



**Acidogenesis/methanogenesis from acid cheese whey in hybrid-UASB reactors**  
**Acidogénesis/metanogénesis de lactosuero ácido en reactores UASB-híbridos**

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### Abstract

The cheese industry produces residues such as acid cheese whey. The latter has a high organic-matter concentration and other nutrients that are severe environmental contaminants. The aim of this study was to evaluate the acidogenesis/methanogenesis of acid cheese whey with short Hydraulic Retention Times (HRT) in Hybrid-UASB reactors, following the evolution of the organic matter, ammonia, and orthophosphate. The acidogenic reactor was operated at an HRT of 0.5 days, while for the methanogenic reactor, this ranged from 1-2 days; both reactors were operated in series. Results showed that the overall HRT of 1.5 days allowed optimal removal (89-96.8%) with organic loading rates of between 2.5 and 10.7 g COD/L-d, while at 14.2-24.9 g COD/L-d, best results were achieved at 2.5 days of overall HRT. Highest methane productivity ranged from 0.99-1.15 L<sub>CH<sub>4</sub></sub>/L<sub>reactor-d</sub> (0.238-0.308 L<sub>CH<sub>4</sub></sub>/g COD<sub>degraded</sub>). Ammonia formation was only significant in methanogenesis (85 mg/L), while orthophosphates showed no change throughout the entire process. In conclusion, Hybrid-UASB reactors operated at short HRT were suitable for the acidogenesis/methanogenesis of this wastewater. Furthermore, methane can be utilized for energy generation, and ammonia and orthophosphate might be employed for the development of high value-added products.

**Keywords:** Acid cheese whey, Acidogenesis/methanogenesis, Hybrid-UASB reactor, industrial effluents, nutrients.

### Resumen

La industria quesera produce residuos como el lactosuero ácido. Este tiene una alta concentración de materia orgánica y otros nutrientes que contaminan al ambiente fuertemente. El objetivo del estudio fue evaluar la acidogénesis/metanogénesis de lactosuero ácido a Tiempos de Retención Hidráulicos (TRH) cortos en reactores UASB-híbridos, siguiendo la evolución de materia orgánica, amonio y ortofosfatos. El reactor acidogénico se operó a un TRH de 0.5 días y el reactor metanogénico de 1-2 días; ambos se operaron en serie. Los resultados muestran que un TRH global de 1.5 días permitió una remoción óptima (89-96.8%) a una carga orgánica volumétrica de 2.5 a 10.7 g DQO/L-d; mientras que de 14.2-24.9 g DQO/L-d los mejores resultados se encontraron a un TRH global de 2.5 días. La mayor productividad de metano fluctuó de 0.99-1.15 L<sub>CH<sub>4</sub></sub>/L<sub>Reactor-d</sub> (0.238-0.308 L<sub>CH<sub>4</sub></sub>/g DQO<sub>degradado</sub>). La formación de amonio únicamente fue significativa en metanogénesis (85 mg/L); los ortofosfatos no mostraron cambio a través de todo el proceso. En conclusión los reactores UASB-híbridos operados a cortos TRH fueron adecuados para la acidogénesis/metanogénesis del agua residual ensayada. Además, el metano puede ser usado para la generación de energía; el amonio y ortofosfatos podrían emplearse para el desarrollo de productos de alto valor agregado.

**Palabras clave:** Lactosuero ácido, acidogénesis/metanogénesis, reactor UASB-híbrido, efluentes industriales, nutrientes.

## 1 Introduction

Cheese whey is an effluent generated after the precipitation and removal of milk casein during cheese

manufacturing (Gelegenis *et al.*, 2007). The process employed for inducing casein precipitation leads to two whey types. The first type is produced when proteolytic enzymes such as chymosin are used; it is called sweet whey and its pH ranges from 6-7.

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The second whey type is known as acid whey; usually, its pH is less than 5. It is generated by different methods, among these, by means of the lactic acid produced during milk fermentation, as well by the addition of organic acids (citric or lactic acid), or by the addition of diluted solutions of mineral acids (Carvalho *et al.*, 2013; Ryan and Walsh, 2016; Badem and Uçar, 2017). Cheese whey is employed as raw material for the manufacture of different products, such as beverages, sausages, yogurt, butter, confectionery items, protein concentrates, crystallized lactose, and others (González-Siso, 1996; Göblös *et al.*, 2008; Ryan and Walsh, 2016; Badem and Uçar, 2017). Despite the multiple uses of whey, approximately 47% of the 115 million tons generated annually worldwide (Kavacik and Topaloglu, 2010) and approximately one million tons produced in Mexico annually (Valencia and Ramírez, 2009) are released into soils, sewers, or bodies of water without receiving treatment, causing serious pollution problems (Gannoun *et al.*, 2008; Ryan and Walsh, 2016; Escalante *et al.*, 2018).

As several studies have pointed out, pollution by cheese whey is not only due to the volumes generated, but also to its physicochemical properties, for example, its high organic-matter concentration, i.e., 50-102 g/L as COD, and 40-60 g/L as BOD (Saddoud *et al.*, 2007; Gannoun *et al.*, 2008; Venetsaneas *et al.*, 2009; Diamantis *et al.*, 2014; Calero *et al.*, 2018a), with the major contributors being lactose (45-50 g/L), soluble proteins (6-8 g/L), and lipids (4-5 g/L) (Chatzipaschali and Stamatis, 2012). This effluent, moreover, contains nutrients such as non-protein nitrogen and phosphorus, as well as mineral salts (Venetsaneas *et al.*, 2009; Saddoud *et al.*, 2007).

Anaerobic digestion has been widely employed for the treatment of effluents with high organic content, e.g., distillery, from wet coffee processing, leachate from composting and landfills, and vinasses in co-digestion with agave bagasse (Sunil *et al.*, 2007; Guardia *et al.*, 2014; Castilla-Hernández *et al.*, 2016; Alzate-Ibanez, 2018; Gómez-Guerrero *et al.*, 2019), with the advantage of low sludge production and potential for energy generation (Jo *et al.*, 2016). Furthermore, anaerobic digestion requires less space (Demirel and Yenigün, 2002), lower operating costs, and the process is simpler compared with other treatment methods (Saddoud *et al.*, 2007). Thus, anaerobic digestion is an excellent alternative for the treatment of cheese whey.

Single-phase anaerobic digestion is carried out within the same physical space and several microbial groups are involved, which, in the absence of oxygen and through several stages (hydrolysis, acidogenesis, acetogenesis, and methanogenesis) degrade polluting organic matter (O'Flaherty *et al.*, 2006), having as end products mainly methane (CH<sub>4</sub>) and carbon dioxide (CO<sub>2</sub>). Therefore, in this syntrophic chain cohabitate microorganism producers of Volatile Fatty Acids (VFA), i.e., acidogenic phase, and the acetic acid/methane producers, i.e., methanogenic phase. Single-phase anaerobic digestion has been utilized for the treatment of cheese whey. However, one of the greatest problems encountered in achieving a successful treatment is the fast acidification of the process (Kalyuzhnyi, 1997), due to the high acidogenic-biomass growth rate, which leads to the accumulation of VFA the principal products of their metabolism. Acidification is intensified due to the low bicarbonate alkalinity in cheese whey (~50 meq/L) (Mockaitis *et al.*, 2006; Gannoun *et al.*, 2008; Diamantis *et al.*, 2014). These aspects exert a negative impact on methanogenesis; because methanogenic archaea are sensitive to low pH environments, and develop better within a range of 6.5-8.2 (Calero *et al.*, 2018b). Another consequence is the loss of the inoculum, due to the difficulty of forming granular sludge (Kalyuzhnyi *et al.*, 1997; Carvalho *et al.*, 2013), generating effluents with a high content of suspended solids, consequently reducing treatment effectiveness (Diamantis *et al.*, 2014).

The anaerobic digestion process in two-phases (acidogenesis/methanogenesis) comprises an alternative to obtain a better treatment. In this configuration, each phase is carried out in independent physical spaces, permitting microorganisms to degrade higher organic loads, diminishing acidification in the methanogenic phase (Saddoud *et al.*, 2007; Göblös *et al.*, 2008) and improving the stability and effectiveness of the overall process. With all this, there remain several aspects that must be addressed, such as treatment at high organic loading rates (i.e., shorter HRT), or treatment systems that may aid in reducing inoculum loss.

Hybrid-UASB reactors are the result of two technologies coupling in a same system: an Upflow Anaerobic Sludge Blanket (UASB) reactor in a lower compartment and an Anaerobic Filter (AF) in an upper compartment (Shivayogimath and Ramanujam, 1999). This strengthens the treatment, improving the characteristics of the high-rate reactor, and promoting biomass fixation and the retention of solids.

According to the packing material utilized in the AF (polyethylene, polypropylene, polyvinyl chloride, zeolite), it is possible to achieve additional benefits. Zeolite has been considered a good filtering medium and has been employed for the removal of ammonium from wastewater (Montalvo *et al.*, 2012). Bearing all of this in mind, this type of reactors can be an option for the treatment of cheese whey.

In this context, the aim of the present study was to evaluate acid cheese whey acidogenesis/methanogenesis using short HRT, in Hybrid-UASB reactors, principally following the organic-matter degradation and the evolution of nutrients such as ammonium and orthophosphate.

## 2 Materials and methods

### 2.1 Origin and composition of cheese whey

Fresh acid whey was collected at a local cheese factory in Tlaxcala, Mexico. Its composition in g/L was the following: COD, 85.4±15.9; BOD<sub>5</sub>, 52.9±10.0; Total Solids (TS), 60.5±15.2; Total Suspended Solids (TSS), 11.2±1.1; fat and oils, 3.6±1.0; total nitrogen, 1.3±0.5; ammonium (NH<sub>4</sub><sup>+</sup>), 0.06±0.03; soluble protein, 5.8±2.6; total phosphorus, 1.2±0.4, and orthophosphate (PO<sub>4</sub><sup>-3</sup>), 1.1±0.3.

### 2.2 Reactors configuration and start-up

Two Hybrid-UASB reactors were installed, including an Acidogenic Reactor (AR) and a Methanogenic Reactor (MR) (Figure 1). Working volume was 2.7 and 4.3 L, respectively. Both were packed with zeolite on top, with a particle diameter ranging from 0.5-13 mm (average, 9 mm). The zeolite was mainly used to promote biomass fixation and the retention of solids, although it can contribute to the removal of the ammonium due to its ion exchange property. The AR and MR were inoculated with 600 and 960 mL, respectively, of anaerobic sludge containing 28.2 g/L of Volatile Suspended Solids (VSS). The inoculum was obtained from an UASB reactor utilized in treating the municipal wastewater of the UAM-Iztapalapa Campus.

The AR was fed with cheese whey at 1.25 g COD/L, pH 4.5, and 0.5 day of HRT during 54 days to select and enrich acidogenic microorganisms. The MR was fed with cheese whey at the same COD level and time period as those of AR, but the pH was adjusted to 7.0 with a 1N solution of sodium

hydroxide (NaOH); the influent was supplemented with 2 g/L of sodium bicarbonate (NaHCO<sub>3</sub>) to improve alkalinity. Mockaitis *et al.* (2006) started their experiments with a concentration of NaHCO<sub>3</sub> equal to the COD concentration fed; however, we added an additional 60% with the purpose of guaranteeing optimal conditions to methanogens. The reactor was operated at 1 day of HRT; on day 50, the specific methanogenic activity of the inoculum was 0.047 L<sub>CH<sub>4</sub></sub>/gVSS-d. During the 54 days, the reactors were operated in parallel.

### 2.3 Acidogenesis/methanogenesis monitoring

To evaluate the acidogenesis/methanogenesis process, both reactors were monitored through analysis of the following: COD; VFA, including acetic, propionic, *i*-butyric, butyric, *i*-valeric, valeric and caproic acids; alcohols (ROH): ethanol and butanol; pH, and CH<sub>4</sub> production. Additionally, NH<sub>4</sub><sup>+</sup> and PO<sub>4</sub><sup>-3</sup> evolution was determined.

COD conversion efficiency into products such as VFA and ROH (also expressed as COD) in the AR was determined using Equation 1. Product rate formation (*rp*) was estimated according to Equation 2 as previously reported by Demirel and Yenigun (2004), where the Products are defined as the sum of VFA and ROH in the effluent or in the influent of the AR, and these are also expressed as COD. For both reactors, removal efficiency and ammonium production were calculated as shown in Equations 3 and 4.

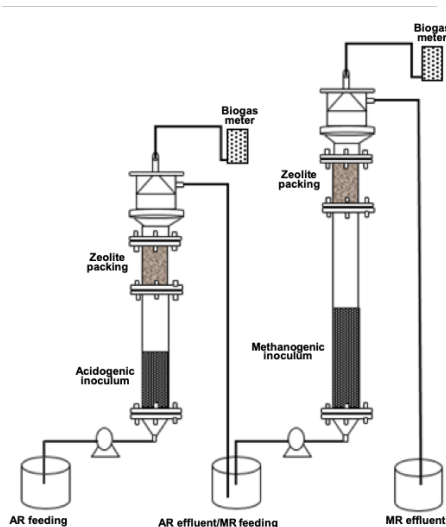


Fig. 1. Scheme of the Acidogenic (AR) and Methanogenic (MR) Hybrid-UASB reactors.

$$COD\ Conversion\ efficiency\ (\%) = \left[ \frac{VFA_{produced}(g/L) + ROH_{produced}(g/L)}{COD_{fed}(g/L)} \right] \quad (1)$$

$$rp(gCOD_{products}/L-d) = \frac{Products_{effluent}(g/L) - Products_{influent}(g/L)}{HRT(h)} \times 24(h/d) \quad (2)$$

$$Removal\ efficiency(\%) = \left[ \frac{COD_{influent}(g/L) - COD_{effluent}(g/L)}{COD_{influent}(g/L)} \right] \times 100 \quad (3)$$

$$NH_4^+ produced (mg/L) = NH_4^+_{effluent}(mg/L) - NH_4^+_{influent}(mg/L) \quad (4)$$

## 2.4 Analytical techniques

Total COD, TS, TSS,  $NH_4^+$ , and  $PO_4^{3-}$  were analyzed according to Standard Methods for the Examination of Water and Wastewater (APHA-AWWA-WEF, 2005). The pH was measured with a Model PC18 Conductronic potentiometer. VFA and ROH quantification was carried out by gas chromatography in a PerkinElmer Clarus 580 GC, utilizing an AT-1000 column and a FID detector. Biogas production was quantified by the displacement of a saturated saline solution and its composition, by means of a Carbowax column and TCD detector in a GOW-MAC Chromatograph. Methane values are reported under STP conditions.

## 3 Results and discussion

### 3.1 Acidogenesis

Evolution of the organic matter during acidogenesis/methanogenesis from acid cheese whey is depicted in Figure 2. The AR was fed at concentrations ranging from 1.2-12.4 g COD/L, which were converted at 37.9 to 49.6% into products such as VFA and ROH (Table 1). Cheese whey due to its fermentable organic content is reported as a good substrate for obtaining VFA (Calero *et al.*, 2018a). The conversion or acidification levels found in this work were near 52.2%, maximal percentage reached at 1 day of HRT in a two-phase treatment coupled to a microfiltration membrane system (Saddoud *et al.*, 2007), and was similar to the 56% reported for a dairy effluent supplemented with nutrients and treated at 0.5 day of HRT and an Organic Loading Rate (OLR) of 9.3 g COD/L-d (Demirel and Yenigun, 2004).

Regarding product rate formation ( $rp$ ), high OLR stimulate VFA production, because supply favorable conditions for acid-forming bacteria

(Demirel and Yenigun, 2004). Figure 3 shows the Pearson correlation analysis ( $r = 0.9926$ ) between OLR and  $rp$ ; it is observed that, when OLR increased,  $rp$  is also favored, reaching 11.2 g COD<sub>product</sub>/L-d (6.49 g product/L-d), exceeding by 2.09-fold the results reported by these authors. Also, the high correlation value could indicate that the acidogenesis process possessed the capacity for the conversion of higher organic loads.

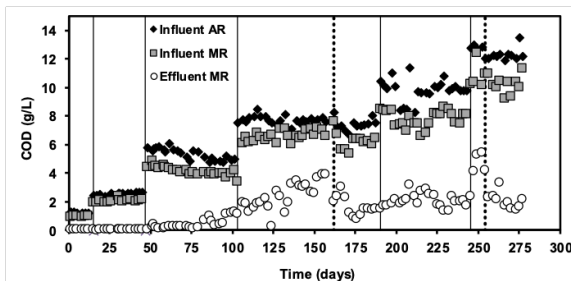


Fig. 2. Organic matter profile during the acidogenic/methanogenic processes. The solid lines indicate an increase in the organic-matter concentration, and the dotted lines, an HRT increase in the MR. Acidogenic Reactor (AR); Methanogenic Reactor (MR).

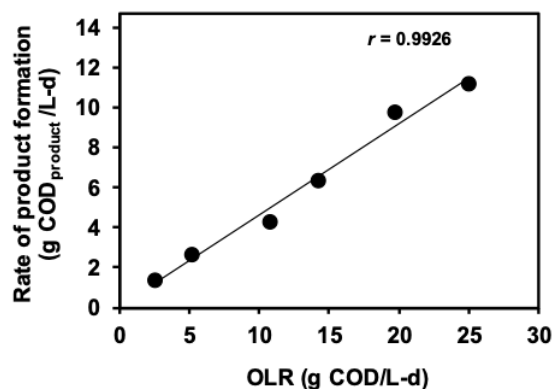


Fig. 3. Increase of Organic Loading Rate (OLR) vs. the rate of product formation ( $rp$ ), during cheese whey acidogenesis.

Table 1. Acidogenic reactor operating condition, COD conversion, and removal efficiency. 0.5 day of HRT.

Time (days)	COD (g/L)	ORL (g COD/L-d)	COD conversion (%)	COD removal efficiency (%)
12-Jan	1.2±0.01	2.5±0.02	48.3±3.9	21.8±3.3
13-45	2.5±0.1	5.1±0.2	49.3±10.1	19.2±3.6
46-101	5.3±0.4	10.7±0.8	37.9±11.9	21.6±4.3
102-187	7.6±0.3	14.2±2.4	43.8±11.5	15.9±8.3
188-243	9.8±0.7	19.7±1.5	49.6±7.0	20.1±5.7
245-277	12.4±0.4	24.9±0.9	45.1±6.9	16.1±6.6

The OLR reported for whey treatment usually ranges from 8.0-22.2 g COD/L-d (Yu and Fang, 2002; Demirel and Yenigun, 2004; Saddoud *et al.*, 2007). Also, using an acidogenic UASB reactor, organic loading rates up to 49.5 g COD/L-d were assayed, but best patterns were found from 15-17 g COD/L-d (Calero *et al.*, 2018b). In the present study, it is probable that the pH control during the feeding to maintain the level above 4.0, along with the configuration of this type of reactor, such as the filter in the upper compartment that aids in avoiding biomass washout and provides a greater surface for its attachment, contributed to that the Hybrid-UASB reactor was able to attain 24.9 g COD/L-d.

The products formed are presented in Figure 4. At all stages, VFA were the major by-products in comparison with ROH, attaining 94.2%. This can be explained because the solventogenesis, that is to say, the production of alcohols and ketones, takes place only under specific conditions, such as high hydrogen partial pressure (Valdez-Vazquez and Poggi-Varaldo, 2009).

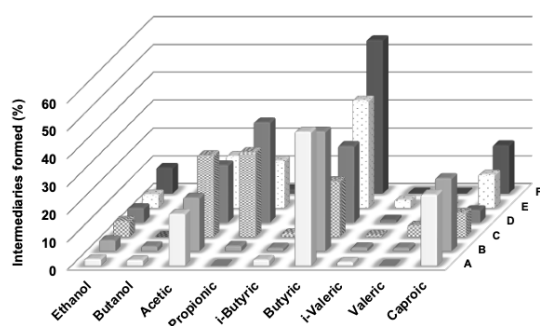


Fig. 4. Percentage of products formed in the acidogenic reactor. Letters in ascending form represent from lowest to highest OLR fed (g COD/L-d): A = 2.5; B = 5.1; C = 10.7; D = 14.2; E = 19.7; F = 24.9.

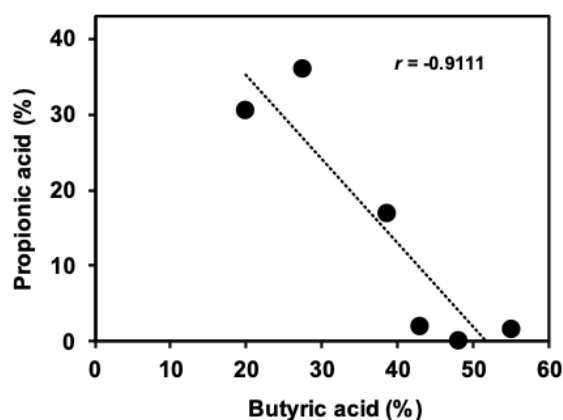


Fig. 5. Pearson correlation between the percentages of propionic acid and butyric acid percentages found in the acidogenic reactor.

In this case, the ethanol formed corresponded to 5.2%, in contrast with that of 10-12% previously reported in another work (Yu and Fang, 2002). Butanol was found at trace concentrations (0.8%).

Among VFA, acetic acid exhibited the most stable formation (20.1±4.9%). Butyric acid was found at a higher proportion (mean, 38.7%), reaching 55.2% in the last OLR fed. Likewise, these results are similar to those reported for whey acidogenesis at pH 5 (Venetsaneas *et al.*, 2009). Butyric acid can be subsequently degraded by acetogenesis and afterward by methanogenesis.

For its part, propionic acid appeared in a considerable proportion from the third OLR fed, increasing to 16.9-35.9%. This acid can derive from lactate degradation that, at your time, is a product of the lactose fermentation. Similar to other studies (Cohen *et al.*, 1984; Calero *et al.*, 2018a), Figure 5 presents a high and inverse correlation between propionic and butyric acids. Reduction in the production of propionic acid takes place

precisely when butyric acid increases, which is favorable because high concentrations of propionic acid could inhibit methanogenesis (Wang *et al.*, 2009). Furthermore, propionic acid showed no significant correlation with acetic acid ( $r = 0.661$ ) due to the accumulation of propionic acid in organic loading rates ranging from 10.7 to 19.7 g COD/L-d, because the acetogenesis of this acid must occur more favorably in the next phase.

Caproic acid was found in  $15.7 \pm 9.0\%$  and might derive from protein fermentation (Yu and Fang, 2002). Also, its occurrence could derive from a chain elongation of ethanol or lactate and acetate, through the reverse  $\beta$ -oxidation pathway (Spirito *et al.*, 2014). This would also perhaps explain the lesser ethanol proportion determined. *i*-Butyric, valeric, and *i*-valeric acids were formed at proportions lower than 3.3

Organic-matter removal efficiency ranged from 15.9-21.8% (Table 1), and was in agreement with that reported in acidogenesis (Saddoud *et al.*, 2007), because acidogenesis consists mainly of COD transformation into available products for the next phase.

### 3.2 Methanogenesis and the overall process

The MR was fed with effluent from the AR at an organic-matter concentration ranging from 1.0-10.5 g COD/L (Figure 2 and Table 2). In this case, in the first two OLR fed, methanogenesis revealed excellent COD removal efficiency, which was reflexed in the overall acidogenic/methanogenic process, reaching around 96% (Figure 6). In the third OLR applied, at the end of this period, slight instability was observed, although removal efficiency remained at acceptable levels, with a mean of 89%.

This level exceeds the 78.4% reported for a similar OLR (Diamantis *et al.*, 2014). Furthermore, and also with a two-phase treatment at 12.8 g COD/L-d, a removal of 68% was attained, which improved (to 80%) when cheese whey previously fermented with *Kluyveromyces lactis* was treated (Göblös *et al.*, 2008). One-way analysis of variance (ANOVA) showed significant differences among the means of the removal efficiencies ( $F = 28.9080$ ;  $p < 0.05$ ), but no statistically significant differences were observed with the multiple comparison Tukey test among the first three ( $p > 0.05$ ), indicating that this OLR can be an option for the treatment.

A further OLR increase affected the removal capacity of the methanogenic consortium; thus, influent alkalinity was augmented by the addition of  $\text{NaHCO}_3$  (3 g/L), since low bicarbonate alkalinity has been reported as a limitation in the treatment of these effluents (Gannoun *et al.*, 2008; Diamantis *et al.*, 2014). This strategy exerted little impact on the recovery of the reactor performance. The acetic and butyric acids probably did not exert an effect during this stage, since these were fed at low concentrations, i.e., 612.3 and 479.4 mg/L, respectively, compared with Wang *et al.* (2009), who reported that at 1,600 mg/L of acetic acid and 1,800 mg/L of butyric acid they reached maximal methane production. For its part, propionic acid could have caused the instability, since the concentration fed was 754 mg/L, which is nearly 900 mg/L, the latter reported by these same authors as a strongly inhibitory concentration for the methanogens. Nevertheless, an increase of HRT from 1 to 1.5 days (first dotted line in Figure 2) led to the rapid recovery of removal efficiency, attaining up to 80%, which was attributed to a greater time to metabolize intermediary products by microorganisms.

Table 2. Methanogenic reactor operating condition, overall COD removal, and methane productivity.

Time (days)	HRT (d)	COD (g/L)		COD OLR (g COD/L-d)	removal efficiency (%)	CH <sub>4</sub> (L/d)	CH <sub>4</sub> (L/L <sub>reactor</sub> -d)	SST (mg/L)
		Influent	Effluent					
12-Jan	1	1.0±0.03	0.05±0.01	1.0±0.01	94.4±1.1	1.08±0.03	0.25±0.01	28.5±4.2
13-45	1	2.1±0.1	0.1±0.02	2.1±0.01	96.8±0.9	2.07±0.82	0.48±0.19	14.9±3.5
46-101	1	4.2±0.3	0.4±0.3	4.2±0.3	89.0±9.1	4.96±1.58	1.15±0.37	97±0.2
102-187	1 - 1.5	6.4±0.6	2.0±1.2	5.1±0.8	66.7±13.8	3.27±0.79	0.76±0.18	129.6±2.7
188-243	1.5	7.8±0.6	2.1±0.5	5.2±0.4	73.1±7.4	4.27±0.81	0.99±0.19	149.3±2.8
245-277	1.5 - 2	10.5±0.6	3.2±0.8	6.0±0.3	73.4±11.8	3.95±0.69	0.92±0.16	175.7±2.4

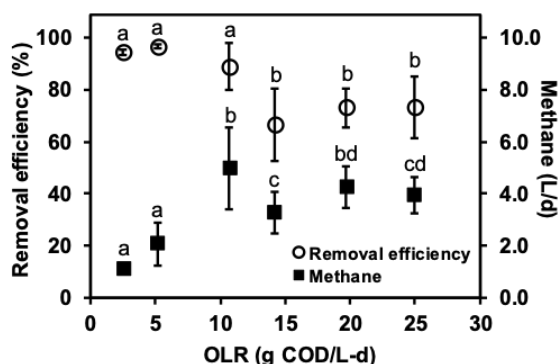


Fig. 6. Organic Loading Rate (OLR) vs. removal efficiency and methane production, in overall treatment. The bars are presenting the Standard deviation. Different lowercase letters on the bars indicate significant differences in Tukey multiple comparison tests.

On day 245, removal efficiency decreased to 60% in response to a new increase in organic matter; therefore, the HRT was established at 2 days (second dotted line in Figure 2), exhibiting a speedy recovery that attained up to 85%, with a mean value for this period of  $73.4 \pm 11.8\%$  (Figure 6). Statistical analysis showed that removal efficiency was similar at OLR above 14.2 g COD/L-d (Tukey,  $p > 0.05$ ); therefore, this HRT was positioned as suitable for the overall treatment of OLR at  $24.9 \pm 0.9$  g COD/L-d.

With regard to methane, its highest production ranged from 3.9-4.9 L/d, equivalent to productivities of 0.92 and 1.15  $L_{CH_4}/L_{reactor-d}$ , reaching similar results between the OLR of 10.7 and 19.7 g COD/L-d (Tukey,  $p > 0.05$ ), followed by those reached at 24.9 g COD/L-d. Productivity fell within the 0.4-1.06  $L_{CH_4}/L_{reactor-d}$  reported for these effluents in a two-phase treatment. In addition, we found similar yields (0.238-0.308  $L_{CH_4}/g$  COD<sub>degraded</sub>) to those reported in a previous work (Diamantis *et al.*, 2014). With pretreated whey, better productivity has been found, 0.42-2.03  $L_{CH_4}/L_{reactor-d}$ , but with low yields, 0.197-0.276  $L_{CH_4}/g$  COD<sub>degraded</sub> (Göblös *et al.*, 2008). In our study, from the methane yield, 3.24 to 4.19 kW-h per kg COD<sub>degraded</sub> could be obtained, that is comparable to achieved by Castilla-Hernández *et al.* (2016) from compost leachates treatment in a two-phase system.

The treated effluent presented a TSS concentration ranging from 14.9 to 175.7 mg/L (Table 2), with a reduction of between 82 and 96%, because these were retained by the zeolite bed. A low content

of solids is a valuable characteristic for the reuse or final disposition of the treated effluent. For the organic-matter degradation of this type of wastewater, the different operational strategies revealed that an overall HRT of 1.5 days is optimal for the acidogenesis/methanogenesis at OLR from 2.5 to 10.7 g COD/L-d in the studied treatment systems, whereas for higher OLR (14.2 to 24.9 g COD/L-d), an overall HRT of 2.5 days is suggested. Both HRT allow for the establishment of compact treatment systems that might be installed in small cheese factories, which generally have limited spaces and insufficient economic resources, for instance those localized in some milk basins in Mexico (Poméon *et al.*, 2006). Furthermore, it is reported that more than 80% of the milk produced in developing countries derives from small producers (Escalante *et al.*, 2018). The necessary dilution of cheese whey for the treatment could be carried out with the plant's washing water as reported by Diamantis *et al.* (2014). An additional benefit of the treatment is the methane generated, which the producers might employ for the production of energy to support part of their requirements.

### 3.3 Ammonium and orthophosphate in acidogenesis/methanogenesis

Cheese whey can contain important protein concentrations, ranging from 2-10 g/L; thus, it is advisable to know the content of the by-products deriving from its degradation, such as ammonium (Göblös *et al.*, 2008). In acidogenesis, the ammonification process was limited, obtaining concentrations from  $2.8 \pm 4.0$ - $16.57$  mg  $NH_4^+/L$ . This could be attributable to that low levels of pH diminish protein solubility and reduce enzymatic activity (Yu and Fang, 2002). Additionally, it has been mentioned that high sugar concentrations, such as those present in these effluents, inactivate proteolytic enzymes (Venkata-Mohan *et al.*, 2008). The contrary took place in methanogenesis in which ammonium increased markedly during all OLR assayed ( $r = 0.9882$ ), attaining up to  $85.0 \pm 6.7$  mg/L (Figure 7a). In this case, the ammonium might derive from protein ammonification. Moreover, in both reactors it was observed that the zeolite exerted no effect on ammonium removal; it is probable that the biomass retained in this support impeded ionic interchange with the nutrient. Thus, an option for the utilization of ammonium was studied (Sánchez-Cárdenas *et al.*, 2017).

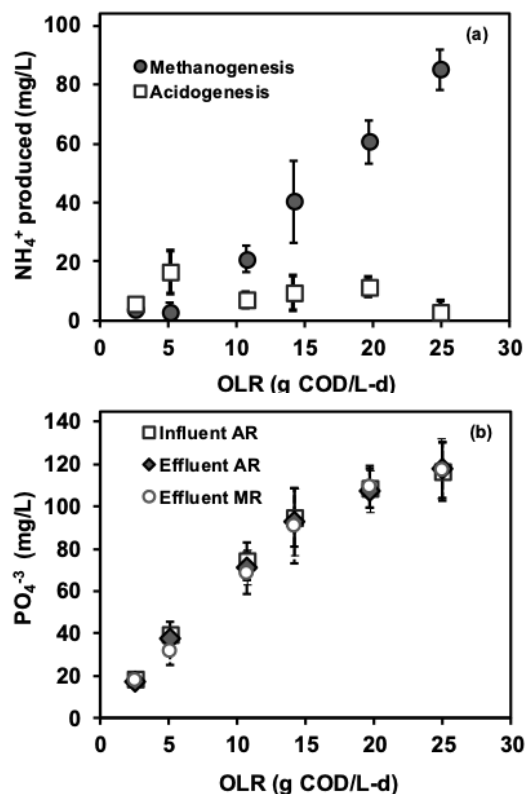


Fig. 7. Production of ammonium (a), and profile of orthophosphate (b) during the increase of OLR in the acidogenic/methanogenic process.

With respect to the orthophosphate pattern during acidogenesis, this increased in proportion to the rise in the OLR ( $8.2 \pm 0.6$  to  $116.8 \pm 13.7$  mg PO<sub>4</sub><sup>3-</sup>/L). Contrary to that found in Hybrid-UASB reactors treating dairy effluents (Rajesh *et al.*, 2007), in neither of the two phases did we observe the formation of orthophosphate (Figure 7b). This is attributed to that the organic substances that make up the raw cheese whey are mostly phosphate-free.

Due to that the ammonium and orthophosphate could promote eutrophication in receiving waters, it is advisable to search alternatives for their use, with the purpose of rendering to this an added value and obtaining a sustainable process. In this regard, the ammonium and orthophosphate of this treated effluent were beneficial for the production of microalgal biomass, such as *Coelastrella* sp., addressed to the generation of biodiesel precursors (Sánchez-Cárdenas *et al.*, 2017). Furthermore, both nutrients might be utilized for the recovery of struvite (MgNH<sub>4</sub>PO<sub>4</sub>·6H<sub>2</sub>O), which can be employed as a slow-release fertilizer (Stolzenburg *et al.*, 2015).

## Conclusions

Adequate COD conversion efficiencies were obtained during acidogenesis and high OLR stimulated VFAs production, with the major products being acetic, propionic, butyric, and caproic acids. The Hybrid-UASB reactors operated to short overall HRT of 1.5 and 2.5 days were optimal for acid cheese whey degradation in the acidogenic/methanogenic process, and the HRT may be set according to the OLR to be treated. Methane production was suitable and can potentially be utilized for energy generation. Furthermore, the low solids contained in the treated effluent is beneficial for its reutilization or final disposition. As for nutrients, ammonium formation was only significant in methanogenesis; orthophosphate exhibited no change throughout acidogenesis/methanogenesis. These nutrients may be employed for the generation of high value-added products.

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