Bioenergy recovery from cattle wastewater in an UASB-AF hybrid reactor
Henrique Vieira de Mendonça, Jean Pierre Henry Balbaud Ometto, Marcelo Henrique Otenio, Alberto José Delgado dos Reis and Isabel Paula Ramos Marques

ABSTRACT
New data on biogas production and treatment of cattle wastewater were registered using an upflow anaerobic sludge blanket-anaerobic filter (UASB-AF) hybrid reactor under mesophilic temperature conditions (37 °C). The reactor was operated in semi-continuous mode with hydraulic retention times of 6, 5, 3 and 2 days and organic loading rates of 3.8, 4.6, 7.0 and 10.8 kg CODt m⁻³ d⁻¹. Biogas volumes of 0.6–0.8 m³ m⁻³ d⁻¹ (3.8–4.6 kg CODt m⁻³ d⁻¹) and 1.2–1.4 m³ m⁻³ d⁻¹ (7.0–10.8 kg CODt m⁻³ d⁻¹), with methane concentrations between 69 and 75%, were attained. The removal of organic matter with values of 60–81% (CODt) and 51–75% (CODs) allowed methane yields of 0.155–0.183 m³ CH₄ kg⁻¹ CODt and 0.401–0.513 m³ CH₄ kg⁻¹ CODs to be obtained. Volatile solids were removed in 34 to 69%, with corresponding methane yields of 0.27 to 0.42 m³ CH₄ kg⁻¹ VSremoved. The good performance of the novel hybrid reactor was demonstrated by biogas outputs higher than reported previously in the literature, along with the quality of the gas obtained in the various experimental phases. The hybrid reactor investigated in this study presents comparative advantages, particularly in relation to conventional complete mixture units, considering economic factors such as energy consumption, reactor volume and installation area.

Key words | anaerobic digestion, bioenergy, biogas, cattle wastewater, UASB-AF hybrid reactor

INTRODUCTION
Milk production in Brazil has increased 37% in the last 20 years (Cavicchioli et al. 2015; Mendonça et al. 2017). Brazilian livestock production accounts for 8.3% of global consumption and the sector aims to capitalise on the growing demand (Oliveira et al. 2016). The recent expansion of cattle raising activity has created a need for intensive feeding in confined areas as opposed to free-range grazing, increasing the generation of cattle wastewater (CWW) (Mouri & Aisaki 2015).

Because of the need to mitigate environmental impacts, the application of anaerobic digestion to treat effluents of this type is an attractive option, with additional advantages of generating energy from biogas and biofertilizers (Mendonça et al. 2017). Other inherent benefits of anaerobic digestion are low consumption of nutrients and formation of low sludge volumes due to the slow metabolism of methanogenic microorganisms.

Various types/models of reactors have been used for anaerobic digestion of agro-industrial wastes and wastewaters, among them the plug flow reactor (PFR) and anaerobic sequencing batch reactor (Nasir et al. 2012). In the last decade, new reactor models have been studied and adapted to improve the yield of the digestion process, making it possible to use smaller units to treat the same effluent volume. Among these are: the anaerobic filter (AF), upflow anaerobic sludge blanket (UASB), fluidized bed anaerobic (FBAR) and oscillatory flow (OFR) reactors. These reactor types accumulate a high concentration of biomass, allowing longer retention of solids even when operating at short hydraulic retention times (HRTs) (Nasir et al. 2012). The choice of reactor model to digest a specific substrate is important. The anaerobic hybrid reactor...
(AHR) has a specific configuration that combines the advantages of the UASB and AF, increasing treatment efficacy.

The anaerobic UASB-AF hybrid reactor, designed and tested in Portugal’s National Laboratory on Energy and Geology (LNEG), proved highly successful in treating olive mill wastewater (OMW, effluent from three-stage olive oil mills). When operating for HRTs of 6 and 7 days and organic load rates (OLRs) of 8 kg COD m⁻³ d⁻¹, it achieved removal rates of 81 to 82% of chemical oxygen demand (COD) and biogas outputs between 3.7 and 3.8 m³ m⁻³ d⁻¹ (65 to 64% CH₄) (Sampaio et al. 2011).

Using the same hybrid reactor to treat an analogous effluent, Gonçalves et al. (2012) applied an organic load of 7.1 kg COD m⁻³ d⁻¹ (HRT of 6 days, 37 °C) and obtained 3.16 m³ m⁻³ d⁻¹ of biogas with 75% methane concentration.

The main characteristic of this UASB-AF hybrid reactor is to carry out the digestion of OMW, which presents all the adverse characteristics to the anaerobic process, such as unbalanced composition (high C/N), high organic content (about 50–200 kg COD m⁻³), acid pH (4–5) and inhibitory/toxic capacity, due to the concentrations of lipidic and phenolic compounds, avoiding any pre-treatment, dilution or chemical correction actions.

This study investigated the behavior and efficiency of the same anaerobic UASB-AF hybrid reactor applied to CWW and compared the results against those reported in other works published in the past 15 years. We also characterize the reactor’s operation along its vertical column and identify the function of the various sections in the overall treatment.

**MATERIALS AND METHODS**

**Experimental set up**

The experiment was conducted with an upflow anaerobic UASB-AF hybrid reactor constructed of polymethyl methacrylate with a usable volume of 1.7 L. The filling medium was a synthetic polymeric mesh, malleable and not subject to corrosion, characterized by providing a void volume of 75%. It was placed in the upper part of the unit, corresponding to only 1/3 of the height of the column. This unit was composed of a feed receiving zone and a settler, at the hybrid base, followed by the ‘sludge blanket’ and ‘AF’ sections and, finally, by the digested flow section. At the hybrid top, the produced biogas is collected in the gas chamber and recorded in a mechanical counter.

The reactor has a water recirculation jacket to keep the operating temperature in the central cylinder at 37 °C (±1 °C). Wastewater was fed into the reactor in a semi-continuous mode, controlled by a peristaltic pump (8 rpm and flow of 1.10 ml/min). The biogas production was measured by a wet mechanical counter and the respective volume was converted for normal temperature and pressure conditions (273.15 K, 1 atm). The UASB-AF hybrid reactor has four sampling points along its vertical column to allow collecting samples in different reaction zones: sludge blanket (P3: 7.5 cm), intermediate zone (P2: 31 cm), filter zone (P1: 41 cm) and effluent zone (P0: 48 cm), (Figure 1).

**Analytic and chromatographic methods**

The total and soluble chemical oxygen demand (COD₃ and COD₄), total solids (TS), volatile solids (VS), fixed solids (FS), total suspended solids (TSS), volatile suspended solids (VSS), fixed suspended solids (FSS), ammoniacal nitrogen (NH₃⁻N), total Kjeldahl nitrogen (TKN), alkalinity, pH, phosphate (PO₄³⁻) and were determined in duplicate, according to Standard Methods (APHA 2022). Volatile fatty acids (VFA) were evaluated by high-performance liquid chromatography (HPLC) with an Agilent model 1100 chromatograph. The concentrations of CH₄ and CO₂ in the biogas were measured by gas chromatography in a Varian model 450-GC chromatograph equipped with a thermal conductivity detector and a Varian Select™ capillary column for permanent gases/CO₂ HR – Molsieve 5A/Porabond Q Tandem #CP7430. The temperatures in the column, injector and detector were 50, 80 and 120 °C, respectively. Helium was used as the carrier gas (52 mL min⁻¹). A total of 0.5 mL of biogas was injected in the chromatograph, collected from the upper part of the reactor. The quantitative acid hydrolysis (QAH) method was used to measure the concentrations of cellulose and hemicellulose (Hoebler et al. 1989).

**Substrate and inoculum**

The substrate was dairy cow effluent collected in a farm of 400 animals in confinement, located in Loures (Portugal). The substrate was initially subjected to gross separation through a sieve with openings of 6 mm (Vanguard V2, AxFlow, Sweden). Later, it was filtered through a 2 mm mesh screen and stored at 4 °C. Table 1 presents the CWW characterization.
The inoculum used to start up the anaerobic UASB-AF hybrid reactor was the CWW itself, obtained in the same dairy exploitation of the substrate (Loures, Portugal).

Start-up and operating mode

The reactor was operated in six phases (Table 2). The first two concern the system start up and the other four the operation in semi-continuous flow to test different HRTs.

The reactor start up was carry out by first filling it with CWW. After 18 days, the chromatographic analysis revealed a small CH₄ concentration, (2% – phase I) and, on the same day, a new start up process was made by injection of a fresh cow manure (400 mL) and CWW (200 mL) mixture containing 45 mg CODₗ L⁻¹ (±5.4). After 12 days of new inoculation (phase II), a methane concentration of 60% (±0.89) was obtained and, subsequently, the UASB-AF hybrid reactor started to be fed under a semi-continuous mode according to Table 2.

RESULTS AND DISCUSSION

Biogas production

The biogas output varied from 0.6 to 1.4 m³ m⁻³ d⁻¹, with methane concentrations between 69 and 75% during the 140 days of the experiment (Table 3).

The production of biogas increased gradually with rising OLR levels, as shown in Figure 2, with daily volumes greater than 1 m³ m⁻³ obtained in the last two phases (V and VI).

In comparison with the data reported in the literature over the past 15 years, the maximum biogas production (1.40 m³ m⁻³ d⁻¹) obtained in phase VI was the most relevant (Table 4).

Values similar to those found in this study were observed by Rico et al. (2011) and Dareioti et al. (2010), in both cases operating a continuous stirred-tank reactor (CSTR) at mesophilic temperature, reporting yields of about 1.5 m³ m⁻³ d⁻¹, after HRTs of 10 and 19 days, respectively. The main advantages of the UASB-AF hybrid reactor...
CODs, soluble chemical oxygen demand; TKN, total Kjeldahl nitrogen; NOorg, organic nitro-
VSS, volatile suspended solids; FSS, fixed suspended solids; TS, total solids; VS, volatile solids; FS, fixed solids; TSS, total suspended solids; CODt, total chemical oxygen demand; Hemicellulose (%)
Cellulose (%) 19.7 (1)
Alkalinity (mg L−1) 4,234 (0.08)
CODs (mg L−1) 20,000 (4.4)
CODs (%) 43.5
CODs (mg L−1) 8,698 (2.7)
CODs (%) 43.5
TKN (mg L−1) 1,246 (0.05)
NH4+ (mg L−1) 522 (0.3)
Norg (mg L−1) 720 (4.1)
NO3− (mg L−1) 0 (0.0)
PO4− (mg L−1) 36 (4.2)
TS (mg L−1) 15,267 (18.4)
FS (mg L−1) 3,793 (61.2)
VS (mg L−1) 11,475 (51.2)
TSS (mg L−1) 10,004 (7.3)
FSS (mg L−1) 100 (7.8)
VSS (mg L−1) 9,900 (4.6)
VFA (mg L−1) 2,035 (8.9)
Cellulose (%) 18.6 (0.84)
Hemicellulose (%) 19.7 (1)

Values in brackets indicate standard deviation. CODs, total chemical oxygen demand; CODt, soluble chemical oxygen demand; TKN, total Kjeldahl nitrogen; Norg, organic nitrogen; TS, total solids; VS, volatile solids; FS, fixed solids; TSS, total suspended solids; VSS, volatile suspended solids; FSS, fixed suspended solids; VFA, volatile fatty acids.

<table>
<thead>
<tr>
<th>Phase/operating</th>
<th>HRT Days</th>
<th>OLR Kg CODt m−3 d−1</th>
<th>OLR Kg VS m−3 d−1</th>
<th>Time Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>I – Start-up</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0–18</td>
</tr>
<tr>
<td>II – Start-up</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>19–30</td>
</tr>
<tr>
<td>III – Semi-continuous</td>
<td>6</td>
<td>3.8</td>
<td>4.3</td>
<td>31–61</td>
</tr>
<tr>
<td>IV – Semi-continuous</td>
<td>5</td>
<td>4.6</td>
<td>5.2</td>
<td>62–85</td>
</tr>
<tr>
<td>V – Semi-continuous</td>
<td>3</td>
<td>7.0</td>
<td>7.9</td>
<td>86–120</td>
</tr>
<tr>
<td>VI – Semi-continuous</td>
<td>2</td>
<td>10.8</td>
<td>12</td>
<td>121–140</td>
</tr>
</tbody>
</table>

OLR, organic load rate as CODt; OLR, organic load rate as VS.

investigated here in relation to CSTR reactors are the absence of mechanical agitation and the need for a smaller usable volume of the reactor for the same volume of substrate to be treated. Demirer & Chen (2005b) treating CW in AHR with HRTs of 10 and 20 days, observed lower values than those achieved by the UASB-AF hybrid reactor in the present work, with biogas outputs of 0.21 and 0.85 m3 m−3 d−1 (63.5% CH4). Ferrer et al. (2011), in Peru, and Resende et al. (2015), in Brazil, investigated horizontal tubular digesters (HTD) at room temperature (Table 4). The first study reported yields of 0.07 and 0.47 m3 m−3 d−1 with HRTs of 60 and 90 days, while the second reported yields of 0.27 and 0.31 m3 m−3 d−1 for HRT of 60 days. The highest biogas production values observed by these two research groups were below the lowest value attained by our UASB-AF hybrid reactor (0.60 m3 m−3 d−1) with only 6 days of HRT. Even in countries with tropical climates, the cooler winter temperatures can impair the performance of anaerobic digestion. Resende et al. (2015) observed temperature fluctuations between 14 and 25°C in the winter and 24 and 35°C in the summer. They reported a decline 14.5% in biogas production when the temperature fell from 29.5 to 19.5°C. Castano et al. (2014) reported that to avoid this impairment and acidification in reactors, the surrounding temperature should be higher than 20°C. Witarsa & Lansing (2015) observed similar results, finding a decline of 70% in methane production when the temperature decrease from 24 to 14°C. As described by the same authors, the most suitable temperatures for anaerobic digestion of CWW are in the mesophilic range (30–35°C) and thermophilic range (50–60°C).

The advantages of using mesophilic rather than thermophilic temperatures, mainly in winter, are the greater ease of avoiding thermal shocks during the process, maintenance of more stable biogas production and lower energy costs to heat the reactor. These observations, together with the results of this study, urge reflection on the advantages of adopting reactors that operate in the constant mesophilic range, in tropical countries like Brazil, where currently the treatment plants operate at room/psychrophilic temperatures (10–25°C), in the winter.

The composition of the biogas produced did not fluctuate greatly during the experiment, as verified in Figure 2. The average methane concentration varied from 69 to 75%, respectively, in phases III and IV, after which it stabilized in the narrow range of 71–72% (phases V and VI: Table 3). For carbon dioxide, concentrations between 20.6% (±1.04) and 23.7% (±1.3) were recorded, in semi-continuous operation. According to Noorollahi et al. (2015), the average CH4 concentration in biogas is typically in the range from 55 to 65%. Other research groups (Comino et al. 2009; Dareioti et al. 2010; Rico et al. 2011), utilizing CSTRs, and Demirer & Chen (2005b), employing an AHR, have found methane concentrations with minimum values of 51 to 64% and maximums of 67 to 69% (Table 4). In comparison, the lowest value found in this study (69% CH4) corresponds...
to the highest value reported in the papers mentioned above (Table 4), indicating that the process in the UASB-AF hybrid reactor produces biogas with higher CH₄ concentration than found in the other reactors.

The higher methane content reached by this hybrid unit may be related to the capacity of concentration of specialized anaerobic clusters (Archaea) that allow good digester functioning even in the presence of complex substrates such as cow effluent.

**Total and soluble COD removal**

As the organic load increased and the HRT decreased, the unit’s removal capacity diminished (Table 3). The highest removal rates were found for operation with longer HRTs (5 and 6 days), where average efficiencies of 76 and 81% COD were attained, respectively. The UASB reactors investigated in the studies of Marañón et al. (2006) and Marañón et al. (2001) reached COD removal rates of 85% and 75.5% when using HRTs of 14 and 22.5 days, respectively (Table 4). Although these values are compatible with those observed for the UASB-AF hybrid reactor, the HRTs used in those previous studies were 2.3 and 3.8 longer, indicating the UASB-AF hybrid unit has better removal capacity than the UASB for this type of wastewater. The removal rates of 55 and 61% obtained by Marañón et al. (2001) when increasing the HRT from 8.9 to 10.6 days and applying loads similar to those tested in the UASB-AF hybrid unit, 4.91 and 4.32 versus 4.6 kg COD m⁻³ d⁻¹ (phase IV), also indicate a clear advantage of the present reactor.

Removal values (79.7% of COD) comparable to those found by us were obtained with a UASB reactor, but it was operated under thermophilic temperature conditions (55 °C) for a longer period of 22.5 days (Castrillón et al. 2005). Further evidence of the high performance of the UASB-AF hybrid system in the treatment of CWW is provided by comparison with another type of unit. Comino et al. (2009), investigating the digestion of a mixture of CWW and whey in a CSTR under discontinuous processing,
obtained COD removal of 74% after operation for 56 days (Table 4).

Reducing the HRT to 3 and 2 days (phases V and VI) did not favor the removal capacity of the UASB-AF hybrid reactor compared to the results obtained in the two preceding phases, where there were decreases of 68 and 60%, respectively. However, using longer HRTs (about 10 days) did not improve the hybrid reactor's performance (Demirer & Chen 2005b) or that of the UASB (Marañón et al. 2001), which when operating with loads of 7 and 4 kg COD m⁻³ d⁻¹, attained removal rates of 64 and 61%, respectively. Furthermore, the use of higher temperatures (53°C) and longer HRTs (10 days) in a jacketed fermenter (mixing aided by a mechanical stirrer set at a speed of 150 rpm) (Abubakar & Ismail 2012) and 17 days in a Biostat B reactor (Germany), in batch treatment (Omar et al. 2008), resulted in removal rates of 49 and 51% COD, respectively, lower than those found by us, with the reactor operating under mesophilic temperature conditions. The lowest removal rates (61 and 55% COD) found by Castrillón et al. (2002) are comparable with the lowest levels attained by the UASB-AF hybrid reactor (phase VI), but with the inherent disadvantages of applying higher digestion temperatures and longer HRTs than we did: thermophilic range, with HRTs of 8.9 and 7.3 days versus mesophilic range and HRT of 2 days.

In comparison with the findings of other researchers, the UASB-AF hybrid reactor tested in this study was able to remove organic matter under more favorable conditions, given its ability to operate at mesophilic temperatures and

<p>| Table 4 | Anaerobic digestion of CM and CWW |</p>
<table>
<thead>
<tr>
<th>Reactor, Temp. Subs.</th>
<th>HRT (d)</th>
<th>OLR (Kg COD m⁻³ d⁻¹)</th>
<th>Biogas m³ m⁻³ d⁻¹</th>
<th>CODr rem. (%)</th>
<th>CH₄ (%)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bach CSTR, 35 CM + WM</td>
<td>56</td>
<td>NR</td>
<td>NR</td>
<td>74</td>
<td>51.4</td>
<td>Comino et al. (2009)</td>
</tr>
<tr>
<td>CSTR, 35 DM 80% + OMW 20%</td>
<td>19</td>
<td>3.63</td>
<td>1.31</td>
<td>63.2</td>
<td>68.9</td>
<td>Dareioti et al. (2010)</td>
</tr>
<tr>
<td>CSTR, 37 CM</td>
<td>10</td>
<td>5.76</td>
<td>1.34</td>
<td>36</td>
<td>67</td>
<td>Resende et al. (2015)</td>
</tr>
<tr>
<td>HTD, 14–35 CM</td>
<td>60</td>
<td>NR</td>
<td>0.27–0.31</td>
<td>NR</td>
<td>53.7–59.2</td>
<td>Castrillón et al. (2002)</td>
</tr>
<tr>
<td>HTD, 20–25a CM</td>
<td>60</td>
<td>NR</td>
<td>0.07</td>
<td>NR</td>
<td>63</td>
<td>Ferrer et al. (2011)</td>
</tr>
<tr>
<td>JF, 53 CM</td>
<td>10</td>
<td>NR</td>
<td>NR</td>
<td>48.5</td>
<td>NR</td>
<td>Abubakar &amp; Ismail (2012)</td>
</tr>
<tr>
<td>PF, 15–30b CWW</td>
<td>32</td>
<td>0.40</td>
<td>0.173</td>
<td>61</td>
<td>65</td>
<td>Mendonça et al. (2017)</td>
</tr>
<tr>
<td>UASB, 55 CM</td>
<td>7.3</td>
<td>5.06</td>
<td>NR</td>
<td>54.8</td>
<td>56–67.7</td>
<td>Castrillón et al. (2002)</td>
</tr>
<tr>
<td>UASB, 37 CM</td>
<td>14</td>
<td>3.7</td>
<td>NR</td>
<td>85</td>
<td>NR</td>
<td>Marañón et al. (2006)</td>
</tr>
<tr>
<td>UASB, 37 CM</td>
<td>5.3</td>
<td>8.63</td>
<td>NR</td>
<td>32.2</td>
<td>64.4</td>
<td>Marañón et al. (2001)</td>
</tr>
<tr>
<td>AHR, 36 DM</td>
<td>20</td>
<td>0.87</td>
<td>0.21</td>
<td>32</td>
<td>63.5</td>
<td>Demirer &amp; Chen (2009b)</td>
</tr>
<tr>
<td>UASB-AF, 37 CWW</td>
<td>6</td>
<td>3.8</td>
<td>0.60</td>
<td>81</td>
<td>69</td>
<td>Present work</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>4.6</td>
<td>0.76</td>
<td>76</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>7.0</td>
<td>1.20</td>
<td>68</td>
<td>71</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>10.8</td>
<td>1.40</td>
<td>60</td>
<td>72</td>
<td></td>
</tr>
</tbody>
</table>

CM, cattle manure; WM, whey mix; OMW, olive mill wastewater; DM, dairy manure; CWW, cattle wastewater; PF, plug flow reactor; CSTR, continuous stirred tank reactor; HTD, horizontal tubular digester; UASB, upflow anaerobic sludge blanket; AF, Anaerobic filter; AHR, anaerobic hybrid reactor; JF, jacketed fermenter (Biostat B); Temp., temperature; Subs., Substrate; NR, not reported; COD rem., chemical oxygen demand removal.

aPilot scale reactors of 2.4 and 7.5 m³.
bFull scale reactor: 540 m³ (the number of dairy cattle confined in the free stall varied from 120 to 150 heads).
shorter HRTs. In terms of construction, this allows a considerable reduction in the cost of reactor implantation and operation.

**Biogas and methane yields by COD removed**

The amount of methane produced (CH₄ yield) as a function of CODᵣ and CODᵣ decreased with higher OLRs and shorter HRTs during the first three phases of operation (Table 3). There was an exception to this pattern in the last period (phase VI), where the methane yield was similar to that in the first two phases of continuous operation (phases III and IV). The best methane yield was obtained in phase V, with HRT of 3 days, attaining levels of 0.183 and 0.515 m³ of CH₄ per kg of total and soluble COD removed, respectively. The results of the present work regarding CH₄ yield (for removed CODᵣ) are within the range of 0.13 and 0.25 m³ CH₄ kg⁻¹ registered by Marañón et al. (2006). Higher values compared to those obtained in this research were achieved by Castrillón et al. (2002). The authors managed to achieve a maximum of 0.30 m³ CH₄ kg⁻¹ CODᵣ removed, even with lower percent methane concentrations in the range of 56 and 67.7%.

The CH₄ yields in relation to CODᵣ were lower than those obtained as a function of CODᵣ (Table 3). This indicates that the HRTs tested in this study were sufficient to degrade the soluble material and that part of the less degradable particulate organic material was retained inside the reactor. Plant residues in CWW not removed in the preliminary treatment were retained in the reactor. These cellulosic wastes are recalcitrant and not biodegradable within a feasible period for operation of the reactor.

The retention of this material in the reactor explains the higher CODᵣ removal in relation to CODᵣ in all the experimental phases. The concentrations of cellulose and hemicellulose detected in the reactor’s outflow and inflow are presented in Supplementary Material 1 (available with the online version of this paper).

The levels of cellulose and hemicellulose detected in the effluent in all phases varied only slightly, suggesting that the upflow speed in this study (0.2 m h⁻¹) was sufficient to prevent excessive drag of these plant residues. Between the reactor’s inlet and outlet, about 15% of the cellulose and 14% of the hemicellulose was retained, with 7.7 (±0.21) and 8.6% (±0.1) of these compounds being determined analytically at point P3 (Figure 1) in phases III and IV. In phases V and VI, about 14% of the cellulose and hemicellulose were retained, with 8 and 8.8% verified at point P3 (sludge blanket zone).

**Solids removal**

The removal rates of TS and VS decreased with shorter HRTs (Supplementary Material 2, available with the online version of this paper). This can be explained by the increase of microbial biomass in the reactor, which promoted the exit of biological material (anaerobic sludge) together with the treated wastewater, concomitantly with the accumulation of material, as well as by the shorter period available for degradation of the substrate. The same pattern was observed for TSS and VSS, as expected.

The removal of VS peaked at 69% in phase III and then successively declined to a minimum of 54.4% in phase VI, for initial HRTs of 6 days and final of 2 days, respectively. Values near these have been reported by various researchers: Demirer & Chen (2005a, 2005b), using conventional slurry anaerobic digestion (CSAD) and Resende et al. (2015), operating a HTD predominantly at mesophilic temperature.

In comparative terms, while the methane yield with HRT of 2 days (phase VI) was higher than all the values listed in Table 5, the methane yield with HRT of 3 days was similar to that found by Ferrer et al. (2011), operating with a HTD at room temperature and with HRTs of 60 to 90 days and Mendonça et al. (2017), operating with a PFR (15–30 °C) and HRT of 32 days. Demirer & Chen (2005b) tested treatment of dairy manure, in a hybrid reactor configuration. For HRTs of 20 and 10 days the authors obtained methane yields of 0.299 and 0.255 m³ kg⁻¹ VSᵣ, with reductions of 44 and 69% of VSs. In this study, the VSs removal rates were similar to those of that previous study, but with shorter HRTs.

**pH, alkalinity and VFAs**

The pH values in the input material were near neutral (between 6.9 and 7.4), indicating suitable conditions for degrading organic material and microbial growth. The anaerobic process promoted an increase in pH in the two experimental phases (III and IV), after which it remained steady or declined slightly in the subsequent phases (Supplementary Material 3, available with the online version of this paper), reaching values of 7.1–7.5 in the digested output.

Likewise, the alkalinity recorded in the substrate increased during phases III and IV, remained identical in phase V and then decreased in the last period. However, the values in the output indicated the achievement of a sufficient buffering effect to maintain the anaerobic process in good operational conditions.
The suitable operation of the UASB-AF hybrid reactor was confirmed by the removal rates in terms of VFA, which varied between 98 and 100% (Supplementary Material 3) in all phases. VFAs did not accumulate in the system in any of the treatment steps, instead being consumed and transformed, mainly into CH₄ and CO₂ (Demirer & Chen 2008). The VFA values at the reactor outlet remained between 50 and 500 mg L⁻¹, a range considered by Gerardi (2005) as secure regarding stability of an anaerobic process. According to Sung & Santha (2005), the alkalinity of anaerobic sludges from a properly operating digester should be able to neutralize the excess VFAs and keep the pH in an ideal interval (6.5–7.5).

This explains the behavior of this reactor in removing VFAs (Figure 3(c)), which happened mainly between the entry point and point P2 (Figure 1), where the sludge blanket is concentrated.

Sampaio et al. (2011), in testing the same UASB-AF hybrid reactor (used in this work) to treat OMW, reported increases in alkalinity of 2.42 to 6.32 kg CaCO₃ m⁻³. The accumulation of carbonated substances together with the sludge is a factor that contributes to increase the alkalinity levels, as observed by various researchers. In the specific case of dairy CWW, consideration should be given to the animals’ diet, given that dairy cows typically receive compounds such as calcium carbonate (CaCO₃), calcitic limestone (also containing CaCO₃) and dolomitic limestone (CaCO₃·MgCO₃). These compounds are eliminated in the animals’ urine/manure and accumulate in the sludge during the anaerobic digestion process. Another aspect is that the production of methane itself raises the alkalinity as a side effect, so under proper operation, the increase of CaCO₃ can be considered a benefit of the treatment process.

The alkalinity increase in the treated outflow, besides being benign regarding neutralization of the VFAs during the digestion process, has another positive effect, because when added to acid soils, it helps control pH.

### Nitrogen and phosphate compounds

The concentration of total nitrogen decreased during the digestion process in all experimental phases, as expected, although the decrease was less pronounced in the phases operating with higher loads: removal rates of 33 and 39% (phases III and IV) and 5 and 8% (phases V and VI). Also as might be expected, the concentration of ammoniacal nitrogen increased after anaerobic digestion while the level of organic nitrogen decreased (Table 6).

The variation of the increase in the concentration of NH₄⁺ was more pronounced after the longest two HRTs (50 and 54%) than after the shorter ones. For phase VI, the concentration of ammoniacal nitrogen on entry of the substrate and exit of the digested material remained virtually unchanged, revealing a drastic reduction of ammonification.

### Table 5 | VS removal and methane yield by anaerobic digestion

<table>
<thead>
<tr>
<th>Reactor, Temp. (°C)</th>
<th>Subs.</th>
<th>HRT (d)</th>
<th>VS (%)</th>
<th>CH₄ yield (m³ kg⁻¹ SVr)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSAD, 36</td>
<td>CM</td>
<td>20</td>
<td>52</td>
<td>0.235</td>
<td>Demirer &amp; Chen (2005a)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>68</td>
<td>0.176</td>
<td></td>
</tr>
<tr>
<td>AHR, 36</td>
<td>CM</td>
<td>20</td>
<td>44</td>
<td>0.299</td>
<td>Demirer &amp; Chen (2005b)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>69</td>
<td>0.255</td>
<td></td>
</tr>
<tr>
<td>CSTR, 35</td>
<td>CM + WM</td>
<td>56</td>
<td>NR</td>
<td>0.21</td>
<td>Comino et al. (2009)</td>
</tr>
<tr>
<td>HTD, 20-25</td>
<td>CM</td>
<td>60</td>
<td>NR</td>
<td>0.32</td>
<td>Ferrer et al. (2011)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>90</td>
<td></td>
<td>0.36</td>
<td></td>
</tr>
<tr>
<td>CSTR, 37</td>
<td>DM</td>
<td>10</td>
<td>38</td>
<td>NR</td>
<td>Rico et al. (2011)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>46</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDT, 27</td>
<td>DM</td>
<td>21</td>
<td>27.9</td>
<td>0.15</td>
<td>Usack et al. (2014)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>36.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDT, 14–35</td>
<td>CM</td>
<td>60</td>
<td>68</td>
<td>NR</td>
<td>Resende et al. (2015)</td>
</tr>
<tr>
<td>PF</td>
<td>CWW</td>
<td>32</td>
<td>40</td>
<td>NR</td>
<td>Mendonça et al. (2017)</td>
</tr>
<tr>
<td>UASB-AF, 37</td>
<td>CWW</td>
<td>6</td>
<td>69</td>
<td>0.27</td>
<td>Present work</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>68.9</td>
<td>0.28</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>53.3</td>
<td>0.36</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>34.4</td>
<td>0.42</td>
<td></td>
</tr>
</tbody>
</table>

CM, cattle manure; WM, whey mix; DM, dairy manure; CWW, cattle wastewater; TPAD, temperature phased anaerobic digestion; CSTR, continuous stirred tank reactor; CSAD, conventional slurry anaerobic digestion; AHR, anaerobic hybrid reactor; HTD, horizontal tubular digester; PF, plug flow reactor; Subs., substrate; VSr (%), volatile solids removal.
related to the low HRT used in this phase. Similar behavior has been reported by other authors. Demirer & Chen (2005a), operating with a conventional anaerobic reactor in a single phase (reactor 1) and another in two phases (reactor 2 – acidogenic and methanogenic), found an increase in ammoniacal nitrogen after digestion in the two operational situations. For the single-phase reactor, increases of 58 and 70% and 20 and 37% (NH\textsubscript{4}\textsuperscript{+}) were observed for HRTs of 20 and 10 days, respectively. For the two-phase reactor, the increases were 22 and 53% (first phase) and 2 and 42% (second phase) for HRT of 10 days. Regarding TKN, the authors detected removal rates up to 34% (R1) and 39% (R2). Demirer & Chen (2005b), using an AHR with HRTs of 10, 15 and 20 days to treat CW, detected an increase in ammoniacal nitrogen only for operation with an HRT of 20 days. The TKN removal efficiencies were between 9 and 36%. Conversion of organic nitrogen (particles, organic molecules, proteins and amino acids) in NH\textsubscript{4} occurred by ammonification during anaerobic digestion.

In relation to phosphate, Castrillón et al. (2002) observed that concentrations of the order of 150 mg L\textsuperscript{-1} in the incoming wastewater were sufficient for adequate operation of a UASB reactor. Although the concentrations recorded in this study (32–45 mg L\textsuperscript{-1}; Table 9) are lower than reported, this did not prevent the development and stability of the process in the reactor. The results obtained indicate that the process can be satisfactorily maintained, even digesting substrates containing lower phosphate concentration ranges.

The analysis of the process in the UASB-AF hybrid reactor indicates that the available phosphorus in the incoming material was used more efficiently in the initial phases of the testing, where removal rates of 65 and 38% were obtained, respectively, with HRTs of 6 and 5 days (phases III and IV). In comparative terms, these values are compatible with those reported in other studies. While Demirer & Chen (2005a) reported similar phosphorus removal rates (61%, R1-conventional reactor, HRT = 2; and 42%, R2-two-phase reactor, HRT = 8), Demirer & Chen (2005b) mentioned lower

### Table 6 | Nitrogen compounds and phosphate

<table>
<thead>
<tr>
<th>Phase</th>
<th>NH\textsubscript{4} mg L\textsuperscript{-1}</th>
<th>NTK mg L\textsuperscript{-1}</th>
<th>N\textsubscript{org} mg L\textsuperscript{-1}</th>
<th>PO\textsubscript{4}\textsuperscript{3-} mg L\textsuperscript{-1}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>In</td>
<td>Ef</td>
<td>In</td>
<td>Ef</td>
</tr>
<tr>
<td>III</td>
<td>560(0.0)</td>
<td>840(0.3)</td>
<td>1,344(0.1)</td>
<td>896(2.5)</td>
</tr>
<tr>
<td>IV</td>
<td>492(0.2)</td>
<td>758(1.2)</td>
<td>1,402(3.2)</td>
<td>852(0.02)</td>
</tr>
<tr>
<td>V</td>
<td>706(1.2)</td>
<td>726(0.06)</td>
<td>1,064(0.6)</td>
<td>1,008(1.2)</td>
</tr>
<tr>
<td>VI</td>
<td>562(1.0)</td>
<td>560(0.2)</td>
<td>1,152(0.7)</td>
<td>1,059(0.1)</td>
</tr>
</tbody>
</table>

In, influent; Ef, effluent.
efficiencies (removal of total phosphorus between 8 and 35%, HRT = 10 and 20 days) than recorded for the UASB-AF hybrid reactor. Subsequently, in phases V and VI the output concentrations increased, by 51 and 60%, respectively.

As mentioned for the other parameters, in the case of phosphate there is also a difference in unit behavior during phases III and IV and the following phases (V and VI). In the first two stages there was a removal of the content available in the administered influents, indicating that the phosphate is used by the microbial population, while in the latter phases there was an accumulation of the component in the medium. This fact does not imply that microorganisms no longer consume, but rather that there is an excessive concentration of phosphate in relation to the needs of the process and that is related to the accumulation of organic material inside the reactor during the experimental time.

Similar to the results for nitrogen, phosphate concentration is a relevant aspect for use of the digested material as an agricultural input in the form of biofertilizer to provide phosphorus as a plant nutrient. However, if the outflow is to be discharged into a water body, it is advisable to perform post-treatment to remove phosphate and nitrogenated compounds.

Behavior of the anaerobic UASB-AF hybrid reactor along the column

The different zones of the UASB-AF hybrid reactor and respective functions and treatment efficiencies are displayed in Figure 3.

The alteration of the values into COD$_t$ along the reactor column, BY function of time, shows an evolution of the unit during the test, with two distinct behavior patterns (Figure 3(a)). In the first 7.5 cm of the column (Figures 1 and 3(a), point P3) no significant differences in behavior were noted during the experimental period, with identical increases in the COD$_t$ concentrations in all phases of the experiment. The increases observed can be attributed to the development of the sludge bed, which as time passed incorporated some particulate organic material with easier biodegradation.

In the next zone (to a height of 31 cm, Figure 3(a), point P2), as expected, the COD$_t$ values were lower than at the previous point. However, the decrease in the COD$_t$ concentration was more pronounced in the first two experimental phases (III and IV) than in the following ones (phases V and VI): 57 and 65% versus 22 and 21%, respectively. When the material undergoing digestion reached the midpoint, at a column height of 41 cm (point P1), there was another decline in the COD$_t$ values, but relatively less accentuated than in phases III and IV than in V and VI, when compared with the values of the previous point (Figure 3(a)). Removal rates near 20 and 15% were noted in the first two experimental phases, where the lowest organic loads were administered (3.8 and 4.6 kg COD$_t$ m$^{-3}$ d$^{-1}$), and about 49 and 51% in the other two phases, when operating with higher loads (7.0 and 10.8 kg COD$_t$ m$^{-3}$ d$^{-1}$).

The results obtained indicate that the UASB-AF hybrid reactor’s performance varies according to the load administered. While on the one hand the base section played a more important role in degrading the substrate when applying low organic loads, the middle zone was more important in determining the degree of treatment when using higher loads. This shows the importance of the means of filling the reactor when applied for anaerobic digestion of substrates with recalcitrant fractions, as observed in the dairy CWW, and in particular when operating at higher loads (>7 kg COD$_t$ m$^{-3}$ d$^{-1}$).

The final segment of the reactor, at the top, did not significantly contribute to alter the concentration of COD$_t$ in the treated material obtained at the outlet, in any of the experimental phases. However, lower loads (phases III and IV) allowed slightly better COD$_t$ removal, of about 8%, in comparison with the result with higher loads (phases V and VI) (Tables 2 and 3). This behavior is associated with the expansion of the sludge bed, which as time passed incorporated the less biodegradable organic material, in response to the increased load. The reactor analyzed in this work is noted for having flexible operation, adjustable to the conditions regarding different substrate concentrations. For lower loads, the middle filling section served only for final polishing of the flow, already substantially degraded, while for higher loads, the middle level acted as a barrier to retention of the sludge blanket and particulate matter included in it, explaining the overall COD$_t$ removal rates higher than 60% under all the operating conditions.

The evaluation of the changes in pH along the column revealed similar behavior during the experimental period to that of COD$_t$ removal. All the initial pH values increased in the first section of the column (point P3, 7.5 cm), for all the operating conditions analyzed, with very similar values (7.4–7.6) regardless of the starting values of the input material (Figure 3(b)). In the next section, until the middle filling point (point P2, 31 cm), no large changes were measured, with all the values being similar (pH ≈ 7.5). In contrast, when the flow undergoing digestion reached the middle zone (point P1), the differences in pH values were substantial between lower and higher loads. In the first case (phases III and IV), the pH values were virtually
identical to those previously recorded and similar to the values obtained in the influent. In the case of operation with higher loads (phases V and VI), the pH declined slightly in the middle zone, and did so more strongly in the final portion, remaining at a neutral value.

The VFAs were almost all consumed in the sections before the middle filling level. In the first section (In-P3, Figure 3(c)), the contribution to total removal was greater than in the next section (P3-P2, Figure 3(c)): 85% versus 68% (III), 84 versus 70% (VI), 74 versus 77% (V) and 72 versus 77% (VI), respectively. The VFA concentration profiles along with stable methane production and yield during the cycles in all the phases indicate that acetogenic and methanogenic reactions occurred appropriately.

**CONCLUSIONS**

The anaerobic UASB-AF hybrid reactor reached the highest biogas productivity levels, 1.20 and 1.40 m$^3$ m$^{-3}$ d$^{-1}$, when applying loads of 7.0 and 10.8 kg COD m$^{-3}$ d$^{-1}$, with HRTs of 3 and 2 days, respectively. Even operating at short HRT, concentrations of CH$_4$ between 69 and 75% were obtained, indicating that none of the phases of the anaerobic digestion process were affected. The pH values were always near neutral and there was no accumulation of volatile organic acids in the system. The organic matter removal with values of 60–81% (COD$_r$) and 51–75% (COD$_d$) allowed methane yields of 0.155–0.183 m$^3$ CH$_4$ kg$^{-1}$ COD$_d$ and 0.401–0.513 m$^3$ CH$_4$ kg$^{-1}$ COD$_d$ to be obtained. VS were removed in 34 to 69%, with corresponding methane yields of 0.27 to 0.42 m$^3$ CH$_4$ kg$^{-1}$ VS$_{removed}$. The removal of COD$_d$ in the reactor’s profile had two distinct behaviors: with higher loads (>7 kg COD$_d$ m$^{-3}$ d$^{-1}$), the AF made a larger contribution than with lower loads (<4.6 kg COD$_d$ m$^{-3}$ d$^{-1}$), and most of the COD$_d$ was removed between the entry and point P2, with the filter only serving to polish the outflow. Because it operates with low HRT, the UASB-AF hybrid reactor provides reduced cost in relation to volume and implantation area.

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