Anaerobic Digestion of High Lipid Content Wastes: FOG Co-digestion and Milk Processing FAT Digestion

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ABSTRACT: The digestion of two different residues with high lipid content was investigated in the present research. The first part of the experimental work focussed on an assessment of the co-digestion of sewage sludge and fat, oil and grease (FOG) residues separated from the grease trap in the biological pre-treatments of wastewater treatment plants, as proposed in the present study. The second part of the experimental work studied the individual digestion of fat recovered from the grease trap of a milk processing plant. The digestion process was performed under batch and semi-continuous operation at mesophilic temperatures. Successful digestion of wastes was attained, with no inhibitory consequences due to the accumulation of long-chain fatty acids. An increase in biogas production was observed under batch digestion of sewage sludge when FOG was added as a co-substrate. However, the small increase reported was in accordance with the limited volume of this residue added. With regard to the digestion of the milk processing fat, although the addition of nutrients may be necessary for maintaining long-term operation, with the long hydraulic retention time tested no foam accumulation was observed and successful operation was achieved.

INTRODUCTION

There are numerous sources of high lipid content wastes, which include waste water treatment plants (WWTPs), animal slaughterhouses, and food processing installations [1]. Co-digestion using these kinds of wastes has become an area of interest to researchers, owing to the multiple advantages that the process offers. Co-digestion is the term used to describe the combined treatment of several wastes with complementary characteristics, this being one of the main advantages of anaerobic technology. [2]. Anaerobic digestion is a complex process which requires strict anaerobic conditions to transform organic matter into biogas, while also allowing the biochemical stabilization of sludge and reducing the amount of solids finally remaining for disposal [3]. Therefore, it is one of the most promising alternatives intended for the treatment of residues and for the production of energy by taking advantage of biogas. The process is considered an environmentally friendly option [4] for the treatment of biowastes, since it not only allows stabilization of organic matter, reducing its potential to putrefy, but also contributes to the reduction of greenhouse gas emissions. Biogas is currently seen as an important future contributor to European energy supplies [3]. The volume of biogas produced is related, among other things, to the content and quality of organic matter fed into the digester. In this way, increases in the concentration of solids in the incoming substrate may lead directly to greater biogas yields.

Lipid-rich waste which can be collected in the grease trap of wastewater treatment plants is also called fat, oil and grease (FOG) waste. This residue presents a great potential for increasing methane yield as noted by several studies [5–8]. Published reports record the benefits obtained from the co-digestion of this waste under mesophilic and thermophilic conditions can result in a doubling, or even higher increase, in methane production. Lipids are one of the major types of organic matter found in food wastes and some industrial wastewaters, such as those coming from slaughterhouses, dairy plants or fat refineries [9]. The addition of FOG, greases, or residues from slaughterhouses with high lipid contents has been evaluated in various different co-digestion processes [7, 10–12]. The addition of the co-substrate has been tested with substrates presenting volatile solid (VS) concentrations as high as 46% and successful results achieved, with no foam accumulation or inhibition due to long-chain fatty acids (LCFAs) [7–10]. The break-down of LCFAs takes place through...
the β-oxidation pathway, which has been reported as the rate-limiting step of the whole anaerobic digestion process [13]. The accumulation of these components during the digestion process may cause inhibition, because of to their known toxicity affecting acetogens and methanogens [13–15]. However, recent studies have stated that inhibition caused by LCFAs could be reversible, with acclimatization being a key factor in avoiding negative effects on microbial communities [8, 16–18]. Cuetos et al. [12] reported successful digestion of high lipid content wastes after a long acclimatization period, whilst recording inhibition problems and foam accumulation when the same residue was treated directly without first submitting micro-organisms to an adaptation period.

Another waste product of similar characteristics is the fat obtained from milk processing installations. The digestion of this type of waste has been studied previously [19, 20]. These studies report the hydrolysis phase of the process as the limiting factor. Under continuous operation, most reactors treating organic wastes with high loads are usually reported to work with long hydraulic retention times (HRTs). In this way, these systems may be particularly suitable for overcoming problems associated with the low hydrolysis rate of fatty milk wastes. With this in mind, the present study aimed to assess anaerobic digestion of FOG and fat obtained from a milk-processing plant.

MATERIAL AND METHODS

The sewage and digested sludge used was obtained from the WWTP of the city of Leon. Primary and secondary sludge (waste-activated sludge) (PS and SS) were used as the substrate for the experiments into digesting sewage sludge under batch and semi-continuous conditions. The grease used as co-substrate was obtained from the grease trap of the WWTP. FOG was added in a proportion of 0.2% V/V, on the basis of data from the WWTP regarding the production of wastes. Later experiments under semi-continuous operation were performed with higher volumetric proportions of the co-substrate (0.8% and 1.8% V/V). Digested sludge from the WWTP digester was used as inoculum. This digester treated a mixture of PS and waste-activated. The temperature of the digestion process was 32°C and the average HRT was 26 days.

The fat used for the second phase of experiments was obtained from a local milk-processing factory. The digestion systems were inoculated with the same digested sludge used in the previous set of experiments. The digestion of this substrate was also evaluated under batch and semi-continuous conditions.

Batch Digestion

Batch experiments were performed to determine the biochemical gas potential of the substrates used in this study. Experiments were carried out until the cessation of gas production was observed. The batch reactors (Erlenmeyer flasks of 250 mL) were filled with inoculum and the corresponding amount of substrate in order to attain the desired proportion of VS between substrate and inoculum. Tap water was added to complete a 250 mL volume in all batch reactors. Two reactors were used for measuring gas production and composition. A batch reactor containing only inoculum was used as blank. The biogas produced by this reactor was subtracted from the corresponding tests. The temperature of digestion was 34°C, this being controlled by a water bath. Agitation was provided by means of magnetic stirrers. The gas volumes were measured using bottle gasometers and corrected to standard temperature and pressure (STP), 0°C and 760 mmHg, respectively.

The PS used in the batch experiment presented a TS concentration of 37.6 g/L with 72% of VS. SS presented a TS content of 24.4 g/L with 75% of VS. The Erlenmeyer flasks were inoculated with 150 mL of digested sludge presenting a TS content of 20 g/L and a VS concentration of 12 g/L. Table 1 shows the characteristics of the grease collected at two different points in the grease trap (denoted FOG_1 and FOG_2). The proportion of VS between the inoculum and substrate

<table>
<thead>
<tr>
<th></th>
<th>FOG_1</th>
<th>FOG_2</th>
<th>GM1</th>
<th>GM2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organic Matter (%)</td>
<td>79.8</td>
<td>83.7</td>
<td>92.5</td>
<td>85.5</td>
</tr>
<tr>
<td>Kjeldahl Nitrogen (%)</td>
<td>3.1</td>
<td>5.8</td>
<td>0.83</td>
<td>4.8</td>
</tr>
<tr>
<td>Grease (%)</td>
<td>17.6</td>
<td>63.5</td>
<td>58.5</td>
<td>10.8</td>
</tr>
<tr>
<td>COD (g/L)</td>
<td>149</td>
<td>92</td>
<td>240</td>
<td>117</td>
</tr>
<tr>
<td>TS (g/L)</td>
<td>133</td>
<td>55</td>
<td>246</td>
<td>109</td>
</tr>
<tr>
<td>VS (g/L)</td>
<td>107</td>
<td>48</td>
<td>232</td>
<td>91</td>
</tr>
</tbody>
</table>

Percentages are expressed on dry basis.
Anaerobic Digestion of High Lipid Content Wastes

for this experiment was 1:1. Digestion systems were denoted, according to the substrate used, either PS or SS, followed by the label for the grease used, in the case of co-digestion systems.

The characteristics of the grease obtained from the milk processing factory (GM1) are also presented in Table 1. Batch digestion for this substrate was performed with several different proportions of VS between the inoculum and substrate, as follows: 0.4, 0.8, 1.0 and 1.5. The Erlenmeyer flasks were inoculated with 200 mL of digested sludge.

Semi-continuous Digestion

The digestion process was carried out in completely mixed reactors provided with mechanical stirrers. The reactor had a working volume of 3L. The systems were kept thermostatically at a temperature of 34°C ± 1°C. Reactors were provided with an outer jacket for circulating heating water that kept the system at a controlled temperature. Each reactor was provided with liquid and gas sampling ports. The reactors worked on a semi-continuous basis, being fed manually once every day. Before the reactor was fed, an equivalent volume was withdrawn. All the samples taken from the reactors were obtained after complete homogenization and previous feeding of the systems. Daily gas production was measured using a reversible device with liquid displacement and a wet-tip counter.

Reactors were inoculated with digested sludge. The PS used in this experiment presented a concentration of 33.5 g/L TS with 72% of VS. The SS sludge presented a TS concentration of 29 g/L with 78% of VS. In this second stage of experimentation, co-digestion with sewage sludge was evaluated using FOG_1 as co-substrate. The co-digestion of sewage sludge and FOG_1 was performed only with a mixture at 0.2% (V/V) of FOG (following the proportions tested in batch experiments). The HRT was set at 30 days. The sewage sludge in this case was composed of a mixture of PS and SS with a volumetric proportion of 30:70 based on the proportions of volumes of sewage sludge produced in the WWTP. A second reactor was used for treating a mixture with a higher volumetric proportion of FOG. The reactor was started up by applying an adaptation period where the feeding substrate had a proportion of FOG of 0.2%. This ratio was gradually increased to 0.8% and finally to 1.8%. This reactor was evaluated with an HRT of 30 days out of 120. Reactors were denoted in accordance with the substrate being digested in each case.

The digestion process for the fat from the milk-processing factory was performed using the same experimental apparatus and conditions as described above. In this phase of experimentation the fat obtained from the industry was denoted GM2. The reactor used was denoted R_GM2 and the HRT applied was 40 days.

Analytical Techniques

Nitrogen concentrations were determined by the Kjeldahl method. Organic matter was analysed in accordance with the Walkey-Black method (Walkey and Black, 1934). Grease content was determined by Soxhlet extraction using Velp Scientifica SER 148/3 in accordance with APHA Standard Methods [21]. COD, TS, VS, ammonium and pH were monitored during the digestion process. These parameters were determined in accordance with the APHA Standard Methods [21]. COD was determined using a Metrohm 862 Compact Titrosampler. The homogenized sample was digested in the presence of dichromate at 150°C for 2 h in a Hanna C9800 reactor. The composition of the biogas was analysed using a gas chromatograph (Varian CP3800 GC) equipped with a thermal conductivity detector. A packed column (HayeSep Q 80/100; 4 m) followed by a molecular sieve column (1m) was used to separate CH4, CO2, N2, H2 and O2. The carrier gas was helium and the columns were operated at a pressure of 331kPa and a temperature of 50°C. Volatile fatty acids (VFAs) were determined on the same gas chromatograph, using a flame ionization detector (FID) equipped with a Nukol capillary column (30 m × 0.25 mm × 0.25 µm) from Supelco. The carrier gas was helium. Injector and detector temperatures were 220°C and 250°C, respectively. The oven temperature was set at 150°C for 3 min. and thereafter increased to 180°C. The detection limit for VFA analysis was 5.0 mg/L. The system was calibrated with a mixture of standard volatile acids from Supelco (for the analysis of fatty acids C2 to C7). Samples were previously centrifuged (10 min., 3500 × g) and the supernatant filtered through 0.45 µm cellulose filters. Gas chromatography was used for the analysis of the long chain fatty acids (LCFAs). Samples for LCFA analysis were extracted as described by Fernández et al. [22]. Samples were mixed with n-heptane, the solution was then centrifuged for 30 min. at 3500 × g and filtered through a 0.2 µm Millipore Millex-FGS filter. The sample was injected into a Perkin-Elmer AutoSystem XL chromatograph equipped with a FID detector and a PEG (100% Polyethylene Glycol) column (15 m × 0.53 mm × 0.5 µm). The carrier gas
was helium. The initial oven temperature of 100°C was maintained for 1 minute, and then increased to 250°C, with a ramp of 5°C per minute, this temperature being maintained for 5 min. Injector and detector temperatures were 250°C and 275°C, respectively. The system was calibrated using a mixture of LCFAs from individual acids with concentrations in the range of 0 to 100mg/L. The detection limit for LCFA analysis was 5.0mg/L. The acids analysed were C6:C24 (with even numbers of carbon atoms) all from Sigma.

RESULTS

Sewage Sludge and FOG digestion

The results obtained from batch digestion assays are presented in Figure 1 for PS and SS systems. Since the addition of co-substrate was limited to the volumetric proportions of production in the WWTP, the benefits of this addition were scarcely noticeable in the minor increase in cumulative methane production in the PS systems. The specific methane production obtained for the individual digestion of PS was 462 mL/g VS, whilst this value increased to 542 mL/g VS (average values for both co-digestion systems). The small addition of complex wastes resulted in a decrease in the biogas production rate, as may be observed from the sigmoid behaviour of the cumulative methane graph. With regard to the SS system, no improvement was observed, all systems presenting an average specific methane production of 358 mL/g VS. Contrary to what was the case for the previous system evaluated, the addition of high lipid content waste did not affect in any significant way the methane production of the co-digestion system. Although an increase in the total volume of biogas produced was obtained in the first case, modification of the rate of biogas production was also observed. This delay may be rationalized as an adaptation of micro-organisms to the presence of the complex substrate, resulting in sigmoid curves of cumulative methane production.

Under semi-continuous operation FOG_1 was selected for the assessment of the digestion process, because of its higher solid contents. Additionally, the residue denominated FOG_2 presented particles of greater size which might render normal operation difficult. Daily biogas production is presented in Figure 2(a). As may be observed, the gas evolution was practically constant with similar values for both reactors evaluated. The addition of the co-substrate in the proportions applied did not translate into an increase in the biogas rate. As it might be expected from batch experiments, the low addition of volatile solids did not represent any significant increase in the organic load supplied to the co-digestion reactor. VS measured during experimentation showed stable behaviour with no significant modifications.

Figure 1. Gas Production from batch digestion test of Sewage Sludge and FOG for (a) PS Systems and (b) SS Systems.
Servable effects were measured. Results obtained for this reactor presented low values for VFA concentrations. Additionally, the LCFAs detected showed low concentrations of octanoic (C8), decanoic (C10), and myristic (C14) acids, with values below 50 mg/L. Increasing the FOG content of the mixture did not result in higher VFA values. This may be rationalized by the mechanisms of inhibition of LCFAs. An accumulation of LCFAs may inhibit anaerobic digestion because of direct toxicity to acetogens and methanogens, the two main groups involved in LCFA breakdown [13]. Another inhibiting mechanism is the result of the adsorption of surface active acids onto the cell wall [23], thus affecting the processes of transportation and protection. Figure 3 shows the LCFA concentrations measured in the reactor treating the FOG mixture at 1.8%. Values obtained here were lower than those reported in the literature as causing inhibition [24]. Thus, the lower gas yield of the reactor was probably due to adsorption of the FOG components onto cell walls.

The addition of fat residues to digesters has been recommended by several authors who have evaluated the co-digestion of greases by applying either continuous supplementation or pulsed addition of waste [11, 25–27]. The addition of high lipid content wastes seems a plausible option for increasing biogas production in already existing digestion systems, as has been demonstrated by the practical implantation of this option in WWTPs [28]. However, in the present study an increase in the FOG concentration resulted in inhibition of the digestion process, highlighting the relevance of testing modifications under pilot scale conditions prior to undertaking operational changes in industrial plants. Another relevant aspect deals with operating considerations which should also be carefully evaluated, to avoid clogging the process piping when delivering this co-substrate [29].

Milk Processing Waste Digestion

Figure 4 shows the results for the gas production obtained under batch digestion of milk-processing waste. The increase in substrate concentration results in an inhibitory effect, as may be observed from the lower production of gas obtained as the Inoculum to Substrate ratio (I/S) increases. Inhibition associated with the concentration of LCFAs has been reported under continuous operation and digestion assays [30–32]. From the results obtained here, it may be rationalized that increasing amounts of the substrate resulted in higher concentrations of LCFAs, which in turn decreased the biogas yield.

Sage et al. [20] studied the degradation of milk fat, reporting a lag phase of several days prior to degradation of fat by anaerobic micro-organisms, with this lag phase before biogas production being mainly due to unsaturated free fatty acids (FFA). Conversion to

![Figure 2](image-url)  
*Figure 2.* (a) Specific methane production of reactor treating the mixture of primary sludge and secondary sludge (PS_SS) and the co-digestion mixture (PS_SS_FOG1) at 0.2 % (b) Daily gas production of reactor treating the mixture at 1.8%.

![Figure 3](image-url)  
*Figure 3.* LCFA concentration measured from the reactor treating co-digestion mixture (PS_SS_FOG1) with increasing proportion of FOG.
biogas occurred at a lower rate for saturated than for unsaturated FFA.

Taking into consideration the low methane yield obtained with the increase in substrate concentration, semi-continuous digestion was evaluated applying a low organic loading rate. Figure 5(a) shows the daily biogas production of the reactor working under semi-continuous operation (R_GM2). The reactor was daily fed with an organic loading rate of 0.65 g VS/L/day with an HRT of 40 days. Under these conditions, steady production of gas was observed, presenting an average methane content of 63%. Data relating to the performance of this reactor are also presented in Table 2. The specific methane production obtained was higher than the value reported for the sludge digestion systems, corroborating the high methane potential of this waste. Although inhibition was probably the cause of the limited production of methane under batch digestion in some of the experiments carried out, this situation may be circumvented under semi-continuous operation by the application of a low organic loading rate to the reactor. In this way, the concentration of LCFAs was low during the period of experimentation, with the main acids detected being palmitic, stearic and arachidic, their average concentrations being 89, 80 and 50 mg/L respectively. Although steady gas production was attained, one of the main problems when considering the digestion of food processing wastes is the concentration of nutrients needed to maintain stable operation of biological treatment processes during long-term performance. In the present study, the concentration of ammonium in the reactor was initially that of the inoculum used for starting-up the digestion process. However, as the time of experimentation grew, a significant reduction was observed, as may be seen in Figure 5(b).

CONCLUSIONS

Digestion of high lipid content waste was successfully attained for both substrates evaluated. The studies undertaken using sewage sludge as the main substrate resulted in no observable modifications to specific methane production with a FOG content of 0.2% (V/V). However, an increase in the addition of FOG to 1.8% resulted in significant detriment to the performance of the process. On the other hand, when digesting fat obtained from a milk-processing factory, the results showed successful operation of the semi-continuous reactor operating with an HRT of 40 days, although at high inhibition was reported from batch tests performed.

ACKNOWLEDGEMENTS

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Figure 5. (a) Daily gas production of reactor treating the waste obtained from the processing milk factory (R_GM2), (b) Total ammonium concentration inside the reactor.

REFERENCES


