





Original Research Paper

Anaerobic treatment of agro-industrial wastewaters for COD removal in expanded granular sludge bed bioreactor

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HIGHLIGHTS

wastewaters

respectively.

GRAPHICAL ABSTRACT



ARTICLE INFO

ABSTRACT

Untreated agro-industrial wastewaters are undesirable in the aquatic environment due to the presence of high organic matter contents. However, they may constitute a large potential for biogas production. The present investigation is focused on three laboratory-scale anaerobic expanded granular sludge bed (EGSB) bioreactors, continuously operated for 60 d under mesophilic condition with the aim of exploring the feasibility of treating three most significant agro-industrial wastewaters in Chiapas, Mexico (i.e., cheese whey, vinasse, and coffee-processing wastewater). The EGSB bioreactors were operated with a hydraulic retention time (HRT) of 6 d under stable conditions (i.e., buffer index (BI) of 0.31, 0.34, and 0.03), generating a maximum chemical oxygen demand (COD) removal efficiency of 91, 74, and 96% with an average methane production of 340, 245, and 300 mL/g COD d for cheese whey, vinasse, and coffee processing wastewater, respectively. According to the obtained results, the EGSB bioreactors could be a sustainable alternative to simultaneously solve the environmental problems and to produce bioenergy.

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1. Introduction

Agro-industries such as cheese factories, distilleries, and coffee plantations represent one of the most important economic activities in Chiapas, Mexico. These industries process agricultural raw and livestock materials generating millions of tons of wastewater and large amounts of by-products, which are much unexploited and in some cases dangerous for the environment (Dareioti et al., 2009). These production facilities are usually scattered throughout the countryside, and the raw materials are processed at different seasonal rates causing significant variations in both quantity and quality of the wastewaters generated during the year.

The most important waste stream of the cheese factories is cheese whey. The characteristics of cheese whey effluents may vary significantly, depending on the final products, system type, and operation methods used in the manufacturing plant (Prazeres et al., 2012). The distillery factories also generate a wastewater called vinasse, containing a variety of organic substances whose composition and characteristics may vary, depending on the feedstock and the process used for distillate production (Robles-González, 2011; Robles-González et al., 2012). The coffee-processing farms generate the coffee-processing wastewater, containing organic matters like pectin, proteins, and sugars (Von Enden, 2002). This wastewater can be further reused for depulping of the same day products, but this causes further increase of its organic content and a decrease in pH (Adams and Dougan, 1987).

It has been well-documented that the wastewaters generated by the abovementioned agro-industries introduce a high concentration of organic pollutants into the environment (De Matos et al., 2001; EPA, 2003; MoEF, 2003; Robles-González et al., 2012). In fact, they could pose serious threats to the surrounding water bodies, aquatic life, and human health if discharged directly into surface waters like rivers, streams or lakes (Haddis and Devi, 2008). Anaerobic digestion in expanded granular sludge bed (EGSB) bioreactors has been proposed as a feasible low cost technology to treat high strength wastewaters, while biogas production from renewable resources could also be targeted (Zhang et al., 2007; Shin et al., 2010).

Therefore, this investigation was focused on simultaneous anaerobic treatment and biogas production from the most significant agro-industrial wastewaters largely found in Chiapas, Mexico, i.e., cheese whey, vinasse, and coffee-processing wastewater using EGSB bioreactors

2. Materials and methods

2.1. Characterization of agro-industrial wastewaters

Cheese whey was obtained from a cheese-processing factory, whilst vinasse and coffee processing wastewater were obtained from a plant of ethyl alcohol and coffee farm, respectively. The samples were stored at -20 °C until used.

The characteristics of the agro-industrial wastewaters such as pH, chemical oxygen demand (COD), biochemical oxygen demand (BOD₅), sedimented solids, total solids (TS), total volatile solids (TVS), total suspended solids (TSS), floating matter, conductivity, color, turbidity, acidity, alkalinity, total nitrogen, total phosphorus, and sulfates were analyzed according to the Standard Methods for Examination of Water and Wastewater (APHA, 2005). The biodegradability index was calculated according to the formula BOD₅/COD (Abdalla and Hammam, 2014) and the total organic carbon (TOC) was determined by the method proposed by Walkley and Black (2006). Additionally, density and viscosity were measured using a viscometer (Anton Paar SVM 300) in triplicate.

2.2. Inoculum

Granular anaerobic inoculum was obtained from a full-scale upflow anaerobic sludge bed (UASB) bioreactor located at the wastewater plant in Tuxtla Gutiérrez, Chiapas, Mexico. The inoculum was regular and spherical in shape (\emptyset , c. 0.5–1mm) and in a grey-green color with TS and TVS contents of 49.75 g/L and 29.5 g/L, respectively.

2.3. Bioreactors set up and operation

The EGSB bioreactors (Fig. 1a) were made of fiberglass with a 3.3 L working volume, a 15.8 height/diameter ratio, and were inoculated with 1000 mL (30% of bioreactors working volume) of the mesophilic inoculum. All the

three bioreactors, R₁ (Cheese Whey), R₂ (Vinasse), and R₃ (Coffeeprocessing wastewater) were operated at a hydraulic retention time (HRT) of 6 d, but different organic loading rates (OLR) of 7.5 kg COD/m³d (R₁), 5.8 kg COD/m³d (R₂), and 3 kg COD/m³d (R₃) were applied. The bioreactors were automatically fed by a peristaltic pump (Master Flex model 7534-04) and the influents were buffered with NaOH and NaHCO₃.

2.4. Effluent analyses

Samples from bioreactors effluents were routinely taken for COD, pH, and temperature measurement according to the Standard Methods for Examination of Water and Wastewater (APHA, 2005). The alkalinity factor was adapted from Speece (1996) according to following procedure: 10 mL of sample was taken and was acidified to a pH of 5.75 and the amount of HCl (0.1M) required was recorded (V₁), corresponding to the part alkalinity (PA). Subsequently, the sample was brought to pH 4.3 and the amount of acid required (V₂) was recorded again, corresponding to intermediate alkalinity (IA) and the total alkalinity (TA) was determined as the sum of both (TA=PA+IA). The buffer index (BI) was then calculated by using the following Equation 1:

$$BI = \frac{IA}{TA} Eq. 1$$

Methane production was measured by the Mariotte bottle technique described by Cruz-Salomón et al. (2017), This technique involves the absorption of CO_2 present in biogas, using an alkaline liquid (3% NaOH solution) with phenolphthalein as indicator. In order to determine *in situ* composition of the biogas produced (methane and carbon dioxide), the same technique but with a solution NaCl (3%) and phenolphthalein, coupled to the equipment Sewerin model Multitec® 540 (Fig. 1b) was used (Cruz-Salomón et al., 2017).



Fig.1. (a) Schematic diagram of the EGSB bioreactor; and (b) Scheme for measuring biogas composition.

3. Results and Discussion

3.1. Physicochemical characterization of wastewaters

The results of the physicochemical analysis of the agro-industrial wastewaters are tabulated in **Table 1**. It is evident that the investigated wastewaters were heavily polluted with organic loads, nutrients, and suspended matters. Organic load was measured as COD and BOD whilst nutrients were measured as phosphate and nitrate contents.

Table 1.

Physicochemical characteristics of the investigated agro-industrial wastewaters.

Parameter	Cheese whey	Vinasse	Coffee-processing wastewater	
pH	4.44	4.53	3.95	
Color (Pt-Co)	9366.7	68700.3	17966.7	
Turbidity (NTU)	416.3	1745.7	1481.7	
Density (g/mL)	1.1512	1.1667	1.1075	
Conductivity (mS/cm)	8.57	15.47	5.21	
Viscosity (mPa.s)	0.9869	1.1301	1.0904	
Floating matter	Р	NP	NP	
Acidity (mg CaCO ₃ /L)	3313	4975	3360	
COD (mgO ₂ /L)	91600	71605	45955	
BOD (mgO ₂ /L)	90083	54861	37944	
Biodegradability index	0.98	0.76	0.82	
Sedimented solids (mL/L)	15	43	380	
TS (g/L)	47.617	64.889	19.593	
TVS (g/L)	44.702	49.82	8.208	
TOC (mg/L)	33400	40400	11400	
Total phosphorus (mg/L)	707.43	110.41	36.43	
Total nitrogen (mg/L)	2200	1600	700	
C/N	15:1	25:1	16:1	
Total sulfate (mg/L)	17	360	10	

Comparing the values of pH, BOD, COD, total solids, phosphate, and nitrate for the cheese whey, vinasse, and coffee-processing with the respective permissible limits for effluent discharge set forth by the World Health Organization (WHO) (WHO, 1995), it was found that the values recorded for all these parameters were at concerning levels. These agro-industrial wastewaters can lead to several serious environmental pollution impacts like anoxia, eutrophication, death of aquatic life, and many severe health problems, if directly discharged into the water bodies without prior treatment (De Matos et al., 2001; Janczukowicz et al., 2008; Robles-González et al., 2012).

It is known that the organic matters contained in agro-industrial wastewaters can be removed by anaerobic digestion provided that they have specific ratios of nutrients (i.e., $15.1 \ge C/N \le 30:1$) and high biodegradability index (i.e., >0.3). When the biodegradability index is less than 0.3, the substrate is not suitable to carry out anaerobic digestion because it does not have enough biodegradable organic matters (Aguirre, 2004). The biodegradability index calculated were 0.98, 0.76, and 0.82 for cheese whey, vinasse and coffee-processing, respectively, marking these agro-industrial wastewaters are excellent substrates to be treated by anaerobic digestion.

The efficiency and stability of treatment depends mostly on the composition (C/N) and the nature of the used agro-industrial wastewaters (Sitorus et al., 2013). High C/N ratio is an indication of a rapid consumption of nitrogen by the microbial population involved in the process and may lead to a slow microbial growth due to subsequent nitrogen deprivation. On the other hand,

low C/N ratios could cause ammonia accumulation, occurrence of pH values exceeding 8.5, which are toxic to microbial complexes, and consequently decreased efficiency of organic matter removal (Yen and Brune, 2007; Sitorus et al., 2013). As presented in Table 1, the C/N ratios were 25:1, 15:1, and 16:1 for vinase, cheese whey and coffee-processing, respectively. Hence, for all wastewaters investigated, this parameter was also within the optimal level for anaerobic digestion.

Another important variable to be taken into account for the anaerobic treatment of agro-industrial wastewater is pH, as this may affect the efficiency of bioreactors. The optimal pH is between 5.5 and 6.5 for acidogenic and between 7.8 and 8.2 for methanogenic phases. Therefore, the overall optimal pH range for microbial anaerobic populations is between 6.8 and 7.4 (Mao et al., 2015) and outside this range, the microbial populations could be inhibited. In this study, the pH values of the agro-industrial wastewaters were not optimum for anaerobic digestion; and therefore, it was necessary to neutralize them with NaOH or NaHCO₃ before feeding into the bioreactors. Overall and according to their physicochemical profile, the studied agro-industrial wastewaters could be regarded as acceptable candidates for anaerobic treatment using EGSB bioreactors.

3.2. COD removal

 R_1, R_2 and R_3 EGSB bioreactors were operated over 60 d under mesophilic condition. The average temperature of the influents was 21.3 ± 0.5 °C. Figure 2 shows the temperature profile of the effluents leaving the EGSB bioreactors.



Fig.2. Variations in the temperature of the effluents leaving the EGSB bioreactors. The values shown are the average values with their standard deviations.

The pH profile in the EGSB bioreactors is presented in Figure 3. Although the pH of the influents was adjusted at 7 using NaOH, it can be observed that in the first 10 d, the EGSB bioreactors suffered from acidification, but when NaHCO₃ was added instead of NaOH, the pH of the bioreactors approached favourable conditions for anaerobic bacteria as reported by Mao et al. (2015).



Fig.3. Variations in the pH of the effluents of the EGSB bioreactors. The values shown are the average values with their standard deviations.

The pH in the anaerobic bioreactors is a very important factor for the optimal functioning of the system. Maintaining pH in an appropriate range depends on the buffering capacity of the reactor, i.e., the present alkalinity as well as the concentration of volatile fatty acids (VFAs). The accumulation of VFAs could result in stress conditions in anaerobic processes; thus, it is necessary to avoid their accumulation and the resultant decrease in pH, which could destabilize the system (Martin-Gonzalez et al., 2013).

A simple way to investigate the conditions in an anaerobic system is through determining the alkalinity supplied by the carbonate/bicarbonate groups as non-protonated forms of VFAs (Londoño and Peñuela, 2015). In this study, the BI was monitored by obtaining the TA, PA, and the IA. The first term is the amount of alkalinity provided by the VFAs and the carbonate/bicarbonate. The second term refers to the alkalinity provided only by the carbonate/bicarbonate. The last term is the difference between TA and PA, is related only to alkalinity provided by VFAs (Björnsson et al., 2001). Figure 4 shows the behavior of these parameters during the processing time. The alkalinity analysis was based on the IA/TA ratio, where the recommended range is 0.2-0.4 to prevent unstable conditions in the system by VFAs accumulation (Rojas, 2004; Pérez and Torres, 2008).



Fig.4. Behavior of the alkalinity factor during the operation of the EGSB bioreactors.

On the other hand, the IA/TA ratio also depends on the type of wastewater, and to achieve a stable process, it needs to be analysed. The IA/TA ratio in the R_1 and R_2 stood at 0.31 and 0.34, respectively, revealing stable conditions, similar to the values previously reported by Cruz-Salomón et al. (2017). However, this ratio was recorded at lower than 0.2 for the R_3 (i.e., average values of 0.03), indicating a potential interference with the buffering capacity of the bicarbonates due to possible VFAs accumulation (Martin-Gonzalez et al., 2013). Nevertheless, the pH value in the R_3 was maintained between 6.5 and 8.0, which showed that no acidification occurred. If pH is over 7, the majority of the VFAs are in anionic form as the pK of VFAs is in the range 4.8-5.0 but when the buffer index is close to zero, then neither anionic nor neutral forms are present. Therefore, a very low buffer index value is indicative of a balanced system without VFA accumulation.

The anaerobic treatment performance in the EGSB bioreactors was determined by monitoring COD removal rates (Figure 5). A dividing line is presented separating the two stages of operation of the bioreactor. The influents were buffered with NaOH and NaHCO₃ in the first and second stages, respectively. As shown in Figure 3, the bioreactors R_1 and R_2 tended to acidification, but when they were buffered with NaHCO₃ in the second stage, an increase in pH and better stability of the bioreactors were observed which also led to a more favourable removal of organic matters after 10 d.

Figure 5a presents the trend of organic matter removal efficiency in the R_1 bioreactor. More specifically, it can be observed that at the beginning, COD removal efficiency decreased due to the acidification caused by higher VFAs production rate (by acetogenic bacteria) than their consumption rate (by the methanogenic archaea). Gutiérrez et al. (1991) have also made similar observations and alkaline supplementation during the start-up period or during the process has been recommended as a strategy to amend the reactor condition (Rodgers et al., 2004). Following buffering with NaHCO₃, a fast pH recovery and increased COD removal were observed. On average, the COD removal efficiency stood at as high as 91%, with the organic matter concentration of the effluent reaching 4105 mg/L.

The R_2 bioreactor was operated in a stable and efficient manner throughout the evaluation period (with suing both NaOH and NaHCO₃), reaching a COD removal rate of 70% (Figure 5b). The organic matter concentration of the effluent was recorded at 8683 mg/L. The low removal rate shows that although the EGSB bioreactor was successful in dealing with the degradable fraction of organic matter in vinasse, it failed to to remove the recalcitrant compounds fraction (e.g., brown polymers also known as melanoidins, etc.). Nevertheless, the removal efficiency obtained in this study was slightly higher than the values reported by Qinglin et al. (2012).

Figure 5(c) presents the trend of organic matter removal efficiency in the R_3 bioreactor. This bioreactor was also operated in a stable and efficient manner throughout the evaluation period, reaching removal rates as high as 96%; with the concentration of organic matter in the effluent reaching as low as 694 mg/L. To the best of our knowledge, this is the first report on digesting coffee-processing wastewater using this type of bioreactor, i.e., EGSB.



Fig.5. Process performance of the EGSB bioreactors, treating agro-industrial wastewaters at mesophilic range (a) R₁, (b) R₂, (c) R₃.

Data concerning the other parameters, analyzed immediately after the bioreactors reached a balanced condition, are shown in Table 2.

Overall, the results obtained showed that the bioreactors were operated properly and resulted in high removal efficiency rates. Nevertheless, the effluents generated still contained a large amount of biodegradable organic matter and nutrients necessitating a second anaerobic treatment, advanced oxidation processes, percolating filters or biodiscs to achieve complete removal of biologically-recalcitrant components and to meet the permissible limits by the WHO for discharging such effluents.

3.3. Methane production

Over the 60 d experiment, methane production was measured at 340, 245, and 300 mL/gCOD d for cheese whey, vinasse, and coffee-processing, respectively. Methane production rates showed statistically significant differences (p<0.05) among different agro-industrial wastewaters. These can be observed in the 24 h kinetics shown in Figure 6.

Table 2.

General analysis of the influent and effluent of the bioreactors.

Parameters	C	Cheese whey (R ₁)		Vinasse (R ₂)			Coffee-processing wastewater (R ₃)		
	Influent	Effluent	E (%)	Influent	Effluent	E (%)	Influent	Effluent	E (%)
COD (mg O ₂ /L)	45213	4105	91	34609	8683	74	19855	694	96
BOD ₅ (mg O ₂ /L)	34101.1	4280	87	26302.8	4550	82	14760	620	95
TS (g/L)	19.558	5.66	71	32.445	12.48	62	10.795	1.46	86
Color (Pt-Co)	4680	2547	46	35333	26366	25	8033	1180	85
Turbidity (NTU)	277	121.4	56	721	430	40	677.6	67.5	90
Phosphorus (mg/L)	240.6	294	-	35.2	46.1	-	18.4	32.3	-
Nitrogen (mg/L)	1090.8	138.3	87	701.7	75.8	89	310.1	18.9	94

E (%) = Removal Efficiency



 ${\bf Fig.6.}$ Methane production in the EGSB bioreactors. The values shown are the media average with their standard deviations.

The higher production of methane from cheese whey could be attributed to the fact that this waste stream is a highly biodegradable substrate as reflected in its biodegradability index presented in **Table 1**. However, the bioreactor fed with cheese when tended to acidify, and therefore, it was necessary to adjust the condition with a buffer solution (e.g., NaHCO₃) for better operation. On the other hand, since the bioreactors fed with vinasse and coffee processing, operated under stable condition (no acidification) throughout the experiment, the lower methane production from these waste streams could be justified by their less biodegradable organic matter contents (lower biodegradability index).

The biogas produced by the R_1 , R_2 , R_3 bioreactors contained 63.5, 70.8, and 80.3% of methane, generating calorific values of 22.80, 25.46, and 28.84 MJ/m³, respectively (determined at normal conditions). Therefore, since biogas with metahne concentration greater than 45% is flammable, these products can be considered of fuel value. Accordingly, these agro-industrial wastewaters hold great potentials for bioenergy production, shifting the paradigm of wastewater management from 'treatment and disposal' to 'treatment and beneficial utilization'.

4. Conclusions

EGSB bioreactors can be a sustainable alternative to solve the environmental problems generated by agro-industrial wastewater. In the present study, COD removal efficiency 91% for R₁ (Cheese Whey), 74% for R₂ (Vinasse), and 96% for R₃ (Coffee-processing wastewater) were achieved. Moreover, this bioreactor type was also proved to be a sustainable alternative for bioenergy production; 340, 245, and 300 mL CH₄/gCOD d with a calorific value of 22.80, 25.46, and 28.84 MJ/m³ from R₁, R₂, and R₃ waste streams, respectively.

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