help to clarify and improve the understanding of anaerobic degradation of lipid rich wastes. The results showed that the addition of lipase enhanced the hydrolysis of lipids. However, the advantages of enzyme addition on the overall process should be minimal due to accumulation of intermediates (LCFA). And these appear to be the key factors of the inhibition of lipid degradation. The study also shows that the effect of inhibition was not permanent. However, long recovery times may be required, which is not desired when operating large scale continuous digesters.

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