Anaerobic waste activated sludge co-digestion with olive mill wastewater
E. Athanasoulia, P. Melidis and A. Aivasidis

ABSTRACT
Co-digestion of waste activated sludge (WAS) with agro-industrial organic wastewaters is a technology that is increasingly being applied in order to produce increased gas yield from the biomass. In this study, the effect of olive mill wastewater (OMW) on the performance of a cascade of two anaerobic continuous stirred tank (CSTR) reactors treating thickened WAS at mesophilic conditions was investigated. The objectives of this work were (a) to evaluate the use of OMW as a co-substrate to improve biogas production, (b) to determine the optimum hydraulic retention time that provides an optimised biodegradation rate or methane production, and (c) to study the system stability after OMW addition in sewage sludge. The biogas production rate at steady state conditions reached 0.73, 0.63, 0.56 and 0.46 lbiogas/lreactor/d for hydraulic retention times (HRTs) of 12.3, 14, 16.4 and 19.7 d. The average removal of soluble chemical oxygen demand (sCOD) ranged between 64 and 72% for organic loading rates between 0.49 and 0.75 g sCOD/l/d. Reduction in the volatile suspended solids ranged between 27 and 30%. In terms of biogas selectivity, values of 0.6 lbiogas/g tCOD removed and 1.1 lbiogas/g TVS removed were measured.

Key words | anaerobic co-digestion, olive mill wastewater, waste activated sludge

INTRODUCTION
The olive agro-industrial activities are of major economic importance in all Mediterranean countries but generate large amounts of by-products, which are totally unexploited and in some cases dangerous for the environment. Since 2000, Greece has produced an annual yield of approximately 377.75 kt of olive oil (Fountoulakis et al. 2001). This corresponds to 2.266 Mt of olive oil wastewater. Olive mill wastewater (OMW) is the liquid fraction of the wastes produced during the olive-oil extraction process. It has a high organic pollutant load (25–162 g COD/l) including various phenolic compounds, of which caffeic acid, thyrosol and hydroxythyrosol occur in the highest proportions. Due to its high organic content, this wastewater is classified among the strongest industrial liquid wastes with concentrations 20–4,400 times higher than the urban wastewater (Xing et al. 2000; Azbar et al. 2009; Sampaio et al. 2011) and consequently among those with the most significant energy potential (Gelegenis et al. 2007). The high concentration of phenols in OMW, reaching up to 10 g/l (Borj et al. 1992), contributes to a high toxicity and antibacterial activity (Capasso et al. 1995). These two features make it impossible to purify OMW anaerobically unless a prior dilution is effected.

Co-digestion between several agro-industrial organic wastewaters (Angelidaki & Ahring 1997; Gelegenis et al. 2007; Azbar et al. 2008; Azaizeh & Jadoun 2010) is a technology that is increasingly being applied for simultaneous treatment of several solid and liquid organic wastes, in which the content of nutrients can thereby be balanced, and the negative effect of toxic compounds on the digestion process may be decreased giving an increased gas yield from the biomass. Moreover, co-digestion may contribute to a more efficient use of anaerobic digestion (AD) reactors and cost-sharing by processing multiple waste streams in a single facility. Recently, there have been several attempts at studying anaerobic OMW co-digestion with other substrates (such as manure, waste activated sludge [WAS], household waste, cheese whey, olive mill solid waste etc.).

Marques (2001), studied the AD of OMW with piggery effluent in an up-flow anaerobic filter. The digester was fed with 83% (v/v) of OMW. The process converted 70–80% of the influent chemical oxygen demand (COD) (20–60 kg
COD/m³), produced 1–3 m³/m³/d (65–75% CH₄) of gas and a more stabilised effluent, with a neutral-basic pH, was produced. Darcioti et al. (2009) examined the biodegradation of a mixture containing 55% OMW, 40% liquid cow manure and 5% cheese whey in a two-stage continuous stirred tank reactor (CSTR) anaerobic process. The average removal of soluble chemical oxygen demand (sCOD) was 75.5% at OLR of 5.5 ± 0.36 g total COD/l reactor/d, while the methane production rate at steady state conditions reached 1.35 ± 0.11 l CH₄/l reactor/d. Angelidaki & Ahring (1997) investigated AD of olive mill effluent with laying hen litter, while biogas production increased 22% when cheese whey was used as co-substrate. Fezzani & Cheikh (2008) studied the mesophilic anaerobic co-digestion of OMW with olive mill solid waste in a batch digester and results show an increase in biogas production and COD removal efficiency from 11.17 ± 2.5 to 30.5 ± 2.5 l/digester and from 44.5 ± 3 to 83.4 ± 2%, respectively.

Only a few studies on co-digestion of OMW and WAS have been reported (Angelidaki & Ahring 1997; Rodríguez et al. 2006). However, these studies are with bench scale reactors, and no results at pilot-scale reactors are reported in the literature. Moreover, there is a lack of knowledge about serial co-digestion of these mixtures. In this study, the effect of OMW on the performance of a pilot scale cascade of two anaerobic CSTRs treating thickened WAS at mesophilic conditions was investigated. The objectives of this work were: (a) to evaluate the use of OMW as a co-substrate to improve biogas production, (b) to determine the optimum hydraulic retention time that provides an optimised biodegradation rate or methane production, and (c) to study the system stability after OMW addition in WAS.

**MATERIALS AND METHODS**

**Feedstock**

The OMW came from a three phase continuous olive oil extraction process in Xanthi, Greece. The main physicochemical compositions in average values are summarised in Table 1. Secondary thickened WAS was obtained from a municipal wastewater treatment plant in Komotini, Greece, which operates at extended aeration conditions.

**Reactor design and operation**

Mesophilic AD of a mixture of OMW and WAS was investigated using a cascade of two CSTRs operating over a range of hydraulic retention times between 12.3 and 19.7 days (Figure 1). The mixture of thickened WAS with OMW was continuously pumped into the first digester, the effluent of which overflowed to the second digester unit. The useful volumes of the two digesters were 40 l and 60 l respectively. Constant sludge volumes were maintained by a level controller. Biogas production was continuously monitored by a volumetric gas meter. The optimal temperature was adjusted to 37 °C by circulating hot water inside the water jacket of the reactors. pH value was maintained constant at 6.8–7.2 by a pH controller.

In order to find the optimum operation conditions of the process, hydraulic retention times of 12.3, 14.0, 16.4, and 19.7 d were examined with influent mixtures of WAS supplemented with 30% (v/v) OMW. All results were compared with the corresponding anaerobic treatment of WAS alone (control). Due to prior experiments that took place in the cascade system, active biomass preexisted and there was no need for starting-up preparation.

**Analytical methods**

**Standard Methods for the Examination of Water and Wastewater** (APHA et al. 1998) were used for the estimation of suspended solids (SS) and volatile suspended solids (VSS) and total and soluble COD concentrations. Biogas production was monitored by a volumetric gas meter. The concentrations of methane and carbon dioxide in the biogas were continuously measured using an on-line gas analyser (BINOS IR, Leybold-Heraeus). A Perkin Elmer gas chromatograph equipped with a capillary column and

<table>
<thead>
<tr>
<th>Parameter</th>
<th>WAS</th>
<th>OMW</th>
</tr>
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<tbody>
<tr>
<td>VSS g/l</td>
<td>19.6 ± 1.69</td>
<td>0.9 ± 0.26</td>
</tr>
<tr>
<td>sCOD g/l</td>
<td>0.58 ± 0.12</td>
<td>38 ± 7.12</td>
</tr>
<tr>
<td>tCOD g/l</td>
<td>28.9 ± 3.7</td>
<td>39.6 ± 4.1</td>
</tr>
<tr>
<td>pH</td>
<td>7.12 ± 0.14</td>
<td>4.8 ± 0.41</td>
</tr>
</tbody>
</table>

Table 1 | Chemical analysis of OMW and WAS characteristics (n=5)
a flame ionisation detector was used to determine the concentrations of volatile fatty acids (Diamantis et al. 2006).

RESULTS

Table 2 summarises four steady state conditions that were examined. In both systems, the daily biogas production rate was observed to increase as the hydraulic retention time (HRT) decreased, as expected due to higher feeding volumes. During the first steady state period (HRT 12.3 d), the average daily biogas production for the mixture was 77 l/d (0.98 l biogas/g sCODin) compared with 30 l/d (0.23 l biogas/g sCODin) for WAS. The increase in biogas production for the co-digestion process was 157% while for biogas yield, in terms of soluble COD that was added, the increase was estimated at 326.1%. During the following steady state periods, biogas production was estimated at 66, 58.5 and 48.4 l/d for the mixture, while the corresponding values for WAS were 3.1, 2.5 and 2.4 times lower. Even though the high toxicity of phenols within the OMW has been reported to inhibit biodegradation – methanogenesis and COD reduction in the anaerobic process (Borja et al. 1992; Boari et al. 1993), we observed that OMW addition to the WAS enhances the biogas production without affecting the system stability.

The biogas production rate observed at different HRTs for the co-digestion of WAS with OMW and digestion of WAS alone, is illustrated in Figure 2. This rate is inversely proportional and shows a linear relationship over the range of HRTs applied. It is observed that biogas production from AD of the mixture is reduced from 0.73 to 0.46 l biogas/l/d as the HRT increases, because of lower feeding volumes. Similarly, biogas production from WAS reduced from 0.32 to 0.21 l biogas/l/d. Moreover, Figure 2 shows that there is a significant increase in biogas production when 30% of OMW is used as co-substrate. In particular, the increase was estimated at more than 2.2 times for all cases.

In addition, Figure 2 presents the average removal of sCOD for all the cases of HRTs that were examined, after 30% addition of OMW in WAS. The average value of sCOD that was added to the system was 9.7 ± 0.4 g and the organic loading rate decreased from 0.75 ± 0.006 g COD/l/d at HRT of 12.3 d to 0.70 ± 0.03, 0.58 ± 0.07 and 0.49 ± 0.05 g COD/l/d at HRTs of 14.0, 16.4 and 19.7 d respectively. The sCOD removal rate was estimated to be high for all cases and ranged between 64 and 72%. This degree of removal is in accordance with previously reported values in the range of 60–80% found as COD reductions during anaerobic degradation of diluted OME (Angelidaki & Ahring 1997; Marques 2001).

Thus, the effluent sCOD of the system was estimated at 2.7 ± 0.12, 3.6 ± 0.24, 3.1 ± 0.26 and 2.6 ± 0.14 g/l at HRTs...
of 12.3, 14.0, 16.4 and 19.7 d respectively. It is important
that the remaining 1.2 ± 0.1 g sCOD/l at the exit of the
system comes from WAS, as this value was measured
when WAS alone was treated anaerobically in the cascade.
The expected reduction of sCOD concentration in the ef-
fluent as the HRT increases is not observed probably due to
differences in sludge characteristics of the in-
fluent mixture.
It is also important to note that as the HRT increases,
higher sCOD removal is taking place in the first stage of
the cascade. In particular, when the HRT of the cascade
was 12.3 d, the corresponding HRT of the first digester
was 4.9 d and the soluble sCOD reduction was 42.9 and
28.8% for the first and the second stage respectively. By
increasing the cascade HRT to 14 d, the HRT of the first
digester simultaneously increased to 5.6 d and the sCOD
reduction increased to 52.7% in the first stage while it
decreased to 10.9% in the second stage of the cascade.
Further increase to the cascade HRT to 16.4 and 19.7 d
increased the HRT of the first stage to 6.6 and 7.9 d respec-
tively with a corresponding increase in sCOD reduction of

### Table 2 | Biogas production and biogas yield at different HRTs (n=10)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>OMW + WAS</th>
<th>WAS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D1</td>
<td>D2</td>
</tr>
<tr>
<td>HRT 12.3 d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biogas production (l/d)</td>
<td>53.9 ± 4.2</td>
<td>23.1 ± 3.4</td>
</tr>
<tr>
<td>Biogas yield (l/g CODin)</td>
<td>0.98 ± 0.06</td>
<td>0.23 ± 0.05</td>
</tr>
<tr>
<td>HRT 14 d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biogas production (l/d)</td>
<td>47.5 ± 2.8</td>
<td>18.5 ± 1.3</td>
</tr>
<tr>
<td>Biogas yield (l/g CODin)</td>
<td>0.91 ± 0.08</td>
<td>0.23 ± 0.07</td>
</tr>
<tr>
<td>HRT 16.4 d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biogas production (l/d)</td>
<td>44.3 ± 1.8</td>
<td>14.2 ± 0.7</td>
</tr>
<tr>
<td>Biogas yield (l/g CODin)</td>
<td>1.02 ± 0.05</td>
<td>0.24 ± 0.05</td>
</tr>
<tr>
<td>HRT 19.7 d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biogas production (l/d)</td>
<td>37.2 ± 1.5</td>
<td>11.2 ± 0.8</td>
</tr>
<tr>
<td>Biogas yield (l/g CODin)</td>
<td>0.93 ± 0.04</td>
<td>0.27 ± 0.06</td>
</tr>
</tbody>
</table>

D = Anaerobic digester; 1,2 = first and second AD for OMW + WAS; 3,4 = third and fourth AD for WAS alone.
56.9 and 65.7% while in the second stage, the sCOD removal rate reduced to 10.1 and 6.2% respectively.

Figure 3 shows the mean values of the biogas selectivity which were calculated for the mixture of OMW and WAS as well as for WAS alone, with the retention times being taken into account. For the case of co-digestion, the selectivity estimated nearly 1.1 l\text{biogas}/g TVS removed. The corresponding value for biogas produced, per gram COD removed was estimated at 0.6 l\text{biogas}/g COD or, taking into account that there is 74% methane in the biogas that is produced, there are 0.44 l\text{methane}/g COD removed. This value is lower than the value of 0.55 l\text{biogas}/g COD removed that Azaizeh & Jadoun (2010) found for 33% OMW and 67% swine manure and a little bit higher than the 0.35 l of methane per gram COD removed that Ubay & Ozturk (1997) estimated during OMW digestion in a UASB reactor. Moreover, according to Figure 3, biogas selectivity of the mixture is considered to be up to two times higher when compared with WAS (0.5 l\text{biogas}/g TVS removed).

In this work WAS was used in order to provide dilution of OMW and to compensate for its potential toxicity. The observed 0.44 l CH\text{4}/g COD removed in the mixture of 30% OMW and 70% WAS in comparison with 0.27 l CH\text{4}/g COD removed that was measured in raw WAS and to 0.35 l CH\text{4}/g COD removed that Ubay & Ozturk (1997) measured when treating OMW alone, shows clearly an improvement of the AD of OMW as well as WAS. Probably this augmentation is due to the contribution of the OMW biodegradable components to the mixture, and the compensation in the OMW composition because WAS provides the important, for the bacterial growth, ammonia and alkalinity to increase the resistance of to acidification (Gelegenis et al. 2007; Sampaio et al. 2011).

Another way of assessing the performance of a digester is to examine the efficiency of the VSS reduction. During the digestion process, volatile solids are degraded to a certain extent and converted into biogas. The VSS reduction was relatively similar at the highest and shortest retention times, a fact that is confirmed by Appels et al. (2008), who claims that for HRT values exceeding 12–13 d (at 35 °C), changes in increasing volatile solids reduction are relatively small. In terms of VSS reduction, the values were 36.8, 40.7, 37.4 and 36.2% for the WAS and 27.9, 26.8, 29.5 and 27.6% for the mixture of OMW and WAS for HRTs of 12.3, 14.0, 16.4 and 19.7 d respectively. According to Dareioti et al. (2010), who co-digested 20% OMW and 80% cow manure, VSS reduction was estimated at 34.2%. VSS reduction is lower for the mixture compared with the corresponding values of WAS, due to the difficult biodegradable compounds that OMW consists of.

VFAs measurement was chosen to predict process instability (Ahring et al. 1995). VFA concentrations of acetic, propionic, iso-butyric, butyric and valeric acid were measured for HRTs of 12.3, 14, 16.4 and 19.7 d. In all cases, iso-butyric and valeric acid were negligible. VFAs in the influent mixture present a remarkable fluctuation during all cases that were examined, due to WAS storage. In the effluent there was no presence of VFAs because of their total conversion to biogas (Figure 4).

The absence of VFAs confirms the stable conditions that the system was running at under all the HRTs that were examined. Borja et al. (1998) suggested that this stability may be attributed to carbonate/bicarbonate buffering due to the production of carbon dioxide in the digestion process that is not completely removed as gas. This buffering effect acts as a protection against the acidification of the system and yields a pH range that is optimal for the methanogens. Further reduction of HRT was not investigated because a destabilisation of the AD would be expected (Appels et al. 2008). As we mentioned above when the HRT of the cascade
was 12.3 d, the corresponding HRT of the first digester was 4.9 d and further reduction of the HRT would affect the methanogenesis (Demirel & Yenigün 2002).

It is important to note that in order to find the most adequate ratio of OMW in the mixture the cascade was fed with 40% OMW. At an HRT of 19.7 d, the system showed almost an immediate increase of biogas production. However due to the sustained high COD loading, a build-up of intermediate VFAs occurred, which led to a substantial pH drop to 4.7, an environment which inhibited methanogenic activity and resulted in an unstable system and failure.

**CONCLUSIONS**

Based on the results of this study, OMW could be digested successfully by combining it with WAS in a two-stage mesophilic CSTR system in a ratio of 30% OMW and 70% WAS. The biogas production rate at steady state conditions reached 0.73, 0.63, 0.56 and 0.46 l biogas/l reactor/d for HRTs of 12.3, 14, 16.4 and 19.7 d. The average removal of sCOD was high for all cases and ranged between 64 and 72% for organic loading rates between 0.49 and 0.75 g COD/l/d. Reduction in the VSS was nearly the same for all cases and ranged between 27 and 30%. In terms of biogas selectivity, values of 0.6 l biogas/g tCODr and 1.0 l biogas/g TVSr were measured. Consequently, OMW could be treated in existing wastewater treatment plants as it increases methane production significantly. However, the feeding rate must not exceed the critical mixture of 30% OMW in order to prevent overloading conditions.

**REFERENCES**


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