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Anaerobic digestion of lipid-rich waste—Effects of lipid concentration

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Abstract

The influence of lipid concentration on hydrolysis and biomethanation of a lipid-rich (triolein) model waste was evaluated in batch. The effect of increasing the concentration of lipid from 5% to 47% (w/w), based on chemical oxygen demand (COD), was investigated. The methane recovery observed was above 93% for all tests. An initial lag phase of approximately 6–10 days was observed for all tests. The methane production rate observed was similar for tests with 5%, 10% and 18% lipid (w/w, COD basis). For higher amounts of lipid (31%, 40% and 47%), a stronger inhibition was observed. However, the process was able to recover from the inhibition. When the effect of addition of lipase on enzymatic hydrolysis of lipids was studied, the results showed that the higher the enzyme concentration, the more accentuated was the inhibition of methane production. The enzyme appears to enhance the hydrolysis but the intermediates produced caused inhibition of the later steps in the degradation process. Since the volatile fatty acid (VFA) profiles presented similar trends for the different concentrations of lipid tested, the major obstacle to methane production was the long-chain fatty acids (LCFA) formation.

Keywords: Anaerobic digestion; Lipid; Hydrolysis; LCFA; Inhibition; Palmitate

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1. Introduction

Lipid-rich waste is produced in considerable amounts each year from the food processing industry, slaughterhouses, the edible oil processing industry, the dairy products industry and olive oil mills. The importance of olive oil mills in the Mediterranean countries is well known. In all lipid-rich wastes from such sources, lipids are not present alone but are usually one of the main components and one of the most problematic ingredients. Lipids cause operational problems in anaerobic digesters due to clogging, and may also cause mass transfer problems for soluble substrates since they become adsorbed to the microbial biomass surface [1]. The flotation of biomass due to adhesion of fat may also cause loss of active biomass because of washout [2]. All these problems limit the operating efficiency of digesters, and a physico-chemical pre-treatment is usually applied in order to remove the lipid fraction before the anaerobic process. Nevertheless, lipids are attractive substrates for anaerobic digestion and co-digestion due to the higher methane yield obtained when compared to proteins or carbohydrates. In this context lipid-rich waste can be regarded as a large potential renewable energy source [3]. Ahring [4] described a significant increase in the yield of methane, from 25 to 50 m^3 biogas/m^3 cattle waste, when fish oil (total concentration 5%) was added to a manure digester. The benefit of adding lipids to a digester to enhance the production of methane is therefore a promising approach which should be better explored. It is therefore important to continue to improve the knowledge of the degradation process of such types of waste.

In an anaerobic environment, lipids are first hydrolysed to glycerol and free LCFAs. This process is catalysed by extracellular lipases that are excreted by the acidogenic bacteria. The further conversion of the hydrolysis products takes place in the bacterial cells. Glycerol is converted to acetate by acidogenesis, while the LCFAs are converted to acetate (or propionate in the case of odd-number carbon LCFAs) and hydrogen through the β-oxidation pathway (syntrophic acetogenesis) [5]. This process depends on the ability of the hydrogenotrophic methanogens to utilise the hydrogen produced during fatty acid oxidation.

The inhibitory effect of lipids is commonly attributed to the LCFAs, with neutral lipids being less inhibitory [6]. However, lipid hydrolysis may be inhibited by product accumulation due to the particularity of lipases, which generally require an interface to be activated. As an interfacial phenomenon, the mechanism of lipid hydrolysis is a function of the "concentration" and quality of the interface [7]. The LCFAs present an amphiphilic structure that will remain at the lipid–water interface, and consequently the physical and chemical properties of the interface may change. Liquefaction of lipids was found to be rate-limiting in slaughterhouse wastewater when high amounts of suspended solids were present due to their low bioavailability [8]. Petruy and Lettinga [9] found similar results when treating a milk-fat emulsion in an expanded granular sludge bed reactor equipped with a sieve-drum at the top of the reactor to prevent floated sludge from being washed out. Sanders [10] suggested that methane production had a positive effect on hydrolysis because it reduces the coagulation of the lipid spheres, thereby maintaining a large lipid–water interface.

The LCFAs have been reported to have an acute toxic effect on anaerobic microbial activity affecting both aceticlastic and hydrogenotrophic methanogens, in some cases in a permanent way [6,11–13]. Recently, Pereira et al. [1,14] found that anaerobic consortia were able to mineralize biomass-associated LCFAs up to 5 g COD-LCFA/g volatile solids,

and demonstrated that inhibition was a reversible phenomenon more likely to be related to physical transport limitations than to metabolic functions. This opens new horizons for the anaerobic digestion of waste and wastewater with high lipid content.

When designing efficient systems for treatment of lipid-rich wastes, all the limiting aspects of their degradation, hydrolysis, slow LCFA degradation and potential inhibition must be taken under consideration. Some studies have been conducted to investigate the influence of lipid concentration on the dynamics of hydrolysis, but in most cases the amount of lipid was lower than 5% (w/v) [2,15,16]. In the case of organic waste, study of the hydrolysis process for a wide range of lipid concentrations would allow a better understanding of the process. Thus, the aim of this study was to investigate the effect of lipid concentration on methane production and to examine whether the biomethanation of lipid-rich wastes under inhibitory conditions can be improved by the addition of lipase.

2. Methods

2.1. Substrate

The model waste was composed of soluble starch (BDH, Poole, UK), whey protein (Arla, Denmark), α-cellulose (Sigma) and triolein (Sigma) as carbohydrate, protein, cellulose and lipid sources, respectively. The amount of lipid was varied, whereas the amounts of the other components were kept constant (Table 1). The amounts of all components are given as COD.

2.2. Inoculum

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 a VS ratio of 1.35 (substrate:inoculum).

2.3. Experimental set-up

The influence of different concentrations of lipid (ranging from 5% to 47%, w/w, COD basis) on the hydrolysis and biomethanation of a model waste was studied. Nutrients with the composition described by Jantsch et al. [17] were added to ensure that no nutrient

Table 1
Substrate compositions tested

Test lipid % (COD basis)	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

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%. The tests were performed in 100-ml vials with a liquid volume of 24 ml and nitrogen atmosphere. The vials were incubated at 37 °C under stirring conditions (150 rpm). Assays were run using 11 replicates. For liquid-phase sampling, one vial was eliminated for analysis. Three replicates were used for gas-phase studies during the experiment and liquid content was analysed at the end. Liquid-phase sampling was performed on days 0, 1, 2, 3, 5, 9, 15, 21 and the final day.

To test whether the enzymatic hydrolysis of lipids was rate limiting for anaerobic digestion, the substrate was treated with a commercial lipase in a separate series of experiments. The lipid concentrations were selected based on the results from the previous experiment. The three triolein concentrations tested were 10%, 18% and 31% (w/w, COD basis) (Table 1). The commercial enzyme used was lipase 80,000 from *Rhizopus oryzae* (Gist-Brocades SA, now owned by DSM, the Netherlands). Each substrate mixture was supplemented with three different enzyme concentrations: 3.6 (E1), 61.0 (E2) and 120.8 (E3) IU/g VS_{added}. Assays with autoclaved enzyme (enzyme inactivated for 40 min at 121 °C) were used as controls. Inoculum and inoculum plus enzyme (active and inactivated) were used as blanks. The incubation conditions were the same as in the first experiment. All tests were run in triplicate.

2.4. Analysis

Biogas production was measured using a pressure transducer [18]. Gas composition was analysed periodically using GC-TCD according to Mshandete et al. [19]. The values of methane production were corrected for the standard temperature and pressure conditions (STP). For liquid-phase analysis, the contents of each serum bottle were centrifuged at 4000*g* for 30 min. Samples from the supernatant were taken for analysis of VFA, lipase activity and soluble COD. The solids were washed twice with 10 ml distiled water, acidified with 5 M HCl to pH 2, and stored at −20 °C for analysis of biomass-associated LCFAs. VFA concentrations were measured using HPLC according to Mshandete et al. [19]. Lipase activity was measured according to Winkler and Stuckmann [20]. COD (total and soluble) was measured according to standard methods [21]. For the characterisation of the substrate fractions, COD was determined using suspensions of each component (1, 2 and 3 g/l), which were homogenised using a Disp 25 homogeniser (20,500 min^{-1}; Inter Med, Denmark). For analysis of biomass-associated LCFAs, aliquots of biomass (ranging from 2 to 4 ml) were dried at 105 °C for 20 h. The total lipids were extracted from the dried solids as described by Bligh and Dyer [22]. Samples (1.5 ml) from the chloroform extract were passed through silica-based columns (Bond Elut®, LRC-Si, 100 mg; Varian, the Netherlands) for separation of the different classes of lipids. The neutral lipids fraction was eluted with 1.5 ml chloroform and the eluate was evaporated under nitrogen. The LCFAs contained in the residue were then methylated. To do this, the eluates were dissolved in the methylation agent, methanol containing 5% (v/v) sulphuric acid, and the reaction was allowed to proceed for 2 h at 50 °C. It was stopped by adding 5 ml of a 5% (w/v) NaCl solution. The methyl esters were finally extracted twice with 5 ml *n*-hexane [23]. The extracts were saved and stored at −20 °C until analysis. Methyl esters were analysed using GC-FID as described by Lyberg et al. [24]. TS and VS were measured according to standard methods [21]. pH was measured with a CG 842 pH-meter (SCHOOT, Geräte

GmbH, Hofheim, Germany) immediately after sampling, to avoid pH changes due to loss of carbon dioxide in the liquid.

3. Results and discussion

The methane recovery was 100% for all the tests except for the one with 31% lipid (w/w, COD basis), for which the methane yield was 93%. The methane production pattern observed (Fig. 1) was similar to the ones reported by Broughton et al. [25] for batch degradation of sheep tallow, and by Salminen et al. [26] for batch degradation of solid poultry slaughterhouse waste. The initial lag phase in methane production observed for all tests could be attributed to the rapid build-up of VFA (Fig. 2) and/or LCFA (Fig. 3), as proposed by Salminen et al. [26]. The methane production rate observed was similar for tests with 5%, 10% and 18% lipid (w/w, COD basis) (Fig. 1). A stronger inhibition was observed for the other tests. For the test with 47% lipid (w/w, COD basis), the lag phase extended to 60 days, but the process recovered.

The profiles of VFA concentrations for acetate, propionate and *n*-butyrate up to day 21 presented similar trends for all the tests except the one with 47% lipid (w/w, COD basis), which always contained higher amounts during the period studied (Fig. 2). Around day 15, acetate and *n*-butyrate concentrations decreased considerably, while propionate remained high. For *i*-butyrate, *i*-valerate and *n*-valerate, no significant differences in concentrations were observed. At the end of the experiment the VFA concentrations were very low in all tests (below 0.2 g/l). The fact that all VFA concentration profiles except for the test with 47% lipid (w/w, COD basis) were similar, and that inhibition was observed for tests with 31% and 40% lipid (w/w, COD basis), indicates that VFAs are not the major cause of inhibition of gas production. The pH values observed also confirm this. The pH values were above 6.2 (day 5) always, except for the test with 47% lipid (w/w, COD basis) which reached 5.7 on day 5—even though bicarbonate was added. If the concentration of VFAs was the major cause of inhibition, the profiles observed should have been correlated to the concentration of lipid present in each test. A more effective methanogenesis in the case of

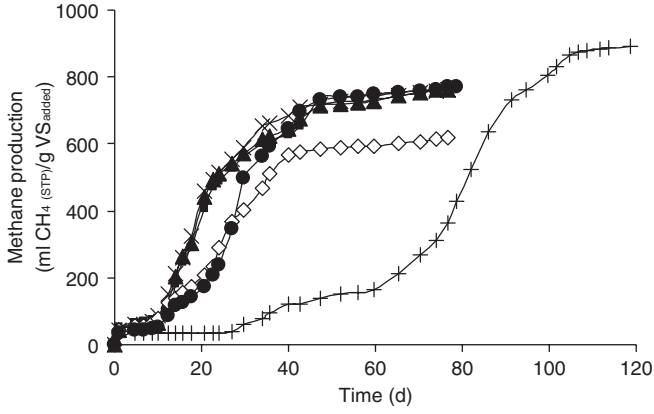

Fig. 1. Cumulative methane production from the model waste containing different amounts of lipid (triolein); -■- 5%, -▲- 10%, -×- 18%, -◇- 31%, -●- 40% and -+- 47%; STP: standard temperature and pressure conditions; inoculum gas production not subtracted.

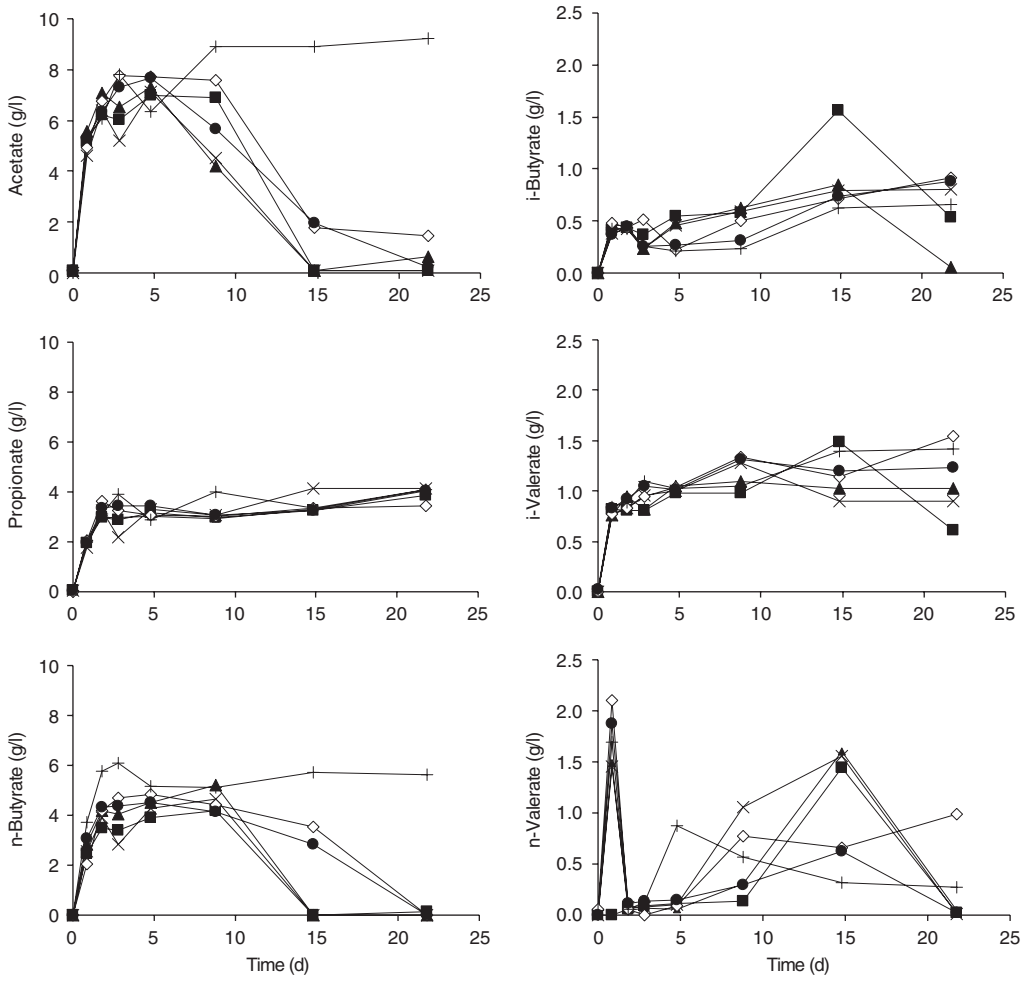

Fig. 2. VFA profiles up to day 21; -■- 5%, -▲- 10%, -×- 18%, -◇- 31%, -●- 40% and -+- 47% lipid.

the tests with a higher concentration of lipid could also explain the similar VFA concentration profiles observed; however, this was not the case, since inhibition of methanogenesis was observed. Thus, the results indicate that the syntrophic acetogenesis was inhibited by the LCFAs up to day 9 for all tests. For the test with 47% lipid (w/w, COD basis), acetogenesis was still inhibited after day 9. Furthermore, considering the similar concentration profiles observed for acetate, for all the tests except the one with 47% lipid (w/w, COD basis), it appears that methanogens were affected by a similar mechanism for all lipid concentrations.

Palmitate was the most abundant LCFA in all tests (Fig. 3). Accumulation of oleate was observed in the first 2 days, for tests with 18–47% lipid (w/w, COD basis). Then there was a decrease in this acid concentration, which correlated with the accumulation that occurred for palmitate. The concentrations of the other acids were always much lower. Stearate concentrations presented about the same values for all tests throughout the experiment,

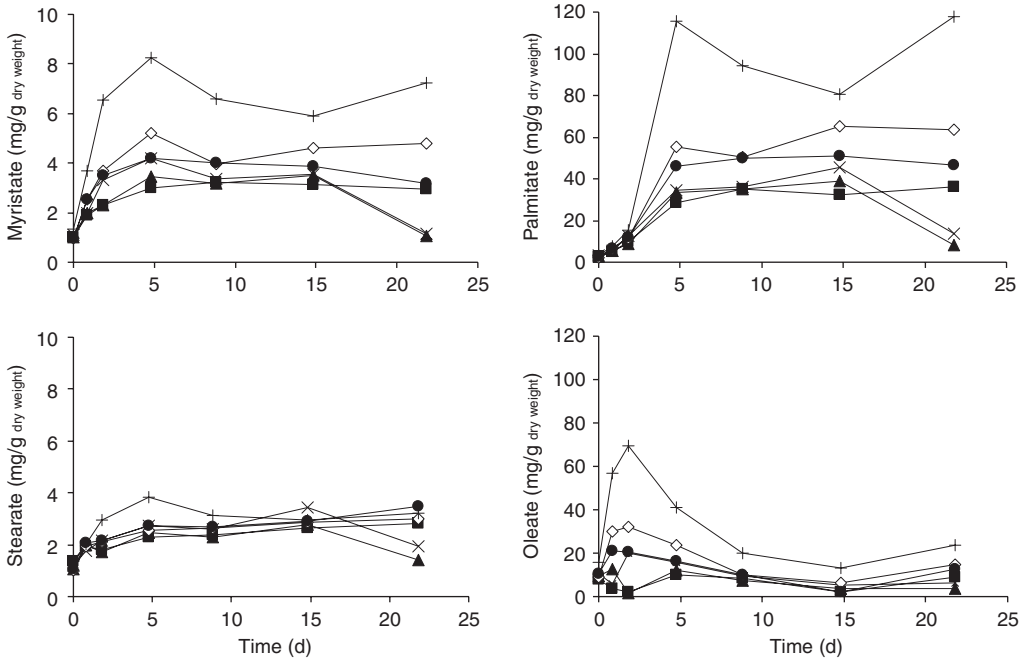

Fig. 3. LCFA profiles up to day 21; -■- 5%, -▲- 10%, -×- 18%, -◇- 31%, -●- 40% and -+- 47% lipid.

indicating that the step of conversion of stearate to palmitate was not limiting. Beccari et al. [27] applied a two-reactor system with partial phase separation for treating olive oil effluents and reported that in the conversion between oleic acid and palmitic acid, the saturation from oleic to stearic was the limiting step, whereas the first step of β-oxidation (stearic to palmitic) proceeded quickly. Also, Lalman and Bagley [28,29] reported that palmitic acid was the main product detected from oleic and linoleic acids, and that stearic acid was not detected. For the tests for which no inhibition of the methanogenesis was noticed besides the initial lag phase, palmitate concentrations in the biomass varied between 28 and 45 $mg/g_{dry\ weight}$ (days 5–15), while higher concentrations (ranging from 50 to 115 $mg/g_{dry\ weight}$) were observed in those for which inhibition was observed. After 21 days of digestion, the concentrations of this acid were still high for these tests. Although between days 10 and 15, biomass-associated LCFAs were nearly constant and palmitate especially was detected in significant amounts up to 80 $mg/g_{dry\ weight}$, the concentrations of acetate and butyrate decreased significantly in all assays except the ones involving 47% lipids (w/w, COD basis) (Fig. 2). This was a clear indication that aceticlastic and hydrogenotrophic methanogens were recovering from the inhibition caused by the LCFAs.

Oleate, which is one of the most toxic LCFAs, was present at concentrations within the ranges for which inhibition of methanogenesis has been reported. Angelidaki and Ahring [6] reported inhibition for oleate concentrations higher than 200 mg/l. Alves et al. [30] observed a reduction of 50% of aceticlastic and hydrogenotrophic methanogens activities at 50 and 200 mg/l of oleate, respectively. Salminen et al. [26] found that palmitate concentrations were inhibitory for degradation of butyrate and propionate, during the degradation of solid poultry slaughterhouse waste. However, this does not seem to have

been the case in the present study since for the tests with 31% and 40% lipid (w/w, COD basis), on day 21 *n*-butyrate concentration was very low while palmitate concentrations were still at the same level as on day 5, and for the tests with lower concentrations of lipid, propionate concentration did not decrease around day 21 when palmitate concentration was already falling. The levels of palmitate on this day were within the same range as those reported by Salminen et al. [26] which, according to these authors, were not at levels considered to be inhibitory for propionate degradation. Sanders [10], studying hydrolysis of palm oil and butter under acidogenic conditions, attempted to establish a relationship between propionate concentration and hydrolysis rate, but did not succeed. The fact that palmitate concentration maintained a stable plateau for the tests which presented inhibition of the methanogenesis after approximately 10 days indicates that palmitate degradation was the key factor, as reported by Pereira et al. [31]. A slight increase in oleate was observed for these tests also, between days 15 and 21. In a recent study by Pereira et al. [1], while studying the mineralisation of biomass-associated LCFAs, a value around 1000 mg COD-LCFA/g VS was proposed as a rough estimation of the specific LCFA load allowing the maximal mineralization rate of the biomass-associated LCFA. This value was obtained using an enzymatic kinetics model considering substrate inhibition. For concentrations below this value, the methane production rate should increase proportionally with the amount of biomass-associated LCFA, and for higher concentrations, a decrease in the methane production rate should occur with increasing concentration of biomass-associated LCFAs. The corresponding maximum mg COD-LCFA/g VS load was calculated for the experiment carried on in the study presented here in an indirect way, based on the initial amount of lipid added and considering that all the oleate released by hydrolysis would be associated with the biomass. The values found were 80, 170, 330, 670, 1,000 and 1340 mg COD-LCFA/g VS for 5%, 10%, 18%, 31%, 40% and 47% lipid (w/w, COD basis), respectively, indicating that for the tests with 40% and 47% lipid (w/w, COD basis), limitations in the ability to mineralise the LCFAs would be expected to be present. For the test with 31% lipid (w/w, COD basis), no limitations would be expected according to the model; however, considering the low correlation coefficient obtained by the authors and the inhibition of methane production observed experimentally, the test with 31% lipid (w/w, COD basis) should be considered as presenting limitations regarding ability to mineralize the LCFA.

In the experiment in which the effect of addition of lipase was studied, the results showed that the higher the enzyme concentration, the more accentuated was the inhibition of methane production (Fig. 4). In the blanks, the maximum yield of methane was higher also for increased concentrations of enzyme added (data not shown). Considering that no enzymatic activity was detected in the inactivated lipase solution, it can be concluded that the enzyme was used as substrate. Rintala and Ahring [32] also reported that after addition of enzymes during thermophilic anaerobic treatment of household waste, the enzymes added were used as substrate.

A significant difference in inhibition of methane production between the tests and controls with inactivated enzyme was observed with increasing lipid content, indicating that the addition of enzyme was beneficial for lipid hydrolysis. If the enzyme was not enhancing the hydrolysis process, similar curves would have been observed for tests and controls with all enzyme concentrations. Furthermore, in the first experiment (Fig. 1), the tests with 10% and 18% lipid (w/w, COD basis) did not show inhibition of methane production after the initial lag phase due to build up of LCFA and VFA, which is

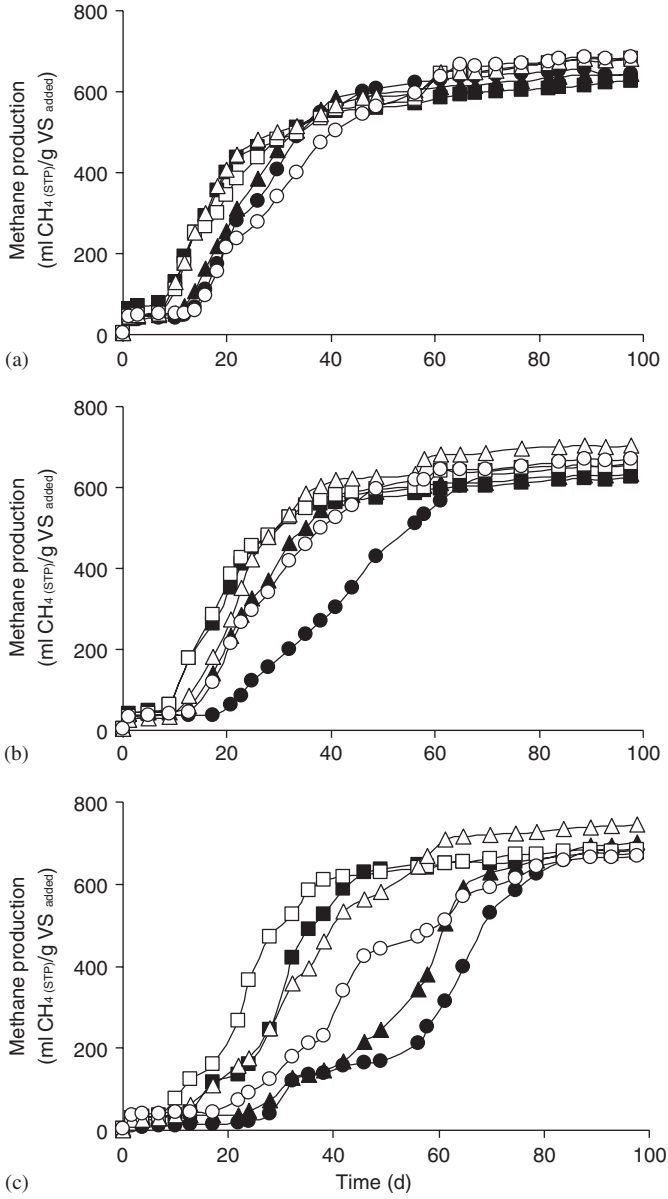

Fig. 4. Cumulative methane production from the model waste containing different amounts of lipid (triolein) and treated with three lipolytic enzyme concentrations. (a) 10%, (b) 18% and (c) 40% lipid; -■- E1 active, -□- E1 inactivated, -▲- E2 active, -Δ- E2 inactivated, -●- E3 active, and -○- E3 inactivated. Enzyme concentrations: E1: 3.6 IU/g VS_{added}, E2: 61.0 IU/g VS_{added}, E3: 120.8 IU/g VS_{added}; STP: standard temperature and pressure conditions; inoculum gas production not subtracted.

contrarily to what was observed in the second one. Moreover, since the VFA profiles presented similar trends in the first experiment for the different amounts of lipid tested in this experiment, the major factor causing inhibition of methane production was the

LCFAs formed, palmitate in particular. This agrees with the observations of other researchers; however, lower concentrations of lipid were tested by them.

4. Conclusions

The results of this study help to improve the understanding of anaerobic degradation of lipid-rich wastes. They indicate that the addition of lipase enhances the hydrolysis of lipids. However, the advantages of enzyme addition on the overall process should be minimal due to accumulation of intermediates (LCFAs), even for high lipid concentrations. The overall results indicate that the different factors involved in degradation of lipids, affected to a certain degree the concentration of the individual intermediate compounds—which is reflected in the methane production rate. LCFAs are the key factors in the inhibition of lipid degradation. The study also shows that the effect of inhibition is not permanent. However, long recovery times may be required, which is not desirable when operating large-scale continuous digesters.

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