Anaerobic treatment of chocolate-processing industry wastewater at different organic loading rates and temperatures

M. Esparza-Soto, A. Jacobo-López, M. Lucero-Chávez and C. Fall

ABSTRACT

The objective of the present study was to determine the optimum operating temperature of laboratory-scale upflow anaerobic sludge blanket (UASB) reactors during the treatment of a chocolate-processing industry wastewater at medium applied organic loading rates (OLR_{appl}). Four UASB reactors were operated at different temperature (15, 20, 25 and 30 °C) and three OLR_{appl} (2, 4 and 6 kg soluble chemical oxygen demand (COD_S)/(m^3 d)). The flowrate and the hydraulic retention time were constant (11.5 L/d and 6 h, respectively). The monitored parameters were pH, temperature, COD_S, and total and volatile suspended solids. The COD_S removal efficiency (RE) and biogas production rate (BPR) were calculated. The 15 °C UASB reactor had the lowest RE (39 to 78%) due to the low operating temperature. Regardless of the OLR_{appl}, the RE of the 20, 25 and 30 °C reactors was high and similar to each other (between 88 and 94%). The BPR of the four UASB reactors had the same behaviour as the RE (BPR of 15 °C: 0.3 to 0.5 L_{biogas}/($L_{reactor}$ d) (L_b/(L_r d)) and BPR of 20, 25 and 30 °C: 0.5 to 1.9 L_b/(L_r d)).

Key words | biogas production rate, chocolate-processing industry wastewater, temperature, UASB reactor

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INTRODUCTION

Anaerobic reactors have been applied to the removal of organic matter from dilute (<1,000 mg chemical oxygen demand (COD)/L) particulate and soluble wastes such as sewage (Sevilla-Espinosa *et al.* 2010). The use of anaerobic reactors for the treatment of low-strength wastewater, including domestic sewage and industrial effluents, has been definitively established in tropical and sub-tropical regions, where wastewater temperatures are above 20 °C (Foresti 2001; Lettinga *et al.* 2001; Turkdogan-Aydinol *et al.* 2011).

Operational temperature is a major factor for the performance of anaerobic reactors. Thermophilic operation has been pointed out as presenting some advantages over mesophilic operation, namely in terms of substrate degradation rates and biogas production. However, mesophilic reactors present a higher operational stability (Couras *et al.* 2014). Also, the biological reactions responsible for anaerobic biodegradation of organic matter is much slower under psychrophilic (<20 °C) conditions than under mesophilic conditions (Bandara *et al.* 2011).

The operation of high-rate anaerobic reactors under low ambient temperatures, which are typical of domestic wastewater (WW), was for many years considered not to be feasible. Moreover, if low-strength domestic WW is treated anaerobically, the subsequent low biogas production will not be enough to heat the reactor to the usual mesophilic operating temperatures (35 °C). Therefore, anaerobic treatment may not be economically attractive for countries with a cool climate. However, researchers had shown that, with appropriate reactor design and operation, successful low-temperature reactor operation is feasible, and the application base of anaerobic treatment has consequently broadened for high-strength industrial WW (O'Flaherty *et al.* 2006).

The upflow anaerobic sludge blanket (UASB) reactor is a widely used anaerobic WW treatment that has numerous benefits over its alternatives, including aerobic WW treatments and other anaerobic reactors (Dutta *et al.* 2018).

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The main benefits of using UASB reactors are low operating costs, and simple design and construction. Because the UASB reactors can withstand the pH, temperature and influent composition fluctuations, which are common in industrial WW, full-scale UASB reactors have been used to treat a variety of WW since their introduction (Dutta *et al.* 2018).

Moreover, the application of a UASB in the treatment of WW shows higher performance with mesophilic temperature than psychrophilic: Sevilla-Espinosa *et al.* (2010), Bandara *et al.* (2011), Farajzadehha *et al.* (2012) and Lu *et al.* (2015) reported COD removal efficiency between 73 and 97% with mesophilic temperatures.

On the other hand, Rizvi *et al.* (2015), El-Kamah *et al.* (2011) and Esparza-Soto *et al.* (2013) obtained lower COD removal efficiency between 62 and 79% at psychrophilic temperature. Bandara *et al.* (2011) mentioned that several studies have focused on anaerobic treatment with low applied organic loading rates (OLR_{appl}) at lower temperatures, but those studies used domestic and synthetic wastewater. For this reason, the objective of this research was to determine the optimum operating temperature of laboratory-scale UASB reactors during the treatment of chocolate-processing industry (CIP) WW at medium OLR_{appl}.

METHODOLOGY

Four laboratory-scale UASB reactors were built with polymerizing vinyl chloride (PVC) pipes (0.05 m diameter). The UASB reactors had a total height of 1.3 m and an effective volume of 2.8 L (Figure 1). The laboratory-scale UASB reactors were inoculated with anaerobic sludge collected from the secondary lamella settler of a low-temperature pilot-scale UASB reactor (Esparza-Soto *et al.* 2013). The laboratory-scale UASB reactors were operated at constant hydraulic retention time (HRT) (6 h) and three OLR_{appl} (2, 4 and 6 kg soluble chemical oxygen demand (COD_s)/(m^3 d)), which were obtained by diluting the raw CIP WW with tap water. After dilution, sodium bicarbonate ($NaHCO_3$, 2 g/L) and sodium hydroxide (8.5 M NaOH) were added to increase alkalinity and to neutralize the pH,

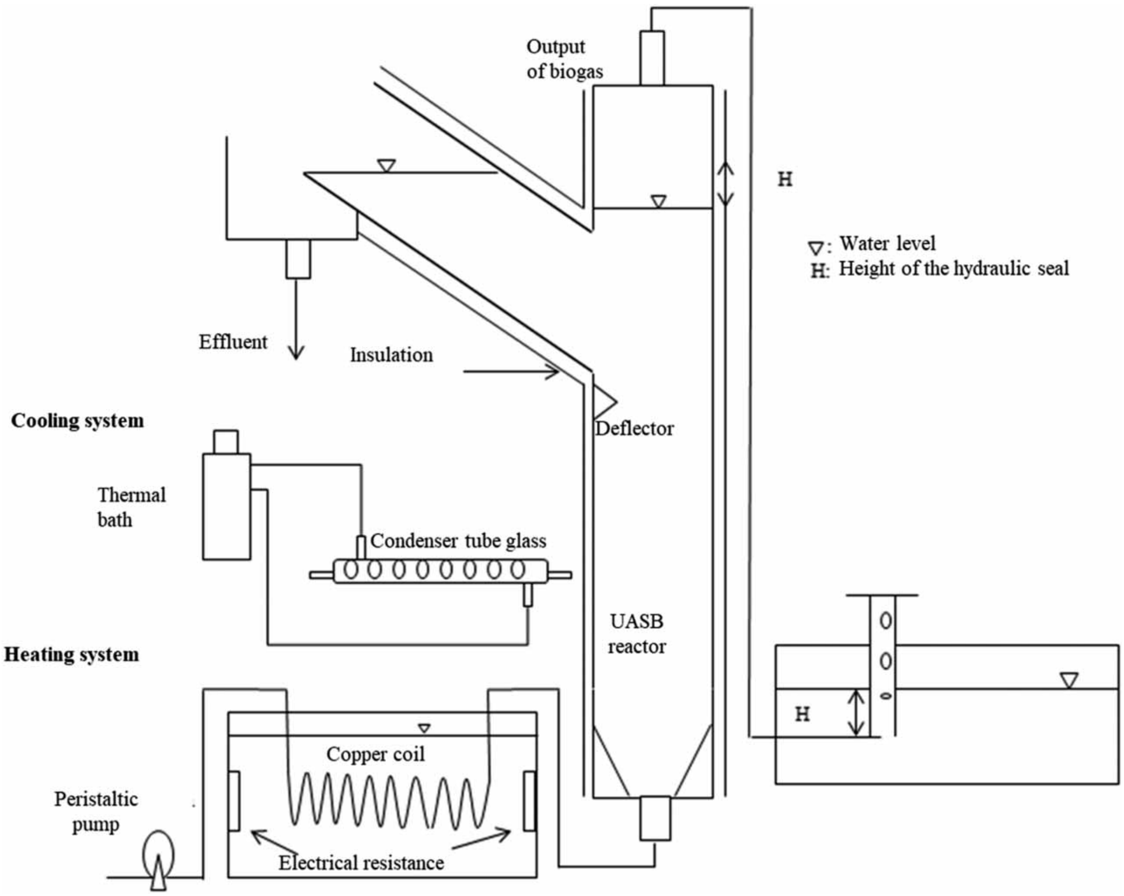

Figure 1 | Scheme of the treatment system of the chocolate-processing industry wastewater.

respectively. The UASB reactors were operated for between 42 and 65 days during each OLR_{appl}. The raw CIP WW was received in the laboratory in 10 m^3 batches every 2 weeks and stored at ambient temperature. The characterization of the raw CIP WW is given in Table 1.

Each UASB reactor was operated at a different temperature (15, 20, 25 and 30 °C). The influent of the 15 and 20 °C UASB reactors was cooled with two immersion thermal baths (Polystat, Cole-Parmer, USA) (Figure 1, cooling system). The influent of the 25 and 30 °C UASB reactors was heated with a 200 W electrical resistance, which was submerged in a 40 L water tank (Figure 1, heating system). The 15 °C UASB reactor was surrounded with silicone tubing (diameter: 0.95 cm, manufacturer: Masterflex) and covered with aluminum foil. The 20, 25 and 30 °C UASB reactors were covered with 0.025 m polystyrene insulation to maintain a constant temperature inside each reactor.

The influent temperature was measured just before entering the UASB reactor by extracting a water sample through a valve. The effluent temperature was measured at the upper part of the UASB reactors, just before leaving the UASB reactor. The temperature, pH, flowrate and daily biogas production were monitored 5 days a week since the beginning of each OLR_{appl}. The daily biogas production (L/d) was measured by the liquid displacement method and corrected for standard temperature and pressure (273.15 K and 1 atm). The biogas production rate (BPR) ($L_{biogas}/(L_{reactor}$ d), $L_b/(L_r$ d)) was calculated by dividing the biogas production by the reactor volume. Once the steady state was reached, the COD_s of the influent and effluent, total suspended solids (TSS) and volatile suspended solids (VSS) of the effluent were determined five times a week. At the end of each OLR_{appl}, the TSS and VSS inside of each reactor were measured. The COD_s, TSS and VSS were analyzed in accordance with *Standard Methods for the Examination of Water and Wastewater* (APHA/AWWA/WEF 2012).

Table 1 | Characterization of raw chocolate-processing industry wastewater

Parameters	Average (n)
pH	4.6 ± 0.5 (7)
Temperature (°C)	21.9 ± 1.3 (7)
COD_T (mg/L)	6,186 ± 1,581 (7)
COD_s (mg/L)	4,624 ± 1,157 (7)
TSS (mg/L)	621 ± 291 (7)
VSS (mg/L)	570 ± 302 (7)
VFA (mg acetic acid/L)	441 ± 206 (7)
Alkalinity (mg $CaCO_3$/L)	521 ± 227 (7)
N-NH_3 (mg/L)	8.7 ± 4 (3)
P_{TOT} (mg/L)	238 ± 67 (3)

n: number of samples, COD_T: total COD, VFA: volatile fatty acid, P_{TOT}: total phosphorus.

The solids residence time (SRT) was calculated with the effluent VSS and the reactor VSS (Equation (1)) (Metcalf & Eddy 2014):

$$SRT = \frac{V_r \cdot X_r}{Q \cdot X_e} \tag{1}$$

where:

V_r: reactor volume (L)
X_r: volatile suspended solids inside the reactor (mg VSS/L)
Q: flow (L/d)
X_e: volatile suspended solids of the effluent (mg VSS/L).

RESULTS

The results of operational parameters of the four UASB reactors are shown in Table 2. The influent and effluent pH was always above 7.0 ± 0.1 in all UASB reactors, which may have prevented inhibition of anaerobic microorganisms inside the reactors. Therefore, the neutral pH may have helped to achieve a stable anaerobic process. Similarly, this pH range has been reported by other researchers such as Li *et al.* (2015), Wang *et al.* (2011) and Turkdogan-Aydinol *et al.* (2011). The temperature oscillated in the range proposed for each UASB reactor (15, 20, 25 and 30 °C), whereas the flowrate was kept as constant as possible to keep the HRT close to 6 h.

The effluent TSS and VSS of the four UASB reactors increased with the OLR_{appl} (Figure 2). However, the effluent TSS and VSS of the 15 °C UASB reactor were always higher than the rest of the reactors, while the 25 °C UASB reactor had lower concentration of effluent TSS and VSS in the three OLR_{appl}. In general, the effluent TSS of the four UASB reactors were less than 200 mg/L (Metcalf & Eddy 2014), whereas the effluent TSS reported by Nacheva *et al.* (2009) and El-Kamah *et al.* (2011) were 154 and 133 mg/L for OLR_{appl} of 4 and 4.7/kg COD/(m^3 d), respectively, which indicated that the TSS were accumulating inside each reactor. The low loss of TSS may have prevented the reactors from emptying and possibly indicated a good granulation inside each reactor (McHugh *et al.* 2003; Abbasi & Abbasi 2012). Therefore, the low concentration of TSS in effluent could have favored the overall performance of the UASB reactors in terms of COD_s removal efficiency (RE)

Table 2 | Results of operational parameters of the four UASB reactors during the OLR_{appl}

Operation period (d)	OLR_{appl} (kg CODs/(m^3 d))	pH Influent	pH Effluent	Effluent temperature (°C)	Flowrate (L/d)	HRT (h)	n
15 °C							
65	2.0 ± 0.3	7.3 ± 0.2	7.6 ± 0.2	16.8 ± 2.4	11.8 ± 0.3	5.7 ± 0.2	22
43	3.6 ± 0.3	7.0 ± 0.2	8.1 ± 0.3	16.7 ± 1.4	10.8 ± 2.8	6.5 ± 1.3	18
52	5.2 ± 0.8	7.0 ± 0.1	7.5 ± 0.3	17.5 ± 2.6	11.0 ± 2.6	6.1 ± 0.5	19
20 °C							
48	2.2 ± 0.4	7.0 ± 0.2	7.7 ± 0.2	20.5 ± 1.2	11.5 ± 2.0	6.0 ± 0.8	25
42	4.3 ± 1.3	7.0 ± 0.2	8.1 ± 0.3	21.8 ± 1.6	11.9 ± 2.8	5.8 ± 0.9	17
52	6.2 ± 1.0	7.0 ± 0.1	7.8 ± 0.5	22.1 ± 1.8	11.9 ± 1.0	5.7 ± 0.5	20
25 °C							
48	2.3 ± 0.6	7.0 ± 0.2	7.9 ± 0.3	24.8 ± 1.1	11.8 ± 1.8	5.8 ± 0.8	22
43	3.9 ± 1.3	7.0 ± 0.2	8.6 ± 0.1	24.9 ± 1.5	10.8 ± 3.0	6.8 ± 2.4	17
52	5.7 ± 0.8	7.0 ± 0.1	7.9 ± 0.5	26.3 ± 1.6	10.9 ± 1.0	6.2 ± 0.8	19
30 °C							
43	2.3 ± 0.9	7.6 ± 0.4	7.9 ± 0.4	28.1 ± 3.0	13.6 ± 3.9	5.3 ± 1.5	34
57	3.6 ± 1.1	7.2 ± 0.3	7.9 ± 0.3	28.9 ± 3.9	11.7 ± 1.3	5.8 ± 0.7	41
48	5.9 ± 1.4	7.0 ± 0.2	8.1 ± 0.2	29.8 ± 2.0	12.2 ± 2.2	5.7 ± 1.4	26

n: number of samples.

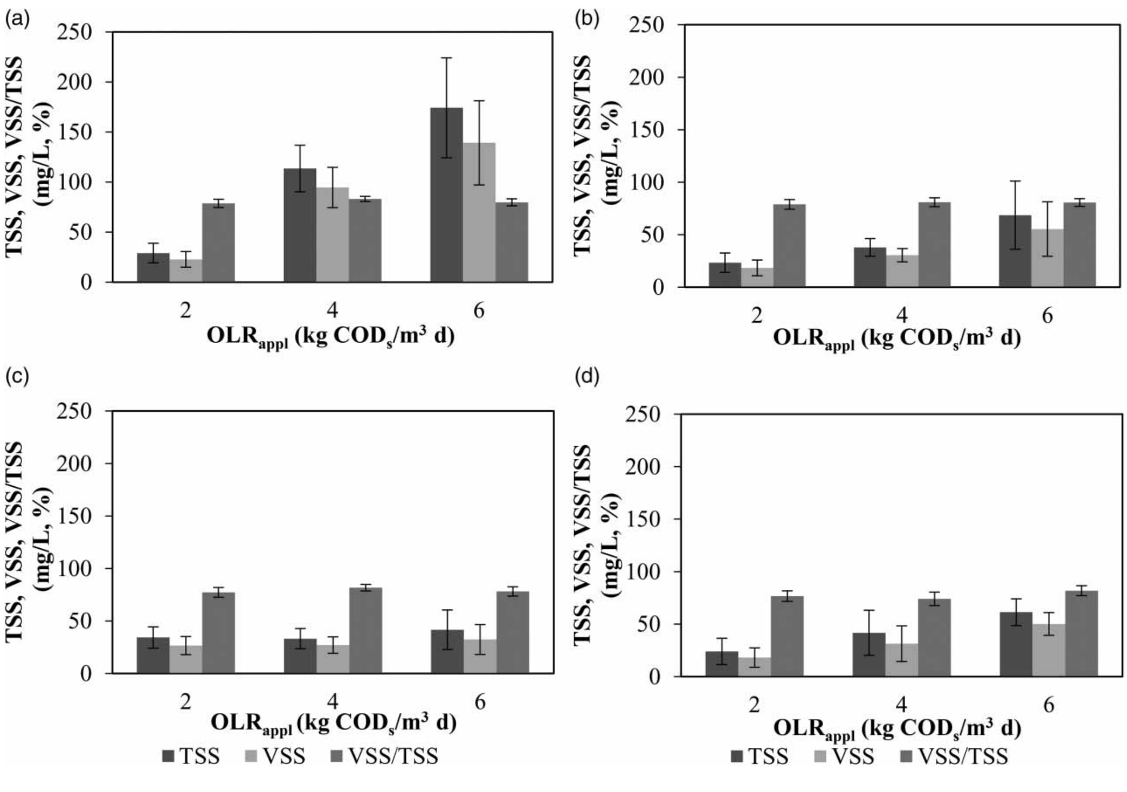

Figure 2 | The total suspended solids (TSS), volatile suspended solids (VSS) and VSS/TSS ratio of the effluent of UASB reactors: (a) 15 °C, (b) 20 °C, (c) 25 °C and (d) 30 °C.

and BPR (McHugh et al. 2003; Abbasi & Abbasi 2012). The effluent VSS/TSS ratio of the UASB reactors was maintained between 74 and 83%. This high VSS/TSS ratio may have indicated that the sludge bed was mostly composed of anaerobic microorganisms and that there was not accumulation of inert suspended solids (Metcalf & Eddy 2014).

The VSS inside the reactors and SRT of the reactors at the four temperatures are shown in Table 3. The VSS inside the reactor does not observe any clear tendency with respect to the OLR_{appl} and temperature except for the 25 °C UASB reactor. The VSS inside the 25 °C UASB reactor increased when OLR_{appl} increased from 3.9 ± 1.3 to 5.7 ± 0.8 kg COD_s/(m^3 d). The increase of VSS inside the 25 °C UASB reactor was possibly due to the low concentration of VSS of effluent.

The SRT of the 15, 20 and 30 °C UASB reactors decreased when the OLR_{appl} (Table 3) and effluent VSS increased (Figure 2), whereas the SRT of the 25 °C reactor practically remained independent of the OLR_{appl}. The SRTs of the 20, 25 and 30 °C UASB reactors are above the minimum recommended value (75 d) (Henze et al. 2008; Metcalf & Eddy 2014). However, the SRT of the 15 °C UASB reactor was above 75 d only during the first OLR_{appl}. This value is the minimum recommended to maintain sufficient methanogenic activity in reactors operated at low temperature (Zeeman & Lettinga 1999). The high measured SRT of the 20, 25 and 30 °C UASB reactors may have allowed their high COD_s RE.

The COD_s (influent, effluent and RE) of the reactors at the four temperatures are shown in Figure 3. The COD_s of influent had fluctuations because a new batch of raw CIP

Table 3 | The volatile suspended solids (VSS) at the beginning and the end of each OLR_{appl} and solids residence time (SRT).

OLR_{appl} (kg COD_s/(m^3 d))	Initial suspended solids (mg VSS/L)	Final suspended solids (mg VSS/L)	SRT (d)
15 °C			
2.0 ± 0.3	7,850	8,431	88
3.6 ± 0.3	17,398	13,492	42
5.2 ± 0.8	11,557	11,760	21
20 °C			
2.2 ± 0.4	16,598	12,929	171
4.3 ± 1.3	12,929	9,435	73
6.2 ± 1.0	9,611	14,493	62
25 °C			
2.3 ± 0.6	16,429	7,080	105
3.9 ± 1.3	7,080	8,462	81
5.7 ± 0.8	9,844	12,310	97
30 °C			
2.3 ± 0.9	17,877	12,700	174
3.6 ± 1.1	12,700	7,990	79
5.9 ± 1.4	7,990	10,029	41

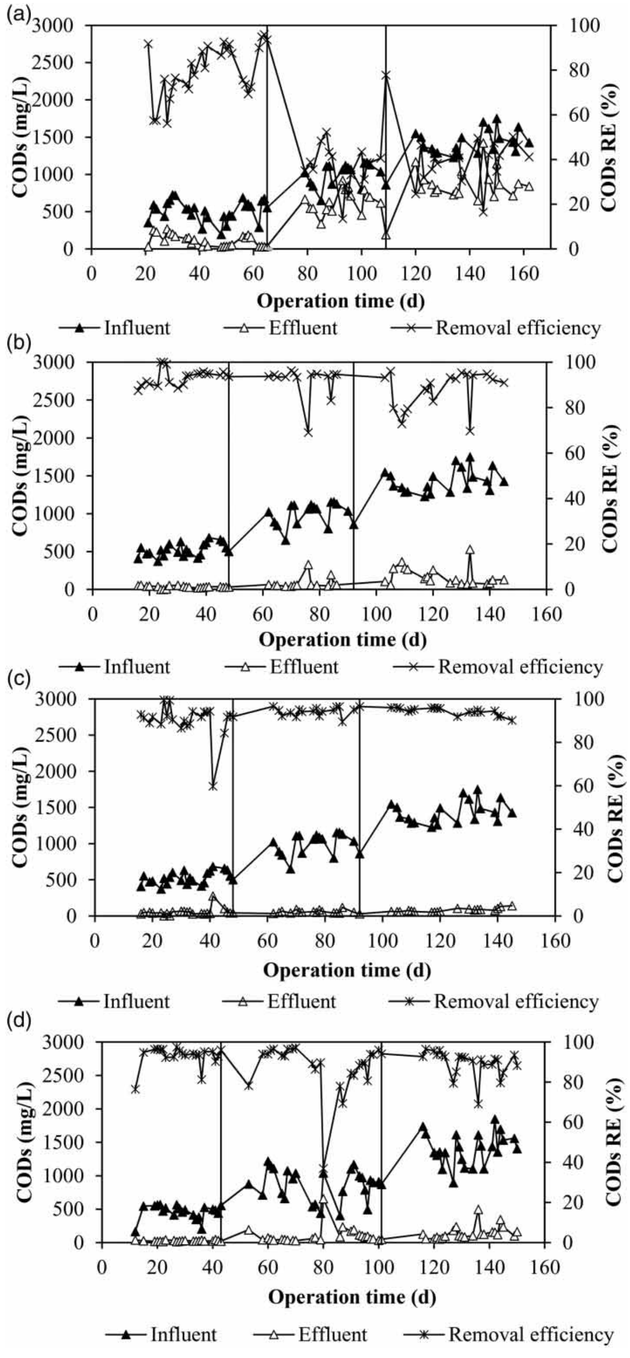

Figure 3 | The COD_s removal efficiency (RE) and influent and effluent COD_s of four laboratory-scale UASB reactors operated at three OLR_{appl} (2, 4 and 6 kg COD_s/(m^3 d)) and different operating temperatures: (a) 15 °C, (b) 20 °C, (c) 25 °C and (d) 30 °C. Vertical lines indicate the transition between OLR_{appl}.

WW was received every 2 weeks and the concentration of raw CIP WW varied significantly between batches. The RE of the 15 °C UASB reactor decreased when the influent COD_s increased (Figure 3(a)). Conversely, the RE of the 20, 25 and 30 °C UASB reactors did not decrease as the influent COD_s increased, but it remained almost constant at approximately 90% (Figure 3(b)–3(d)). The stable effluent COD_s concentration was observed in the 20, 25 and 30 °C UASB reactors even with some fluctuations in influent COD_s concentration, whereas the 15 °C UASB reactor was not so. As discussed above, the low concentration of TSS in effluent and long SRT probably allowed a high and stable RE to be obtained in the 20, 25 and 30 °C UASB reactors (McHugh *et al.* 2003; Abbasi & Abbasi 2012).

The RE of the 15 °C UASB reactor decreased from 78 ± 12% to 39 ± 8.6% as the OLR_{appl} increased from 2 to 6 kg $COD_s/(m^3\,d)$, respectively. This loss of RE as the OLR_{appl} increased may indicate that the best performance of the 15 °C UASB reactor may be at an OLR_{appl} lower than 2 kg $COD_s/(m^3\,d)$, according to the permissible OLR_{appl} for this temperature by Henze *et al.* (2008). Although low, the RE of the 15 °C UASB reactor was superior to that reported by Rizvi *et al.* (2015) and Álvarez *et al.* (2006) with OLR_{appl} of about 2 kg $COD_s/(m^3\,d)$ (Table 4). The higher performance of the 15 °C UASB reactor compared with the literature could be due to the fact that the anaerobic sludge was previously adapted to the type of WW and psychrophilic temperature. This high RE may indicate that the 20, 25 and 30 °C UASB reactors were under-loaded and that they can be operated at higher OLR_{appl} without reducing their RE.

The performance of the 20, 25 and 30 °C UASB reactors was better and comparable to that reported in the literature for reactors operated at similar conditions of OLR_{appl} and temperature (Table 4). For an operating temperature between 20 and 30 °C, the RE was statistically similar (87.7 ± 12.5% to 94.3 ± 2.0%), regardless of the OLR_{appl} (Figure 4). Therefore, the 20 °C UASB reactor was as stable as the 25 and 30 °C reactors, thus saving the energy investment required to increase the operating temperature by 5 and 10 °C, respectively. The anaerobic sludge previously adapted to the low operating temperature and type of industrial WW (CIP WW) perhaps allowed the 20 °C UASB reactor to obtain RE similar to the 25 and 30 °C UASB reactors.

The RE obtained for an OLR_{appl} close to 6 kg COD/ $(m^3\,d)$ at 15, 20 and 25 °C increased as the temperature increased: 39 ± 8.6, 88 ± 8.1 and 94 ± 1.7%, respectively (Figure 4). The RE showed a trend that coincides with that obtained by Lew *et al.* (2004); at an OLR_{appl} of 5 kg COD/ $(m^3\,d)$ at 10, 14, 20 and 28 °C, the RE was 48, 70, 72 and 82%, respectively. Lew *et al.* (2004) indicated that their results suggest a much lower biodegradability at lower temperatures of a range of compounds comprising the heterogeneous composition of domestic WW. The RE in this study was lower than the RE obtained by Lew *et al.* (2004) for the temperature close to 15 °C, probably because CIP WW is more complex than domestic WW.

The BPR of the four UASB reactors had the same behavior as the RE (Figure 5). The BPR of the 15 °C UASB reactor (0.3 to 0.5 $L_b/(L_r\,d)$) was always lower than that of the 20, 25 and 30 °C UASB reactors (0.6 to 1.9 $L_b/(L_r\,d)$), regardless of the OLR_{appl}. The BPR of the 20, 25 and 30 °C UASB reactors increased with the OLR_{appl}, which was expected because more COD_s was removed. On the other hand, it was expected that the BPR of the 30 °C UASB reactor would be higher than the rest of the UASB reactors, but it was similar to the BPR of the 20 and 25 °C UASB reactors (Figure 5). Nevertheless, the BPR of the 30 °C UASB reactor obtained in this study was comparable with Subramanyam & Mishra (2008) (0.95 $L_b/(L_r\,d)$ with OLR_{appl} of 4.6 kg $COD_s/(m^3\,d)$), whereas the BPR of the 20 °C UASB reactor was higher than that reported by Esparza-Soto *et al.* (2013) (0.9, 0.7 and 1.2 $L_b/(L_r\,d)$ at OLR_{appl} of 4.1–7.0 kg $COD_s/(m^3\,d)$). The BPR of the 30 °C UASB reactor was similar to the 20 and 25 °C UASB reactors because the effluent TSS and VSS were low in the three reactors. This could have benefited the BPR.

CONCLUSIONS

The average effluent TSS of the four UASB reactors was less than 200 mg/L. The low effluent TSS of the four UASB reactors could prevent the reactors from emptying and possibly indicated a good granulation inside the UASB reactor. The effluent VSS/TSS ratio of the UASB reactors was maintained between 74 and 83%. This high VSS/TSS ratio may have indicated that there was not accumulation of inert suspended solids.

The RE of the 15 °C UASB reactor decreased from 78 ± 12% to 39 ± 8.6% as the OLR_{appl} increased from 2 to 6 kg $COD_s/(m^3\,d)$, respectively. The 15 °C UASB reactor removed less COD_s than the rest of the UASB reactors. On the other hand, the RE of the 20, 25 and 30 °C UASB reactors did not decrease as the influent COD_s and OLR_{appl} increased, but it remained almost constant at approximately 90%.

Table 4 | Operating conditions and COD removal efficiency in UASB reactors reported in the literature and in this study

Reference	Type of wastewater	Temperature (°C)	OLR_{appl} (kg COD_T/(m^3 d))	Removal efficiency (%)
This study	Industrial	16.8 ± 2.4	2.0 ± 0.3*	79.8 ± 11.8
		16.7 ± 1.4	3.6 ± 0.3*	39.0 ± 13.5
		17.5 ± 2.6	5.2 ± 0.8*	39.0 ± 8.6
		20.5 ± 1.2	2.2 ± 0.4*	94.0 ± 3.0
		21.8 ± 1.6	4.3 ± 1.3*	92.0 ± 7.2
		22.1 ± 1.8	6.2 ± 1.0*	88.0 ± 8.1
		24.8 ± 1.1	2.3 ± 0.6*	90.3 ± 7.5
		24.9 ± 1.5	3.9 ± 1.3*	94.3 ± 2.0
		26.3 ± 1.6	5.7 ± 0.8*	94.3 ± 1.7
		28.1 ± 3.0	2.3 ± 0.9*	93.1 ± 5.1
		28.9 ± 3.9	3.6 ± 1.1*	87.7 ± 12.5
		29.8 ± 2.0	5.9 ± 1.4*	90.0 ± 6.4
Rizvi *et al.* (2015)	Municipal	17	1.9	62 y 57
		20		68 y 61
El-Kamah *et al.* (2011)	Industrial	21	4.7 ± 1.9	56 ± 18
			7.4 ± 2.7	44 ± 15
Álvarez *et al.* (2006)	Municipal	20	1.4	39.4
			1.7	46.6
Tawfik *et al.* (2008)	Dairy and domestic	20	4.5	69
Esparza-Soto *et al.* (2013)	Industrial	18	5.6 ± 0.5*	59 ± 6
			4.1 ± 0.3*	79 ± 5
			7.6 ± 0.6*	78 ± 3
Ghangrekar *et al.* (2005)	Synthetic	24–32	1.5–3.0	93.5–96.5
			3.9–4.9	95.6–95.0
Bandara *et al.* (2011)	Synthetic	25	3.6	85
Farajzadehha *et al.* (2012)	Municipal	30	3.6	73
			4.8	77
			5.8	84
			7.2	85
Lu *et al.* (2015)	Synthetic	35	2*	90
			4*	58
			6*	63

OLR_{appl}: applied organic loading rate; COD_T: total chemical oxygen demand; *asterisk values are given as soluble chemical oxygen demand (COD_S) instead of COD_T; RE: removal efficiency of COD_S.

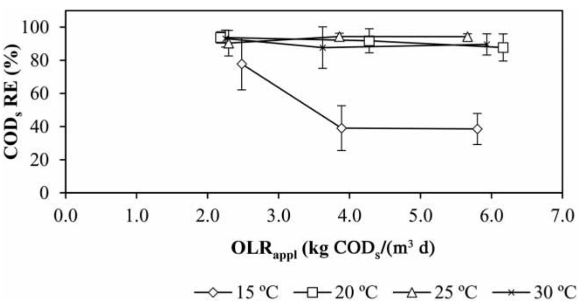

Figure 4 | COD_s removal efficiency (RE) of four reactors by OLR_{appl}.

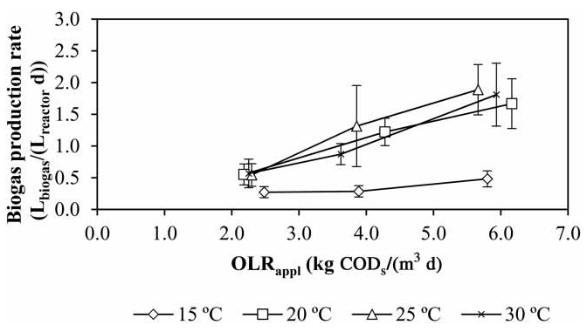

Figure 5 | Average biogas production rate of the four UASB reactors operated at three OLR_{appl}.

The RE of the present study showed that the 20 °C UASB reactor was comparable to the 25 and 30 °C UASB reactors for CIP WW with OLR_{appl} up to 6 kg COD_s/(m^3 d) without the need of an external source of energy for heating the reactor. The performance of the

20 °C UASB reactor was possibly because the anaerobic sludge was previously adapted to the psychrophilic temperature.

The BPR of the four UASB reactors had the same behavior as the RE. The BPR of the 15 °C UASB reactor (0.3 to 0.5 $L_b/(L_r\ d)$) was always lower than that of the 20, 25 and 30 °C UASB reactors (0.6 to 1.9 $L_b/(L_r\ d)$), regardless of the OLR_{appl}.

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