Anaerobic digestion of sunflower oil cake: a current overview
M. A. De la Rubia, V. Fernández-Cegrí, F. Raposo and R. Borja

ABSTRACT
Due to the chemical and physical structure of a lignocellulosic biomass, its anaerobic digestion (AD) is a slow and difficult process. In this paper, the results obtained from a batch biochemical methane potential (BMP) test and fed-batch mesophilic AD assays of sunflower oil cake (SuOC) are presented. Taking into account the low digestibility shown during one-stage experiments the methane yield decreased considerably after increasing the organic loading rate (OLR) from 2 to 3 g VS L⁻¹ d⁻¹, SuOC was subjected to a two-stage AD process (hydrolytic-acidogenic and methanogenic stages), in two separate reactors operating in series where the methanogenic stage became acidified (with >1,600 mg acetic acid L⁻¹) at an OLR as low as 2 g VS L⁻¹ d⁻¹. More recently, BMP assays were carried out after mechanical, thermal, and ultrasonic pre-treatments to determine the best option on the basis of the methane yield obtained.

Key words | batch assay, biochemical methane potential (BMP), fed-batch assays, pre-treatment, sunflower oil cake (SuOC), two-stage anaerobic digestion

INTRODUCTION
Sunflower oil cake (SuOC) is the part of whole sunflower seeds which remains after the oil extraction process. It is an agro-industrial residue generated in Spain in great quantities (about 4–5 million tons per year). This extracted flour is mainly composed of fibre and protein (Vioque et al. 2001). This flour has been generally used for cattle feed (Szabo et al. 2001; Torrijos et al. 2008); nevertheless it represents one of the reservoirs of proteins with major potential for the food industry (Vioque et al. 2001). Other applications for the extracted sunflower flour have been in the preparation of antibiotics (Kota & Sridhar 1999) and some enzymes (Proteases) (Pandey et al. 2000). Nevertheless, the scarce and limited applications of the different methods of re-use of these wastes and their high production justify the study of other processes or alternatives that enable their utilization and reuse.

An aerobic digestion (AD) is the most effective process for the treatment and stabilization of organic wastes such as SuOC, offering the advantage of a net energy gain by producing methane. Moreover, the AD process can be improved by means of a process in two stages (De la Rubia et al. 2009), as the stability of the global process remains awkward when imbalances take place between the activity of the groups of microorganisms that carry out the first phase of hydrolysis of the high molecular weight compounds and acidification of the resulting monomers (acidogenic stage) and those that, in the second phase, metabolize the acids formed to methane (methanogenic stage). On the other hand, owing to the refractory structure of the lignocellulosic biomass the efficiency of AD to treat agriculture residues is limited. Although cellulose and hemicellulose can be degraded under anaerobic conditions, lignin (undegradable in biogas processes) prevents enzyme accessibility to cellulose (Zhu et al. 2008). While hemicellulose serves as a connection between the lignin and the cellulose fibres and gives the whole cellulose–hemicellulose–lignin network more rigidity. Therefore, only a low fraction of lignocellulosic biomass can be converted into biogas.

Hence, the pre-treatment of the lignocellulosic biomass is crucial to remove lignin and hemicellulose and make cellulose more accessible to the enzymes that convert carbohydrate polymers into fermentable sugars (Mosier et al. 2005; Pérez et al. 2007) and, therefore, to increase the biogas potential. Some physical, physico-chemical, chemical, and biological processes have been used for the...
pre-treatment of lignocellulosic materials, not only to remove the inhibitory lignin complex but also to reduce cellulose crystallinity, which is a major limit for cellulose hydrolysis (Jeihanipour et al. 2010).

Since 2005, the ‘Reuse of Wastes and Wastewater Treatment Group’, of the Instituto de la Grasa (IG) of the Spanish National Research Council (CSIC) has been studying the anaerobic stabilization of SuOC. During these years batch and fed-batch (one and two stage) experiments have been carried out. Recently, a combination of thermal, mechanical and ultrasonic pre-treatments and batch anaerobic assays has been assessed. Finally, the best option (ultrasound pre-treatment) has been chosen to study a combined ultrasound pre-treatment and one-stage AD of SuOC, which is currently being carried out. In this paper the most relevant results obtained during the above-mentioned experiments are summarized.

**MATERIAL AND METHODS**

**Raw material**

SuOC was collected from a sunflower oil factory located near Seville (Spain). Prior to using the substrate, it was sieved to give a fraction with a particle size lower than 2 mm (around 90% of the total particles of the SuOC had this size). The full composition and main features of the SuOC used have been described elsewhere (Raposo et al. 2008a).

**Inocula**

Two kinds of inocula were used in the different assays conducted.

Granular sludge (GS) was taken from an industrial upflow anaerobic sludge blanket (UASB) reactor which treats brewery wastewater. The main characteristics of this anaerobic sludge were: pH, 7.6 ± 0.1; total solids (TS), 60 ± 3 g L⁻¹; volatile solids (VS), 45 ± 2 g L⁻¹.

Sewage sludge (SS), a mixed anaerobic culture, was collected from a municipal wastewater treatment plant which operates in the anaerobic stabilization of primary and waste activated sludge. The main characteristics of this digested sludge were: pH, 7.6 ± 0.1; 33 ± 2 g L⁻¹ of TS, and 18 ± 1 g L⁻¹ of VS.

**Experimental design**

The experiments carried out have been summarized in Table 1 and/or in the following list:

1st: Biochemical methane potential (BMP) using SuOC as a substrate and GS as inoculum. The effect of inoculum to substrate ratio (ISR), expressed as VS basis, was studied in this set of experiments.

2nd: One-stage fed-batch experiments using SuOC as a substrate and the two previous inocula described (GS and SS). Organic loading rates (OLRs) of 1, 2 and 3 g VS L⁻¹ d⁻¹ were assayed.

3rd: Hydrolytic-acidogenic (H-A) fed-batch experiments using SuOC as a substrate and the inoculum GS. Six

<table>
<thead>
<tr>
<th>Experiment</th>
<th>One-stage fed-batcha</th>
<th>Hydrolytic-Acidogenicb</th>
<th>Two stages</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMP</td>
<td>OLR g VS L⁻¹ d⁻¹</td>
<td>HRT d</td>
<td>OLR g VS L⁻¹ d⁻¹</td>
</tr>
<tr>
<td>ISR</td>
<td>0.5</td>
<td>1</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>7</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>9</td>
<td></td>
</tr>
</tbody>
</table>

*aExperiments were developed at different OLR but at the same HRT.

*bEvery OLR (4, 5, 6, 7, 8, 9) was assayed at every HRT (8, 10, 12, 15).
different OLRs from 4 to 9 g VS L\(^{-1}\) d\(^{-1}\) and four hydraulic retention times (HRTs) of 8, 10, 12 and 15 days were studied.

4th: Two-stage (H-A and methanogenic) fed-batch experiments using SuOC as a substrate and the two inocula previously described. After optimizing the H-A stage (OLR of 6 and 8 g VS L\(^{-1}\) d\(^{-1}\) and HRT of 10 days) the methanogenic reactors were fed with the effluent obtained in the first stage. OLRs of 1, 1.5, 2.5 and 2.5 g VS L\(^{-1}\) d\(^{-1}\) were assayed in this second methanogenic stage.

5th: BMP using pre-treated SuOC as a substrate and the two inocula mentioned above. The following pre-treatments were assayed:

- Mechanical (sieve): The SuOC ≤ 2 mm was sieved and three different fractions: 0.355–0.55 mm, 0.71–1.0 mm, and 1.4–2.0 mm were chosen to be assayed.
- Thermal: A 2% (w/v) SuOC suspension was treated for 4 h at ambient temperature (AT), 100, 150 and 200 °C.
- Ultrasound: A 2% (w/v) SuOC suspension was treated with an ultrasound frequency of 20 kHz and a supplied power of 120 W. Five specific energies (SE) were supplied, ranging from 24,000 to 597,600 kJ kg TS\(^{-1}\) and obtained by increasing the operation time.

**Equipment**

**BMP assays**

The experimental design consisted of a multiflask batch system which was fully described elsewhere (Raposo et al. 2008a). The reactors, which were maintained at 35 ± 1 °C in a temperature-controlled water bath, were initially charged with the inoculum by keeping a concentration of 15 g VS L\(^{-1}\). The ISR was maintained at 2, except in the experiments to study the effect of ISR. A stock mineral medium solution whose composition has been described elsewhere (Raposo et al. 2006) was also added, and finally distilled water was added to achieve the desirable working volume of 250 mL. Reactors were flushed with N\(_2\) in order to achieve and maintain anaerobic conditions.

The methane released was measured by volume displacement (the carbon dioxide was removed previously by flushing the gas through a 2N NaOH solution), and expressed at standard temperature and pressure conditions. Methane production was monitored daily and calculated by subtracting the amount of methane produced by the blank controls (endogenous tests, with the inoculum alone added) from the methane production of each fed reactor.

All the experiments were run for 7–8 days, until no significant gas production was observed, suggesting that biodegradation was essentially completed, as a control of cellulose (~310 mL CH\(_4\) g\(^{-1}\) COD\(_{added}\)) also confirmed. Each experimental setup was performed in triplicate.

**Fed-batch assays**

Experiments were carried out in four completely mixed glass digesters, each one with a total volume of 2.5 L and a working volume of 2 L. The reactors were mixed using magnetic bars and an adjustable stirrer at 700 rpm. The digesters, maintained at 35 ± 1 °C in a temperature-controlled water bath, were started with an inoculum concentration of 17 g VS L\(^{-1}\). Nitrogen gas was used and sparged to maintain anaerobic conditions before starting the experiments and after each feed.

**Analytical methods**

The chemical compositions of the raw material, inocula and digestates were determined:

- **Raw material:** The following parameters were analysed in the substrate: TS and VS, according to the Standard Methods 2540B and 2540E (APHA 1998), respectively; total chemical oxygen demand (CODt) was determined using the method proposed by Raposo et al. (2008b). Total Kjeldahl nitrogen (TKN) determination was also described elsewhere (Raposo et al. 2009).
- **Inocula:** The inocula and digestates were characterized by direct sampling. The pH (using a pH meter model Crison 20 Basic), TS and VS were determined (APHA 1998).
- **Soluble fraction:** The supernatant obtained after centrifuging the inocula and digestates for 15 min at 10,000 rpm was filtered (0.45 μm) and used to characterize the following parameters: (i) soluble chemical oxygen demand (CODs), using the closed digestion and colorimetric Standard Method 5220D (APHA 1998); (ii) total alkalinity, which was measured by pH titration to 4.3; (iii) soluble ammonia nitrogen determined by distillation and titration according to the standard method 4500E (APHA 1998); and (iv) the volatile fatty acids (VFA) concentration determined using a gas...
RESULTS AND DISCUSSION

One stage

BMP assays of untreated SuOC

In order to determine the BMP of SuOC, the influence of ISRs and the evolution and variation of the chemical control parameters of the process with digestion time, different batch assays were conducted.

The results from this study suggest that SuOC is a potential substrate for AD. Batch experiments carried out at mesophilic temperatures and at ISRs of 3.0, 2.0, 1.5, 1.0, 0.8 and 0.5 demonstrated that the ultimate methane yield decreased considerably from 193 ± 19 mL CH₄ g⁻¹ COD added to 91 ± 9 mL CH₄ g⁻¹ COD added when the ISR decreased from 3.0 to 0.5, showing a marked influence of this parameter on the methane yield. However, the net VS removed only varied from 42 to 36% when the ISR decreased from 3.0 to 0.5. A considerable increase in CODs due mainly to an accumulation of VFA in the digestates was observed at ISRs of 0.5 and 0.8, which demonstrated a clear imbalance of the process, typical of stress on methanogenic microorganisms. The lower the ISRs, the greater the accumulation of the longer chain VFA, and only the ISRs of 2 and 3 were allowed to obtain digestates with no residual VFA at the end of the digestion time, as can be seen in Figure 1. Therefore, on the basis of the results obtained in the BMP test, an ISR over 2.0 is suggested and recommended in order to prevent acidification and an imbalance of the AD process of this substrate (VDI 4630 2006; Raposo et al. 2008a, 2012).

Fed-batch anaerobic digestion of SuOC

Once it was determined that SuOC was a potential substrate for AD, fed-batch anaerobic experiments at OLRs of 1, 2 and 3 g VS L⁻¹ d⁻¹ and HRT of 25 days were carried out. After the start-up step, the reactors were subjected to a programmed steady-state operation, using the mentioned OLRs. The attainment of the steady-state was verified after a period equivalent to 2–3 times the HRT by checking whether constant effluent characteristic values (TS, VS, COD and VFA levels) were achieved. The sampling during each steady-state period was performed for five consecutive days.

Taking into account the results obtained during this study, shown in Table 2, it can be stated that the activity of acidogenic microorganisms exceeded the activity of the methanogenic organisms when the OLR was increased from 2 to 3 g VS L⁻¹ d⁻¹, because VFA were accumulated and reached values higher than 1,500 mg acetic acid L⁻¹. The reactor was overloaded: to be specific, the methane yield diminished from 149 ± 5 mL CH₄ g⁻¹ COD added to 101 ± 5 mL CH₄ g⁻¹ COD added when OLR was increased from 2 to 3 g VS L⁻¹ d⁻¹. Because acidification occurred, the feeding was stopped before reaching a total imbalance of the process.

As Demirer & Chen (2005) stated, conventional one-stage digestion was not an effective system for wastes containing high solid concentrations, as SuOC.

Two stages

Acidogenic microorganisms and the methanogens constitute two very different groups in terms of their
growth kinetics, requirements for nutrients, optimum pH and capacity to support and maintain their ideal conditions before situations of overloading or ‘stress’ occur. Moreover, it is generally accepted that hydrolysis is the rate-limiting step in the AD of vegetable solid waste. On this basis, a process carried out in two stages can optimize the operative conditions of every step and give major stability to the global process.

By means of these experiments the suitable values of the HRT and OLR, which resulted in maximum efficiencies of elimination of organic matter accompanied with a maximum production of VFA in the first reactor and maximum methane yield coefficients in the second, were obtained.

A relevant feature of the two-stage AD approach is that when a high solid containing waste is introduced into the first stage, it is liquefied along with acidification.

**Hydrolytic-acidogenic stage**

In this study the effect of the variations of HRT and OLR on CODs and VFA production to improve the H-A step of the AD of SuOC was studied (De la Rubia et al. 2009).

During the mesophilic acidogenic fermentation of SuOC, variations in the HRT did not affect the COD solubilization of this substrate within the HRT range (15–8 days) studied. Variations in OLR affected the organic matter liquefaction slightly, with the highest value (30.1%) being reached at an HRT of 10 days and an OLR of 8 g VS L\(^{-1}\) d\(^{-1}\). The organic matter liquefaction or hydrolysis yield can be defined by the following equation:

\[
\text{Hydrolysis yield} = \frac{S_S}{S_I} \times 100
\]

where \(S_I\) is the initial total substrate concentration (calculated by means of the quotient: (COD\(_t\) g SuOC)/(volume related to the corresponding HRT) where \(\text{COD}_t\) is the COD concentration of solid substrate: 1.1 g COD g\(^{-1}\) TS) and \(S_S\) is the soluble output COD.

The acidification yield increased with an OLR of up to 6 g VS L\(^{-1}\) d\(^{-1}\), the highest value (83.8%) being achieved for an HRT of 10 days and an OLR of 6 g VS L\(^{-1}\) d\(^{-1}\). However, higher OLR produced a decrease in the acidification yield, probably due to the fact that the acidogenic bacteria could have been affected and inhibited at the highest OLR studied.

**Methanogenic stage**

The effluents obtained under the optima OLR (6 g VS L\(^{-1}\) d\(^{-1}\)) and HRTs (8 and 10 days) of the H-A stage were treated in the methanogenic reactors to determine the optimum operational parameters. With the effluent of reactor H-AI, operated at an OLR of 6 g VS L\(^{-1}\) d\(^{-1}\) and HRT of 8 days, the methanogenic reactor MI was fed. Four different ORLs were assayed for this second stage: 1, 1.5, 2 and 2.5 g VS L\(^{-1}\) d\(^{-1}\), at HRTs of 45, 28, 21 and 16 days, respectively, as can be seen in Table 2. The reactor MII was fed with the effluent of H-AII (operated at OLR of 6 g VS L\(^{-1}\) d\(^{-1}\) and 10 days of HRT); with this reactor three ORLs and HRTs were used: 1, 1.5 and 2 g VS L\(^{-1}\) d\(^{-1}\), and 36, 22 and 16 days, respectively.

The best results were obtained when the methanogenic reactors were operated at HRT between 21 and 28 days, and OLR of 1.5 and 2 g VS L\(^{-1}\) d\(^{-1}\) (Table 2). At an HRT of 16 days, the methanogenic activity was clearly inhibited. This was shown by the methane yield drop, for both methanogenic reactors, and the high VFA concentration achieved, which varied between 1,600 and 5,200 mg acetic acid L\(^{-1}\), for OLR of 2 and 2.5 g VS L\(^{-1}\) d\(^{-1}\), respectively.

Consequently, neither the one-stage nor the two-stage mesophilic AD processes were able to efficiently degrade SuOC at an OLR higher than 2 g VS L\(^{-1}\) d\(^{-1}\).

**Table 2**: Methane yield and total VFA (TVFA) concentration for each experiment conducted in one and two stages*

<table>
<thead>
<tr>
<th>OLR g VS L(^{-1}) d(^{-1})</th>
<th>CH(<em>4) ml g(^{-1}) COD(</em>{added})</th>
<th>TVFA mg C(_2) L(^{-1})</th>
<th>OLR g VS L(^{-1}) d(^{-1})</th>
<th>CH(<em>4) ml g(^{-1}) COD(</em>{added})</th>
<th>TVFA mg C(_2) L(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>136 ± 8</td>
<td>214 ± 27</td>
<td>1</td>
<td>109 ± 13</td>
<td>652 ± 131</td>
</tr>
<tr>
<td>2</td>
<td>149 ± 5</td>
<td>585 ± 87</td>
<td>2</td>
<td>141 ± 6</td>
<td>342 ± 43</td>
</tr>
<tr>
<td>3</td>
<td>101 ± 5</td>
<td>1,566 ± 195</td>
<td>2.5</td>
<td>48 ± 7</td>
<td>5,215 ± 52</td>
</tr>
</tbody>
</table>

*Average values from five trials ± standard deviation of the mean values (p < 0.05).
**Pre-treatments**

Pre-treatments are frequently used to facilitate the methane production by overcoming the limitation of hydrolysis, which includes the solubilization and biodegradation of hemicellulosic and lignin fractions of the substrates. Taking into account the above-stated difficulty of SuOC to be anaerobically degraded, combinations of mechanical, thermal, and ultrasonic pre-treatments and AD processes in batch mode were assessed.

To evaluate the efficiency of the above-mentioned pre-treatments, with the aim of achieving a maximum solubilization level by comparing their capacity for converting the complex organic compounds present in the waste into simpler compounds that can be easily biodegradable by AD processes, BMP experiments were carried out.

Thermal and ultrasound pre-treatments involve the addition of water to the substrate to be pre-treated; therefore after pre-treatments of SuOC two fractions are obtained: a water-insoluble solid fraction and a liquid fraction. Both of these fractions were separated and evaluated individually. The results are compared in Table 3.

**Mechanical pre-treatment**

Batch AD experiments of SuOC with different particle sizes (0.355–0.55, 0.71–1.0 and 1.4–2.0 mm) revealed that this parameter affects methane yield. In this way, the largest size (1.4–2.0 mm) within the range studied (0.355–2.0 mm) resulted in the highest methane yield, 175 ± 7 mL CH4 g⁻¹ CODadded when compared with particle sizes of 0.355–0.55 and 0.71–1.0 mm, for which 143 ± 3 and 155 ± 2 mL CH4 g⁻¹ CODadded respectively, were reached. This could be attributed to the different initial chemical composition of the different fractions (De la Rubia et al. 2011). Therefore, optimizing the size reduction of SuOC could potentially improve the methane yield of the AD process of this substrate.

**Thermal pre-treatment**

- **Solid fraction:** The highest methane production was obtained for SuOC pre-treated at AT (114 ± 9 mL CH4 g⁻¹ CODadded). This is because at this low temperature some soluble compounds still remained in the solid fraction, which can be degraded during BMP assays. The lowest methane yield was obtained at 200 °C (53 ± 8 mL CH4 g⁻¹ CODadded). Therefore, the higher the temperature applied, the lower the methane yield obtained for this fraction.

- **Liquid fraction:** In this case the best results were obtained at 100 °C (310 ± 4 mL CH4 g⁻¹ CODadded). The sample treated at AT resulted in 276 ± 6 mL CH4 g⁻¹ CODadded while at 150 and 200 °C the methane yield decreased to 220 ± 15 and 247 ± 10 mL CH4 g⁻¹ CODadded respectively. Hence, temperatures above 150 °C produced the formation of non-degradable or toxic compounds, which brought about a potential inhibition for the growth of bacteria and Archaea due to their lethal nature.

From the results obtained it can be stated that 100 °C is the best temperature to thermally pre-treat SuOC before AD.

**Ultrasound pre-treatment**

- **Solid fraction:** SuOC pre-treated by ultrasound obtained the highest methane production of 111 mL CH4 g⁻¹ CODadded for an SE of 24,000 kJ kg⁻¹ TS. A higher SE brought about a lower methane yield.

*Table 3 | Ultimate CH4 yield obtained after the different pre-treatments studied*

<table>
<thead>
<tr>
<th>Pre-treatment</th>
<th>Mechanical – particle size (mm)</th>
<th>0.355–0.55</th>
<th>0.71–1.0</th>
<th>1.4–2.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>mL CH4 g⁻¹ CODadded</td>
<td>Thermal (°C)</td>
<td>Fraction</td>
<td>AT</td>
<td>100 °C</td>
</tr>
<tr>
<td>Solid</td>
<td>145 ± 3</td>
<td>155 ± 2</td>
<td>175 ± 7</td>
<td></td>
</tr>
<tr>
<td>Liquid</td>
<td>0.71–1.0</td>
<td>114 ± 9</td>
<td>105 ± 7</td>
<td>82 ± 7</td>
</tr>
<tr>
<td>1.4–2.0</td>
<td>276 ± 6</td>
<td>310 ± 4</td>
<td>220 ± 15</td>
<td>247 ± 10</td>
</tr>
<tr>
<td>Liquid</td>
<td>0.355–0.55</td>
<td>111 ± 5</td>
<td>107 ± 4</td>
<td>103 ± 4</td>
</tr>
<tr>
<td>0.71–1.0</td>
<td>330 ± 16</td>
<td>297 ± 8</td>
<td>270 ± 10</td>
<td>312 ± 11</td>
</tr>
</tbody>
</table>

*Average values are from three trials ± standard deviation of the mean values (p < 0.05).
Liquid fraction: the methane yield obtained for this fraction ranged between 270 ± 15 mL CH₄ g⁻¹ COD_added (for SE of 597,600 kJ kg⁻¹ TS) and 330 ± 16 mL CH₄ g⁻¹ COD_added (for SE of 24,000 kJ kg⁻¹ TS), showing that an increase in the ultrasound time did not improve the solubilization of compounds which are not easily degraded.

The final values of the TVFA were very low for both the solid and liquid fraction digestates after the three pre-treatments studied, with values in the range of 5–16 mg acetic acid L⁻¹. This means that the overall anaerobic process was conducted satisfactorily and a correct balance of the process occurred. Moreover, results from the ultrasound study, and when compared with the other two pre-treatments studied, demonstrate the suitability of the ultrasonic pre-treatment of SuOC for increasing the anaerobic biodegradability of this substrate and methane yield coefficient.

The different pre-treatments used may promote methane production because the AD of SuOC without pre-treatment is a slow and difficult process which becomes acidified at a low OLR, even when the H-A and methanogenic stages are separated in two different reactors that operate in series.

Conclusions and recommendations

Although the results obtained after BMP assays suggest that SuOC was a potential substrate for AD, neither the one-stage nor the two-stage mesophilic AD process was able to efficiently degrade SuOC at an OLR higher than 2 g VS L⁻¹ d⁻¹.

A temperature of 100 °C for thermal pre-treatment and an SE of 24,000 kJ kg⁻¹ TS for ultrasound pre-treatment were the best conditions among those assayed, obtaining similar mean methane yields (average of the solid and liquid fractions): 208 and 220 mL CH₄ g⁻¹ COD_added for thermal (100 °C) and ultrasound (24,000 kJ kg⁻¹ TS) pretreatment, respectively. The energetic cost necessary to treat SuOC by thermal pre-treatment (4 h at 100 °C) is much higher than that needed for ultrasound, where only 16 min and 120 W of power are necessary. Therefore, these ultrasound conditions were chosen to conduct fed-batch experiments with pre-treated SuOC.

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