Biogas production from cheese whey wastewater: laboratory- and full-scale studies
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ABSTRACT
A two-phase system for biogas production from cheese whey wastewater (CWW) was designed, set up and operated at laboratory and full scale for a whole cheese production season (8–9 months). The high efficiency and stability of the laboratory-scale system was demonstrated under various organic loading rates (OLRs) reaching 13 g chemical oxygen demand (COD) L\(^{-1}\) d\(^{-1}\) and producing up to 9 L L\(^{-1}\) d\(^{-1}\) of biogas (approximately 55% in methane). The COD removal was above 95% and the pH was maintained above 6.3 without any chemical addition. The full-scale system was operated at lower OLRs than its normal capacity, following the good response and high stability in disturbances of the laboratory-scale unit.

Key words | biogas, cheese whey wastewater, full scale, two-phase

INTRODUCTION
The food industry is a major production sector generating large quantities of wastes. Stabilization of wastes is imperative prior to their disposal to the environment according to national legislation of many countries including Greece. In the case of wastes of high organic content, such as food wastes, anaerobic digestion is often the core technology in the treatment scheme. Although the process development aims primarily at meeting the environmental specifications for safe waste disposal, provision could be taken to utilize the energy produced in the form of biogas.

A typical type of waste produced in agricultural areas of Greece is cheese whey wastewater (CWW) from cheese manufacture. There are numerous and small cheese manufacturing units scattered in Greece, producing more than 160,000 t of cheese (data for 2007 by Hellenic Statistical Authority, ELSTAT 2009–2010). There are three main types of CWW depending on the origin of production: from (a) primary cheese, (b) secondary ‘soft’ cheese and (c) washing processes (Prazeres et al. 2012). The volume of CWW produced is large (2.5 m\(^3\) CWW are produced per ton of milk processed). CWW contains mainly lactose (39–60 g L\(^{-1}\)) and its chemical oxygen demand (COD) is high (50–70 g L\(^{-1}\)). Other constituents are proteins (1.4–8 g L\(^{-1}\)) and fats (0.99–10.58 g L\(^{-1}\)) (Prazeres et al. 2012).

Anaerobic digestion is an alternative technology to other methods employed in CWW management such as animal feeding, and protein and other compound recovery (Prazeres et al. 2012). The adoption of these options requires the presence of farmers and industries in the proximity of the cheese manufacturing plant to minimize transport costs. On the other hand, a biogas plant could be installed on-site and the biogas produced could be exploited to cover the thermal or other energy requirements of the cheese manufacturing plant.

CWW is rich in lactose, and therefore easily fermentable to acids and subsequently to biogas. The rapid conversion of CWW to acids, however, combined with the low alkalinity of the mixed liquor, causes an abrupt drop in the pH, and the methanogenic population is adversely affected (Kalyuzhnyi et al. 1997; Janczukowicz et al. 2008). As a result, the application of pH control is often regarded as necessary, via addition of chemicals such as lime (Patel & Madamwar 1997; Gannoun et al. 2008), sodium hydroxide (Yang et al. 2005) and bicarbonate salts (Ergüder et al. 2001; Frigon et al. 2009).

Both single- and multi-stage systems have been studied for CWW treatment. Single-stage bioreactors should operate under high hydraulic retention times (HRTs) and/or require...
the application of pH control (Wildenauer & Winter 1985; Yan et al. 1993; Gavala et al. 1999). It is a common practice to separate the acidogenesis from the methanogenesis phase in order to enhance the stability of the process. In this way, the rapid acidification and the pH reduction in the same reactor where the sensitive group of methanogens grow would be avoided. The objectives addressed in the present study were to develop a stable anaerobic process in a simple two-phase configuration at laboratory and full scale, and operate it on a minimum demand for pH control. Several studies on CWW digestion have been conducted in two bioreactors in series (Demirel & Yenigun 2002; Antonopoulou et al. 2008; Erdirencelebi et al. 2011), the results of which are compared with the results of the present study.

**MATERIALS AND METHODS**

**Wastewater**

The CWW was obtained from a small cheese manufacturing plant in Thiva (Greece) where the full-scale biogas plant was installed. It corresponded to the wastewater after the soft cheese recovery. The CWW was diluted with wash-out wastewater (from operations aimed at meeting the hygiene requirements of the cheese plant), tap water or the methanogenic reactor effluent. The main characteristics of CWW and the wash-out wastewater were: 54.4 ± 5.9 g COD L⁻¹, 5.93 ± 0.8 g total suspended solids (TSS) L⁻¹, electrical conductivity 12.47 ± 4.15 mS cm⁻¹; and 2.3 ± 2.0 g COD L⁻¹, 0.24 ± 0.15 g TSS L⁻¹, electrical conductivity 3.03 ± 1.21 mS cm⁻¹ respectively. The diluted mixture was also supplemented with trace metals (in mL L⁻¹ undiluted CWW: 2 (NH₄)₂Fe(SO₄)₂ · 6H₂O 20 mM, 1 NiCl₂ · 6H₂O 10 mM, 1 CoCl₂ · 6H₂O 10 mM, 1 Na₂MoO₄ · 6H₂O 10 mM) and stored at 4 °C.

**Configuration**

The experimental set-up consisted of a 2 L laboratory-scale continuously stirred tank reactor (CSTR) followed by a clarification tank and finally a 6.3 L upflow anaerobic sludge bed (UASB) reactor (Figure 1). The CSTR, fed on the CWW, was stirred via a magnetic stirrer and operated without pH control. The effluent was allowed to settle in a clarification tank. The acidogenic biomass from the bottom of the tank was partially recycled to the CSTR. The supernatant of the clarification unit was introduced into the recirculation stream of the UASB. The recirculation rate was set at 4–6 L h⁻¹ resulting in an upflow liquid velocity of 2–3 m h⁻¹. The UASB reactor was designed with a pH control system, dosing a 10% NaOH solution into the recirculation flow, when the pH was lower than 6.5. Temperature control was accomplished by using a thermal bath (Lauda Thermo-star C12) with water recirculation through the reactors’ double jacket. The experiments were conducted at ambient temperature (20 °C) in the first stage and at 31 °C in the second stage.

The same configuration was applied in the full-scale plant too. The operating volumes of the CSTR and UASB were 12 and 35 m³ respectively. Heating of both reactors to the desired temperature was accomplished via heat exchangers using the biogas for thermal energy production.

**Analytical methods**

Samples were taken from the feeding tank, the supernatant of the clarifier (accounting for the effluent of the CSTR and the influent of the UASB) and the recycling stream of the UASB (accounting for the effluent of the UASB) in the configurations at both laboratory and full scale. The parameters determined and presented in this work were the COD (according to APHA (1989)), the pH (using a WTW 192 pH meter), the biogas production rate (by a wet-gas meter, Ritter Kunststoff-werk KWU B), the biogas composition (using an IR gas analyzer, BINOS, Leybold-Heraeus GmbH) and the cations of Na, Ca and Mg (using ion chromatography; ICS-3000, Dionex).

**Experimental conditions**

The indigenous acidogenic microflora taken from a storage tank of cheese whey was used for inoculating the CSTR. The UASB was inoculated with granular sludge (TSS concentration: 35 g L⁻¹, volatile solid content: 53%, mean settling velocity: 9.5 m h⁻¹) obtained from a full-scale...
UASB reactor treating brewery wastewater. The volumes of the inoculums were 2 L and 10 m³ in the laboratory and full scale respectively.

The laboratory-scale configuration was operated under various operating conditions. The operation period is separated into phases to facilitate the description of the experiment (Table 1). Each phase is characterised by the dilution ratio (in terms of volume of CWW to the volume of the feeding mixture), the dilution medium, the total COD of the influent streams of the bioreactors and the HRT in each bioreactor.

## RESULTS AND DISCUSSION

### Laboratory-scale unit

The anaerobic system set up for biogas production of CWW has been operated for 8.5 months under various loading rates. CSTR was the acidogenic reactor, operated at an HRT of 0.5–0.8 d (Table 1). Due to the high production of organic acids, consisting mainly of lactic acid (Stamatelatou et al. 2002), the pH was extremely low (3.27 ± 0.26) to allow methanogenesis to take place. As expected, practically no COD removal was observed in the first stage and both bioreactors were fed on a similar organic load, in terms of total COD concentration (Table 1). Any difference between the influent COD concentrations of the bioreactors at each phase can be attributed to the fact that the UASB influent was the supernatant of the clarification tank and, therefore, free of most of the particulate COD, while the CSTR influent contained most of the solids of the CWW.

CSTR is a suitable bioreactor for processes with fast dynamics such as acidogenesis. Moreover the combination of CSTR with the clarification tank and the biomass recirculation allowed the CSTR to be operated at a low HRT. It produced an acidified stream (TSS < 1.5 g L⁻¹) fed to the UASB reactor. The TSS concentration inside the CSTR was maintained at 5 g L⁻¹.

Figure 2(a) shows the time evolvement of the specific methane production rate (MPR) following the variations in the organic loading rate (OLR). The start-up phase (phase 1) was short since the UASB was capable of handling OLRs up to 15 g COD L⁻¹ d⁻¹. The HRT was decreased to 1.2 d within 12 days and kept to 1.56 d until the end of this phase. The fast adaptation and response of the UASB during start-up was remarkable, indicating that a fast start-up of the full-scale UASB was possible.

The influent COD of the UASB varied mainly between 10 and 15 g L⁻¹ (Figure 2(b)). The COD level was the result of the dilution ratio selection and the variable organic load of the CWW coming in batches from the cheese manufacturing plant. In two phases (2 and 7) the COD of the influent was increased to 24.8 and 20.7 g COD L⁻¹ respectively. During phase 2, the UASB did not respond satisfactorily, increasing the COD of the effluent to 4.2 g L⁻¹. However, during phase 7, the effluent was sustained well below 0.8 g COD L⁻¹, indicating the good adaptation of the biomass. Other parameters determined in this phase were the volatile fatty acids (0.56 ± 0.26 g L⁻¹ in COD equivalents), with acetic acid being the

### Table 1 | Operation phases of the anaerobic system treating CWW

<table>
<thead>
<tr>
<th>Phase</th>
<th>D.R.</th>
<th>D.M.</th>
<th>COD (influent of CSTR) (g L⁻¹)</th>
<th>HRT in CSTR (d)</th>
<th>COD (influent of UASB) (g L⁻¹)</th>
<th>HRT in UASB (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
<td>1:4</td>
<td>w.w.</td>
<td>17.97 ± 1.29</td>
<td>0.50 ± 0.03</td>
<td>15.19 ± 2.49</td>
<td>1.56 ± 0.15</td>
</tr>
<tr>
<td>#2</td>
<td>1:2</td>
<td>w.w.</td>
<td>24.60 ± 1.20</td>
<td>0.58 ± 0.03</td>
<td>24.80 ± 0.24</td>
<td>1.84 ± 0.14</td>
</tr>
<tr>
<td>#3</td>
<td>1:4</td>
<td>w.w.</td>
<td>13.47 ± 1.68</td>
<td>0.57 ± 0.03</td>
<td>11.20 ± 2.08</td>
<td>1.81 ± 0.19</td>
</tr>
<tr>
<td>#4</td>
<td>1:4</td>
<td>w.w.</td>
<td>10.6 ± 0.93</td>
<td>0.29 ± 0.02</td>
<td>7.9 ± 1.27</td>
<td>0.91 ± 0.03</td>
</tr>
<tr>
<td>#5</td>
<td>1:4</td>
<td>w.w.</td>
<td>14.68 ± 1.03</td>
<td>0.57 ± 0.07</td>
<td>10.80 ± 0.25</td>
<td>1.79 ± 0.21</td>
</tr>
<tr>
<td>#6</td>
<td>1:3</td>
<td>w.w.</td>
<td>16.97 ± 3.45</td>
<td>0.56 ± 0.06</td>
<td>14.60 ± 1.62</td>
<td>1.78 ± 0.19</td>
</tr>
<tr>
<td>#7</td>
<td>1:2</td>
<td>t.w.</td>
<td>18.94 ± 1.89</td>
<td>0.56 ± 0.06</td>
<td>20.74 ± 1.08</td>
<td>1.80 ± 0.09</td>
</tr>
<tr>
<td>#8a</td>
<td>1:3</td>
<td>t.w.</td>
<td>13.31</td>
<td>0.54 ± 0.06</td>
<td>12.07 ± 2.00</td>
<td>1.87 ± 0.50</td>
</tr>
<tr>
<td>#8b</td>
<td>t.w.</td>
<td></td>
<td>16.13 ± 1.67</td>
<td>0.42 ± 0.03</td>
<td>13.13 ± 1.27</td>
<td>1.32 ± 0.08</td>
</tr>
<tr>
<td>#9a</td>
<td>1:3</td>
<td>t.w.</td>
<td>16.77 ± 0.18</td>
<td>0.70 ± 0.12</td>
<td>10.75 ± 1.81</td>
<td>2.27 ± 0.33</td>
</tr>
<tr>
<td>#9b</td>
<td>ef</td>
<td></td>
<td>12.34 ± 1.41</td>
<td>0.76 ± 0.05</td>
<td>11.62 ± 1.12</td>
<td>2.36 ± 0.08</td>
</tr>
</tbody>
</table>

D.R.: dilution ratio.
D.M.: dilution medium (w.w.: wash-out wastewater, t.w.: tap water; ef: UASB effluent).
predominant compound (Stamatelatou et al. 2012). This means that the UASB was kinetically limited under the operating conditions of phase 7 and further increase in the OLR would decrease the performance.

The effect of increasing the OLR via reducing the HRT or increasing the COD of the influent was studied. Halving the HRT from 1.8 d to 0.9 d (phase 4) did not affect the good performance of the UASB, while the transition to the new steady state was fast. The COD in the effluent remained approximately at 0.3 g L\(^{-1}\) under an OLR of 8.7 ± 2.8 g COD L\(^{-1}\) d\(^{-1}\). Similar OLR was imposed during phase 6 (8.1 ± 1.0 g COD L\(^{-1}\) d\(^{-1}\)) achieved by keeping the HRT at its nominal value (1.8 ± 0.2 d) and increasing the COD of the influent (14.6 ± 1.6 g COD L\(^{-1}\)). Although the COD of the effluent in both cases was low, the effluent in phase 4 contained half the COD in phase 6; 0.3 ± 0.1 g COD L\(^{-1}\) compared to 0.6 ± 0.14 g COD L\(^{-1}\) respectively. This indicates that, under similar OLR, the operation at a low HRT facilitates the mass transfer of the organic matter and removes any inhibitory parameter at a higher rate.

The fate of specific cations Na\(^{+}\), Ca\(^{2+}\) and Mg\(^{2+}\) in the influent of the CSTR, and the influent and effluent streams of the UASB was studied (Figure 3). It was observed that Na\(^{+}\) remained at the same levels in all three streams, while Ca\(^{2+}\) and Mg\(^{2+}\) were lower in the effluent as a result of the possible precipitation of the salts formed with carbonate. These salts may influence the functionality of the granules due to the formation of inorganic layers (van Langerak et al. 1998). The low dilution ratio did not reduce the cation concentration in the feed although tap water was used (phase 7). Tap water was effective under a dilution ratio of 1.3 (phase 8a versus phase 6).

Another option tested for the dilution medium was the UASB effluent (phase 9b). During this last phase, the cation concentration in the feed was lower than in phase 6 but slightly higher than in phase 8a (under the same dilution ratio). Moreover, the pH increased and affected the methane percentage in the biogas by increasing it slightly (from 55 to 60%). However, in the long term, the utilization of the effluent as a dilution medium would result in the slow increase of the salinity in the feed and the possible inhibition of the process. The conductivity of the effluent was 9–10 mS cm\(^{-1}\) compared to that of the wash-out wastewaters (3.03 ± 1.21 mS cm\(^{-1}\)), while the conductivity of raw CWW was approximately 12 mS cm\(^{-1}\).

It should be noted that during the operation of the biogas unit, the pH was self-sustained above 6.3 in the UASB and pH control was not activated. The pH in the CSTR was below 4 due to the acidification process (with the exception of phase 9b when the UASB effluent was used as the dilution medium).

**Full-scale unit**

The start-up period was short in this case too and the MPR followed the OLR variations as well (Figure 4(a)). The profiles of the COD values in the influent and effluent streams of both stages were at similar levels for the case of the laboratory-scale configuration (Figure 4(b)). However, the unit had to be operated at 30% of its design capacity.
due to the limited CWW supply, with short intervals of increasing it up to 75%. This accounted for HRT values of 2 and 7 d (on average) applied to the CSTR and UASB reactor respectively (Figure 4(c)). The pH was self-sustained at 3.2–3.4 and 6.3–6.6 in the CSTR and the UASB respectively (Figure 4(c)).

Performance evaluation

The correlation of specific MPR of the methanogenic UASB with the OLR in both laboratory- and full-scale configurations was linear (Figure 5). The line inclination expresses the methane yield with respect to the influent COD and was close to the maximum value (0.35 L g⁻¹ COD at STP).

This indicated that a very high COD removal was achieved under a wide variety of OLR imposed, demonstrating the high stability of the proposed configuration.

Alkalinity addition was necessary according to researchers who operated the methanogenic bioreactor under higher OLRs (13–25 g COD L⁻¹ d⁻¹) using undiluted CWW (Table 2). In the present work, the methane yield estimated for two operating phases was high and corresponded to a low HRT (1.8 d) and a fairly high OLR (8.1–11.5 g COD L⁻¹ d⁻¹) without any chemical addition for pH control.

Conclusions

The two-phase biogas unit was operated under 8.1 g COD L⁻¹ d⁻¹ (COD_influent: 14.6 g L⁻¹, HRT: 1.8 d). The operation

<table>
<thead>
<tr>
<th>Description</th>
<th>COD (g L⁻¹)</th>
<th>OLR (g COD L⁻¹ d⁻¹)</th>
<th>Yield* (L CH₄/g COD_influent)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 UASB in series treating acidified CWW</td>
<td>Influent: 27, Effluent: 7.5</td>
<td>HRT: 4</td>
<td>OLR: 6.75</td>
<td>0.252</td>
</tr>
<tr>
<td>UASB treating CWW with alkalinity addition</td>
<td>Influent: 15, Effluent: 3.5</td>
<td>HRT: 2.3</td>
<td>OLR: 25.22</td>
<td>0.337</td>
</tr>
<tr>
<td>PARBb treatingacidified CWW with alkalinity addition</td>
<td>Influent: 57, Effluent: 2.2</td>
<td>HRT: 4.2</td>
<td>OLR: 12.25</td>
<td>0.33</td>
</tr>
<tr>
<td>PARBb treating acidified CWW with alkalinity addition</td>
<td>Influent: 58, Effluent: 0.5</td>
<td>HRT: 4.4</td>
<td>OLR: 13.18</td>
<td>0.34</td>
</tr>
</tbody>
</table>

*Estimated from the COD removal and the theoretical yield at STP: \( \frac{\text{COD}_{\text{influent}} - \text{COD}_{\text{effluent}}}{\text{COD}_{\text{influent}}} \).  
*PARB: periodic anaerobic baffled reactor.
of the unit under higher OLR (11.5 g COD L\(^{-1}\) d\(^{-1}\)) did not avert the process from stability. The dilution medium used to adjust the influent COD and lower the calcium and magnesium cations was the wash-out wastewaters from the cheese manufacturing plant, but in cases of shortage of this kind of dilution medium, lower dilution ratio can be applied or the methanogenic effluent can be used. These options, however, should not be followed for a long time, since they lead to the increase of the influent COD concentration (causing mass transfer limitations) and the accumulation of the calcium, magnesium and salinity levels in the feed in the long term. No chemical addition was necessary for pH control.

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