Performance and stability of an expanded granular sludge bed reactor modified with zeolite addition subjected to step increases of organic loading rate (OLR) and to organic shock load (OSL)

T. Pérez-Pérez, I. Pereda-Reyes, E. Pozzi, D. Oliva-Merencio and M. Zaiat

ABSTRACT

This paper shows the effect of organic shock loads (OSLs) on the anaerobic digestion (AD) of synthetic swine wastewater using an expanded granular sludge bed (EGSB) reactor modified with zeolite. Two reactors (R1 and R2), each with an effective volume of 3.04 L, were operated for 180 days at a controlled temperature of 30 °C and hydraulic retention time of 12 h. In the case of R2, 120 g of zeolite was added. The reactors were operated with an up-flow velocity of 6 m/h. The evolution of pH, total Kjeldahl nitrogen, chemical oxygen demand (COD) and volatile fatty acids (VFAs) was monitored during the AD process with OSL and increases in the organic loading rate (OLR). In addition, the microbial composition and changes in the structure of the bacterial and archaeal communities were assessed. The principal results demonstrate that the presence of zeolite in an EGSB reactor provides a more stable process at higher OLRs and after applying OSL, based on both COD and VFA accumulation, which presented with significant differences compared to the control. Denaturing gradient gel electrophoresis band profiles indicated differences in the populations of *Bacteria* and *Archaea* between the R1 and R2 reactors, attributed to the presence of zeolite.

Key words | anaerobic digestion, denaturing gradient gel electrophoresis (DGGE), expanded granular sludge bed (EGSB) reactor, organic shock load (OSL), volatile fatty acids (VFAs), zeolite

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INTRODUCTION

Pig meat production has increased significantly at large scale in recent decades. Therefore, the resulting high-strength wastewater, which is produced in large amounts, and its disposal in small areas demands an easy and environmentally friendly technology. Anaerobic digestion (AD) offers not only a technical solution but also an environmental solution to this problem (Pereda-Reyes *et al.* 2015). AD is an efficient and feasible approach because it relies on bioenergy and produces biofertilizer.

Among the high-rate technologies for high-strength wastewater treatment, the expanded granular sludge bed (EGSB) reactor, a modification of the up-flow anaerobic sludge bed reactor (UASB), seems to meet the needs of large-scale industries that produce pig meat. Compared to the UASB, the EGSB reactor operates at higher up-flow velocities due to the recirculation of effluent, and it is designed at a greater height/diameter ratio of 20 (López & Borzacconi 2011). The EGSB reactor was developed to have increased biomass to substrate contact and reduced dead zones, preferential fluxes and shortcuts (Fuentes *et al.* 2011). Lately, the attention directed toward the EGSB has increased due to its ability to operate at higher organic loading rates (OLRs) while still favoring the hydrodynamics of EGSB reactors (Puñal *et al.* 2003). As a UASB, EGSB reactors are based on the ability of microorganisms to form aggregates by self-immobilization.

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EGSBs have been studied for the efficient treatment of diverse effluents (Chen *et al.* 2014; Xu *et al.* 2014) but less so for swine wastewater treatment (López-Fernández *et al.* 2011; Lee & Han 2012). As is very well known, AD can suddenly fail due to the accumulation of volatile fatty acids (VFAs) and other important parameters (Pagés-Díaz *et al.* 2015). However, zeolite has been widely used as microorganism support material in fixed and fluidized bed configurations for biological processes in order to retain a high active biomass concentration with a significant decrease in the hydraulic retention time (HRT) (Milán *et al.* 2010). Zeolite also serves as a mineral source for bacteria and archaea (Pobeheim *et al.* 2011). More recently, it was demonstrated that the addition of 5 cm in height of a static bed of zeolite exerted a positive effect on EGSB hydrodynamics without affecting the fluid pattern of this technology.

Considering the capacity of EGSB reactors for the efficient treatment of different sewage and the results obtained with the use of zeolite in different investigations, the objective of the present paper was to study the influence of this material on the operational and efficiency parameters while treating synthetic swine wastewater, and the dynamics and microbial composition when the OLR is increased and an organic shock load (OSL) is applied during the operational period.

METHODS

Operational strategy

The laboratory experiments were conducted in two acrylic EGSB reactors (R1 and R2), each with a 0.05 m-diameter tube and 0.11 m-diameter separator. The heights of the tube and separator were 0.96 m and 0.11 m, respectively (Figure 1). The effective volume of each reactor was 3.04 L. Small crystal balls were placed at the bottom of each reactor to guarantee a homogeneous distribution of the effluent. In the case of R2, 120 g of zeolite was added, for 5 cm of static bed and a final concentration of 40 g of zeolite per litre of total effective volume of reactor. The mean particle diameter of the zeolite was 0.1511 mm. R1 was operated as a control reactor.

The reactors were operated in a chamber with a mesophilic temperature controlled at 30 ± 1 °C. A Gilson peristaltic pump

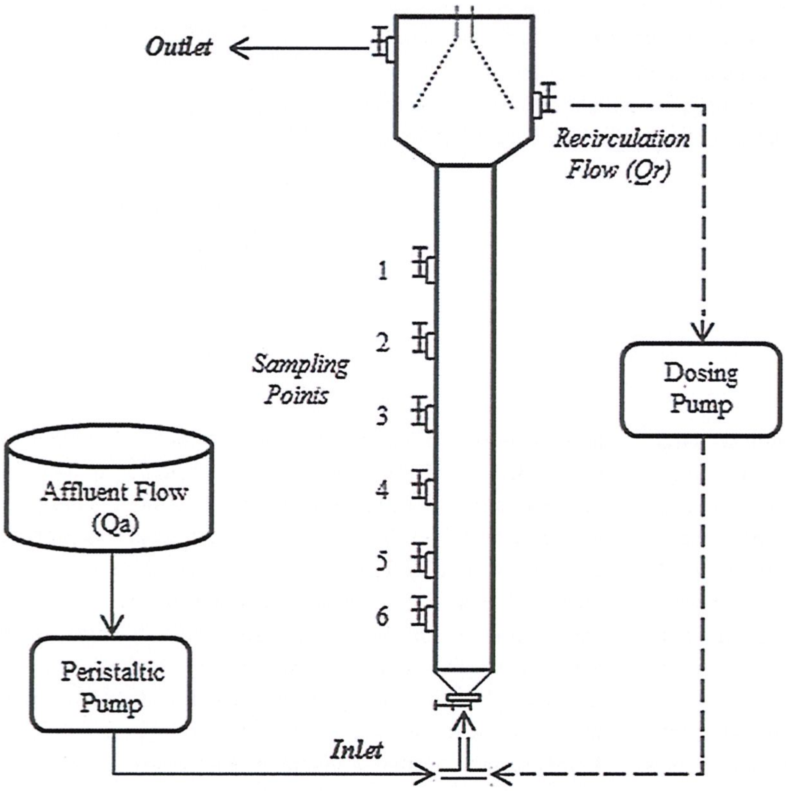

Figure 1 | Schematic of the experimental unit.

fed the effluent into the reactor at the bottom of the column. A dosing pump (Prominent) was used for recirculation.

The theoretical HRT was held constant and equal to 12 hours. The feeding flow was approximately 4.0 mL/min. The recirculation flow was approximately 200 mL/min, resulting in 6.0 m/h of up-flow velocity. The reactors were operated in continuous mode for 180 days. The adaptation period was operated at low concentrations of chemical oxygen demand (COD) with an OLR of 0.5 kgCOD/m^{3}d for 30 days. After this period, the OLR was increased from 1 to 6 kgCOD/m^{3}d for 100 days. Due to irregularities with the inoculum, a further re-inoculation was applied at 6 kgCOD/m^{3}d. From that moment, a continuous OSL was applied over a period of 6 h (according to Amorim *et al.* 2005) with step increments of the OLR as described in Table 1. The feeding COD concentration was step increased from 581.2 ± 34.8 to 14654.0 ± 248.3 mg/L, corresponding to an OLR applied of 1.0–32.0 kg COD/m^{3}d (Table 1).

Inoculum and feeding

The reactors were initially inoculated with granular sludge collected from a UASB reactor treating poultry slaughterhouse waste (Pereiras Poultry, São Carlos, Brazil). The inoculum had a concentration of 43.4 g/L of total solids (TS) and 36.2 g/L of total volatile solids (TVS). Considering the volume of the reactor, 20% of the effective volume was filled with inoculum. Before inoculation, the sludge had a mean diameter of 1.53 ± 0.46 mm.

The feeding consisted of a synthetic effluent simulating the characteristics of swine wastewater according to Bergmann *et al.* (2000). The organic fraction regarding the mass ratio of carbohydrates:proteins:lipids was 1.75:1:0.75. The protein fraction was simulated with meat extract; the carbohydrate fraction was composed of sucrose, starch and cellulose in a mass ratio of 1:3:1, respectively. The lipid fraction was simulated with soybean oil emulsified with commercial detergent.

At day 130 (100 days of operation) with 6.0 kgCOD/m^{3}d of OLR, the reactor was re-inoculated with granular sludge obtained from a full-scale UASB reactor treating poultry slaughterhouse waste (Dacar Poultry, Tieté, São Paulo, Brazil). The TS concentration of the inoculum was 36.91 g/L with 26.53 g/L of TVS and a mean diameter of 1.94 ± 0.51 mm.

Natural zeolite

The zeolite was derived from the Tasajeras ore in Villa Clara Province, Cuba. It is characterized by 70% clinoptilolite-heulandite, 5% mordenite, 15% anorthite and 10% quartz.

Chemical analysis

Determination of TS, TVS and COD was in accordance with *Standard Methods for the Examination of Water and Wastewater* (APHA 2005). The pH was determined with a two-point calibrated (4 and 7) QX 1500 pH-meter. The total Kjeldahl nitrogen (TKN) concentration was measured according to the Kjeldahl method with a Marconi MA 036 meter by employing digestion followed by distillation and titration (APHA 2005). The alkalinity was assessed according to the methodology proposed by Ripley *et al.* (1986). VFAs, namely, acetic (HAc), propionic (HPr), butyric (HBu), isobutyric, isovaleric and valeric (HVa) acids, were analyzed with a Shimadzu GC/FID-2010 gas chromatograph coupled with an HP-INNOWAX capillary column (30 m × 0.25 mm × 0.25 mm).

Statistical analysis

One-way analysis of variance and Tukey's multi-comparison test were conducted to statistically compare the efficiency in

Table 1 | Values of applied OLR and OSL

OLR (kgCOD/m^{3}d)	COD$_i$ (mgCOD/L)	TS (g/L)	Operation days	OSL (kgCOD/m^{3}d)	COD$_o$ (mgCOD/L)
1	581.2 ± 34.8	0.712 ± 0.011	30		
4	2,558.0 ± 117.7	2.749 ± 0.108	40		
6	3,113.8 ± 71.6	5.198 ± 0.227	40	12	5,849.5
8	4,139.1 ± 54.0	5.416 ± 0.111	10	24	12,139.7
12	6,291.5 ± 67.1	7.647 ± 0.093	10	36	17,983.3
24	10,290.7 ± 228.6	12.200 ± 0.158	10	48	24,128.3
32	14,654.0 ± 248.3	14.183 ± 0.209	10		

COD$_i$, i: initial concentration of organic matter; COD$_o$, o: organic overload.

terms of COD and the concentration of VFAs between the two reactors. The software Statgraphics Centurion XV was used for the statistical analysis development.

Microbiological analysis

Samples were withdrawn from Point 2 in both reactors, located at a distance of 13.5 cm from the bottom of the reactor, after successive shock loads and at the end of the process, to analyze the effect of different phases on the microbial community using denaturing gradient gel electrophoresis (DGGE). The samples were immediately frozen at −20 °C.

Deoxyribonucleic acid (DNA) extraction was performed with a direct method using glass beads and a mixture of phenol:chloroform:buffer (1:1:1 v/v) following a procedure described by Griffiths *et al.* (2000) that was modified for this study. The 16S rRNA (ribosomal ribonucleic acid) fragments were amplified by polymerase chain reaction (PCR) using primers 968FGC-1401R for the bacteria domain and primers 1100FGC-1400R for the archaea domain. The 16S rRNA fragments were then subjected to DGGE (Muyzer *et al.* 1993).

Using denaturing gradient concentrations of 45% and 65%, DGGE was prepared for either the bacterial or archaeal community, respectively. The electrophoresis conditions were 75 V and 60 °C for 16 h. The DGGE band profiles were obtained using an Eagle Eye III TM (Stratagene) at 254 nm and Eagle Sight software. Jacard's similarity coefficients for the band profiles were calculated by BioNumerics software version 3.5. The dendrograms were constructed in accordance with the UPGMA (unweighted pair group method with arithmetic average) method.

Biomass samples were analyzed by optical microscopy techniques in an Olympus BX60 microscope coupled to an Evolution QE camera and Image-Pro Plus 4.5 software. Samples were collected from Point 2 and from the effluent of both reactors at 70 days of operation (6.0 kgCOD/m^{3}d of OLR) and at the end of the applied OLR of 8.0 kgCOD/m^{3}d (20 days after re-inoculation).

RESULTS AND DISCUSSION

EGSB reactor operation

Because AD has a good buffer capacity, pH and partial alkalinity are good indicators of process imbalance. During the operation of the reactors, it was verified that the pH was maintained close to neutral. Before re-inoculation, the mean value of pH for each OLR was 7.18 ± 0.16 in R1 and 7.50 ± 0.13 in R2; R1 displayed lower values than the feeding (7.37 ± 0.10). However, after re-inoculation, the effluent showed higher values than the feeding, and for all applied OLRs, the pH values in R2 (7.5–7.83) were higher than those in R1 (7.28–7.59), and therefore not indicating signs of acidification. There were no significant differences between reactors or between OLRs despite changes in VFA levels. According to Ward *et al.* (2008), the optimal pH value for AD ranges between 6.8 and 7.2, and an excessively alkaline pH provokes granule disintegration. In the present paper, both reactors showed stable operation despite the high values of pH.

Several studies include the intermediate and partial alkalinity (IA/PA) ratio for the control of AD (Ferrer *et al.* 2010). The mean IA/PA ratio values obtained for the effluent were 0.79 for R1 and 0.72 for R2 (Figure 2). According to Ripley *et al.* (1986), if the IA/PA relationship is greater than 0.3, this indicates the existence of disturbances in the process of AD. However, Moterani (2010) remarked that due to the particularities of each effluent, some reactors show no irregularities in the process although the relationship exceeds this value.

The suggested values for this relationship are numerous, and they are dependent on the substrate and reactor configuration (Martín-González *et al.* 2013). Furthermore, Moterani (2010) observed a medium value for an IA/PA ratio of 0.63 in swine wastewater treatment in a UASB reactor, and Pereira *et al.* (2009) reported values of 0.62. Neither study showed a negative effect on AD efficiency. Clearly, these values exceed twice the recommended value. In the present study, lower values were obtained only at an OLR of 6 kgCOD/m^{3}d with 0.56 and 0.46 for R1 and R2, respectively. Due to the relevance of this indicator, it is highly recommended that further research be developed to re-establish the optimal criteria for the IA/PA ratio according to waste streams and different configurations, as studied by several authors.

After the adaptation period, during the first applied OLR, the removal efficiency in terms of COD was stable for both reactors. However, when the OLR was increased to 4 kgCOD/m^{3}d, the values of the IA/PA ratio for R1 (1.04 ± 0.15) and for R2 (0.88 ± 0.15) were higher, and the removal efficiency decreased prominently with a significant difference in comparison with 1 kgCOD/m^{3}d. This behavior is probably due to the step-wise increase in the OLR at the beginning of the reactor's operation. At 6 kgCOD/m^{3}d, it

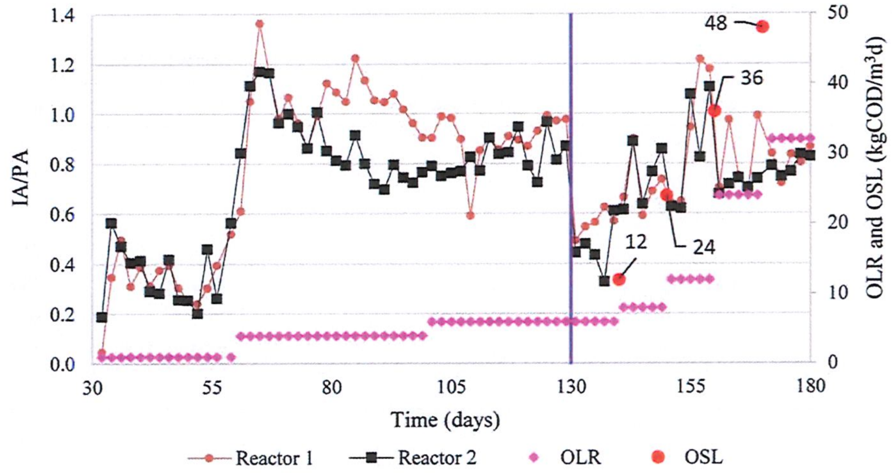

Figure 2 | Variation of IA/PA relationships in both reactors during AD.

was decided to re-inoculate the reactors due to the tendency of COD removal efficiency to decrease, with values lower than 80%. After the start-up period, at 6 kgCOD/m^3d, the removal efficiency in terms of COD ranged between 78.9 and 83.5% for R1 and between 84.3 and 86.8% for R2, as observed in Figure 3. It is important to remark that significant differences were found both after and immediately prior to re-inoculation ($p < 0.0001$) for both reactors, which proved the lack of activity of the previous inoculum and the necessity of re-inoculating the reactors.

Table 2 shows the average COD removal efficiency values for each OLR after the OSLs were applied in each reactor. At an OLR of 32.0 kgCOD/m^3d, significant differences were observed between the two reactors with p values <0.01. These results could be attributed to the presence of zeolite, which showed higher robustness of the studied anaerobic system as the OLR increased.

These results are in agreement with those obtained by Delforno *et al.* (2014) and Yu *et al.* (2014), who used EGSB reactors to treat high-strength wastewater but conducted the operation at OLR values lower than those applied in the present study. It is important to note that effluent recirculation contributes to high values of COD removal efficiency due to its ability to dilute the chemical products present in high-strength wastewater.

When the organic load applied to the system is increased, the methanogenic consortium is no longer able to completely degrade the produced VFAs. Therefore, the efficiency and the stability of the reactor are negatively

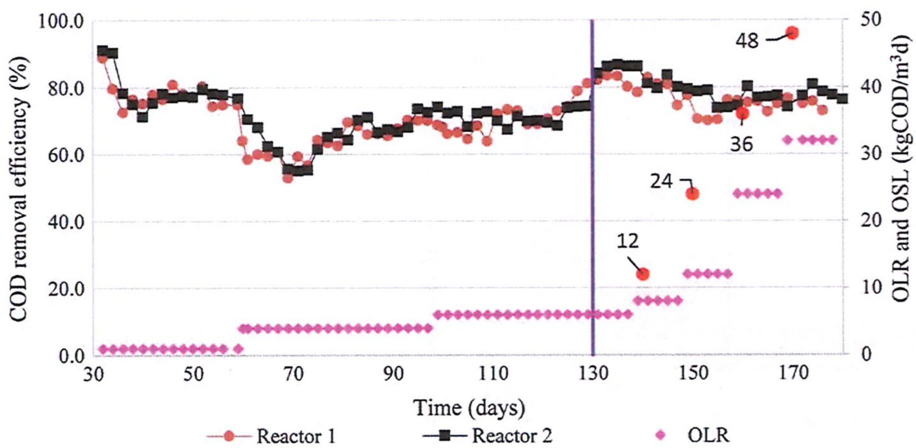

Figure 3 | COD removal efficiency in R1 and R2 during the operational period. The perpendicular line marks the date of re-inoculation.

Table 2 | Average values of COD removal efficiency for each OLR and after OSL

	Removal efficiency (%)			Removal efficiency (%)	
OLR (kgCOD/m³d)	R1	R2	OSL (kgCOD/m³d)	R1	R2
1	77.7 ± 4.1	78.8 ± 5.4			
4	63.8 ± 5.0	65.7 ± 5.8			
6*	69.4 ± 4.9	71.2 ± 3.2			
6**	81.9 ± 1.9	86.0 ± 1.0	12	80.2	81.2
8	80.7 ± 1.6	80.9 ± 1.7	24	74.5	78.8
12	72.6 ± 3.4	76.1 ± 2.7	36	76.2	80.3
24	75.2 ± 1.5	77.2 ± 2.2	48	75.0	77.4
32***	75.1 ± 1.4	78.2 ± 1.7			

*Before re-inoculation.
**After re-inoculation.
***Significant differences between R1 and R2 with p values <0.01.

affected (Franke-Whittle *et al.* 2014). Because reactor stability is essential for obtaining a high removal efficiency, many researchers have correlated it with the VFA concentration in the reactor (Akuzawa *et al.* 2011). As observed in Figure 4, while increasing the OLR, the accumulation of acetic and propionic acid increased in both reactors without reaching inhibitory levels for methanogen activity.

In the case of HAc, the values obtained after the organic loading shocks showed that the highest accumulation in all cases was in R1, which is attributable to the absence of zeolite in this reactor. Significant differences between the two reactors for the last two applied OLRs were observed. Nevertheless, in the case of HPr there was no significant difference between R1 and R2 while increasing the OLR, but with OSL application, the behavior was similar to that obtained for HAc.

The higher acid conversion in R2 could be due to the robustness of the system in the presence of zeolite, which is consistent with the report by Lin *et al.* (2013). These researchers added zeolite to batch experiments while treating swine manure, resulting in high degradation velocities, especially for HPr and HBu. With the increase of the OLR from 8.0 kg COD/m^{3}d and greater values, acid accumulation began to increase, with HAc being predominant in both reactors with ~44% for R1 and ~45% for R2 (data not shown). Nevertheless, neither the high OLR applied nor the OSL provoked the accumulation of VFA to inappropriate levels or resulted in the instability of the anaerobic process.

In the case of butyric and valeric acids (Figure 5), accumulation was observed after the application of 12.0 kgCOD/m^{3}d, mainly for the HVa isomers. For HBu,

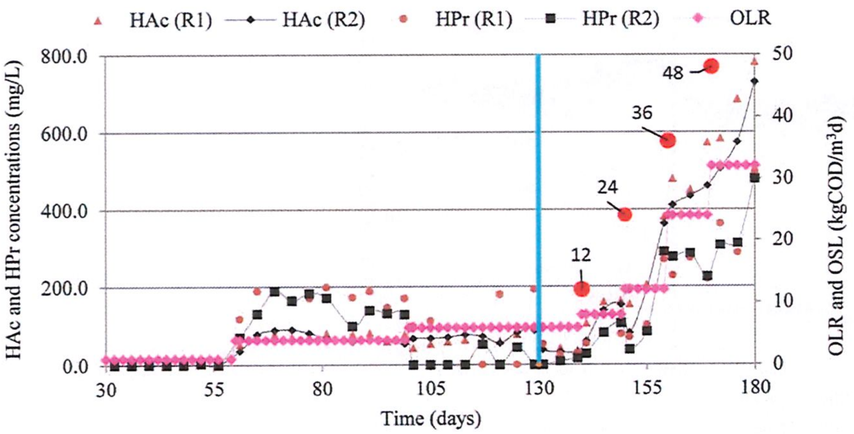

Figure 4 | Variation of HAc and HPr concentrations in the two reactors during AD.

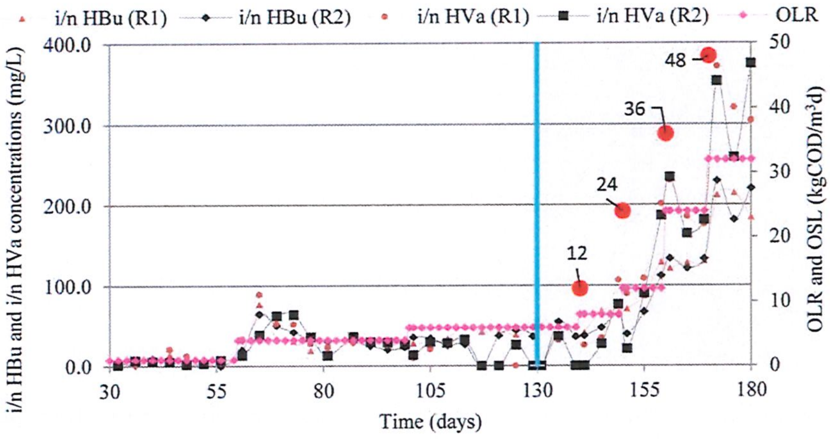

Figure 5 | Variation of i/n HBu and i/n HVa concentrations in the two reactors during AD.

despite slight increases at the highest OLR, the concentrations of this acid nonetheless remained lower than the inhibitory levels (Wang *et al.* 2009).

Total Kjeldahl nitrogen

Nitrogen is essential for microorganism growth, although at higher levels it can act as an inhibitor. During the operation of the reactors, it was verified that TKN was maintained stably in the two reactors (Figure 6). Nevertheless, after increasing the OLR to 12 kg COD/m^{3}d and above, slight differences appeared, presenting at lower concentrations in R2. This could be attributed to the high adsorption ability and selectivity of zeolite from its ammonium ions (Montalvo *et al.* 2014). Liu *et al.* (2015) found similar results in the co-digestion of animal manure and wheat straw with the addition of zeolite. Despite this, there was no significant difference in the nitrogen concentrations between the two reactors, with values below 2,000 mg/L not affecting the archaea community. In addition, the nitrogen concentrations did not exceed the inhibitory level (1.7 g/L) (Rajagopal *et al.* 2013).

Microbial analysis

PCR/DGGE analysis was used to compare the band profiles of the bacterial communities (Figure 7) and archaeal communities (Figure 8) in the control reactor (R1) and the reactor with zeolite (R2) during the operational period.

Although this technique is not quantitative, differences between the intensities of the bands in the profiles were observed. For the bacterial domain (Figure 7), when comparing R1 and R2, it was noticed that the similarity coefficient was 20%, indicating a significant difference between the

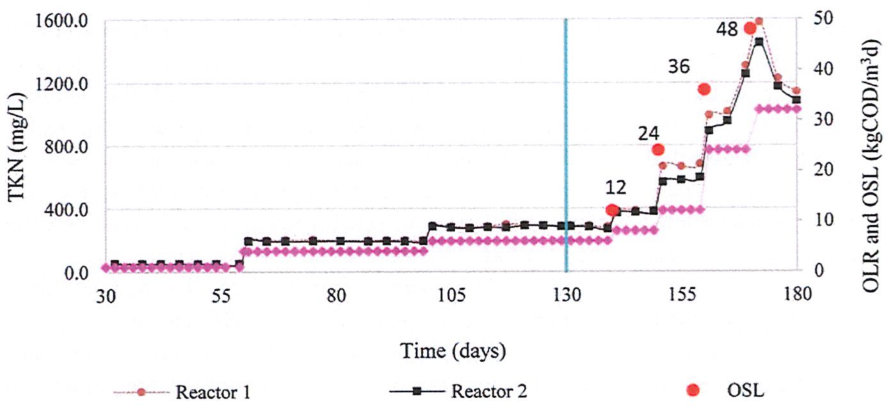

Figure 6 | Profiles of TKN in the two digesters during the operational period.

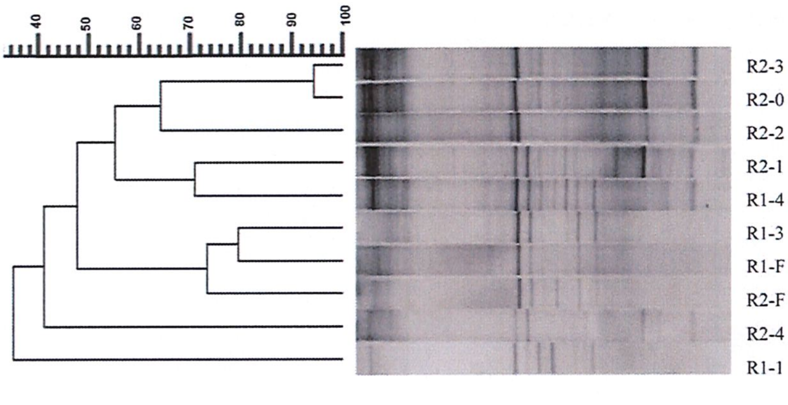

Figure 7 | Analysis of similarity coefficients (Jaccard correlation) for the DGGE band profiles related to the bacterial domains of the samples from the control reactor (R1) and reactor with the addition of zeolite (R2). Legend: Ri-j, i: reactor nomenclature; j: order of shock loads, F: end of the process.

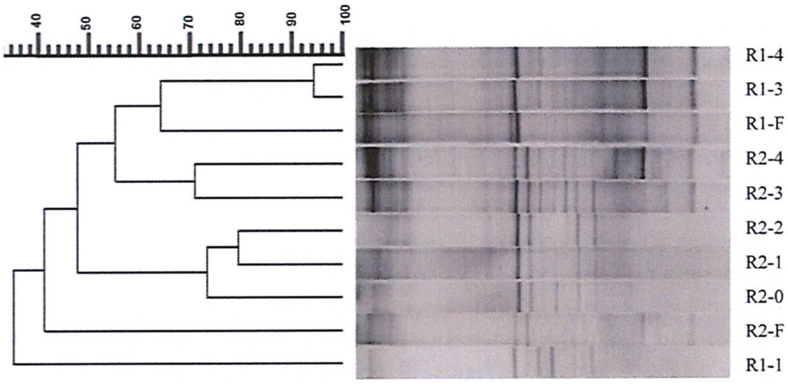

Figure 8 | Analysis of similarity coefficients (Jaccard correlation) for the profile of the DGGE bands related to the archaeal domain of the samples from the control reactor (R1) and reactor with the addition of zeolite (R2). Legend: Ri-j, i: reactor nomenclature; j: order of shock loads, F: end of the process.

microbial populations that colonized the two reactors. Considering all of the experimental conditions, R2 had a higher bacterial microbiota similarity (35%) than R1 (20%), indicating that the bacterial community in R2 was less sensitive to alterations of organic load. Meanwhile, increasing the organic load maintained similarities of 50% between R1-3, R1-4 and R1-F and 48% between R2-0, R2-1, R2-3 and R2-4. Apparently, the load shocks did not promote significant differences in the similarity coefficients of the bacterial communities when comparing R1 and R2. At the end of the experiment in R2, with higher organic load (R2-4 and R2-F), some selection of the bacterial microbiota for conditions more critical to them has occurred, based on the similarity coefficient of 62%.

The similarity coefficient for the archaeal domain (Figure 8) was 34% between R1 and R2. Comparing all conditions in R1, there is 34% similarity, and the conditions in R2 have a similarity of 46%, indicating that the archaeal community was less sensitive to the alterations of organic load in R2. These values indicate that the changes in the microbial communities occurred mainly in the bacterial populations (Figure 7).

As with the bacterial community, the increase of organic load caused a 62% similarity between R1-3, R1-4 and R1-F and 38% between R2-0, R2-1, R2-3 and R2-4. It is possible that increasing the organic load caused the archaeal domain similarity coefficients (62% in R1 and 38% in R2) to decrease more markedly than those of the bacterial domain (50% in R1 and 48% in R2). As a result, the archaeal community was more sensitive to the organic load increases than the bacterial community.

These differences in biomass formation may be due to the hydrodynamic regime in the EGSB reactor.

As mentioned before, the first 100 days of operation were not considered in the discussion of the results due to irregularities found with the inoculum. Based on microbiological analysis, it was proved that the first inoculum used for the experiments had curved rod shapes and vibrio bacilli with various dimensions; furthermore, the microbes had intracellular granules similar to an elemental sulfur precipitate, as observed in Figure 9 (Figure 9(a) and 9(b) for Point 2 and Figure 9(c) and 9(d) for effluent). In addition, fluorescent bacilli appeared, suggesting the presence of hydrogenotrophic methanogens. Nevertheless, in the effluent, there were higher amounts of phototrophic anoxic microorganism-like cells in comparison with the amounts at Point 2. A lack of *Methanosaeta*-like cells and *Methanosarcina*-like cells was observed. These microbiological results reinforced the low COD removal efficiency obtained during that period and the need for re-inoculation.

After re-inoculation (Figure 10), more microorganisms were present. According to Carballa *et al.* (2015), *Methanosaeta* species predominate when anaerobic reactors are operating under stable conditions. It could be possible that the observed increase of microorganisms was linked to the presence of *Methanosaeta*-like cells. The predominance of *Methanosaeta* was related to the low concentrations of VFAs (mainly acetic acid) detected in the reactor compared with those in other similar studies (De Vrieze *et al.* 2012).

The predominance of these species, which is typical of the methanogenesis stage in the AD process, led to a higher COD removal efficiency and superior yields of methane (data not shown).

Estimation of apparent kinetic parameters

By considering the reactor as a complete mixing tank, the use of first-order kinetics and a pseudo-homogeneous approach in which the kinetic constant is apparent (*k*app) and embodies the mass transfer fluxes, *k*app can be estimated for each operation under different OLRs, as:

$$k\mathrm{app} = \frac{Ef}{HRT\,(1 - Ef)} \tag{1}$$

In Equation (1), *Ef* is the COD removal efficiency and *HRT* is the hydraulic retention time. So, for each applied OLR, the values of *k*app are presented in Table 3.

According to the evaluation of the biodegradation process, it is possible to conclude that the addition of 40 g/L of zeolite, i.e., the mass of zeolite per total effective volume of reactor, favored the kinetics of the process with significant differences at 6 $kgCOD/m^3d$ (p value = 0.0039),

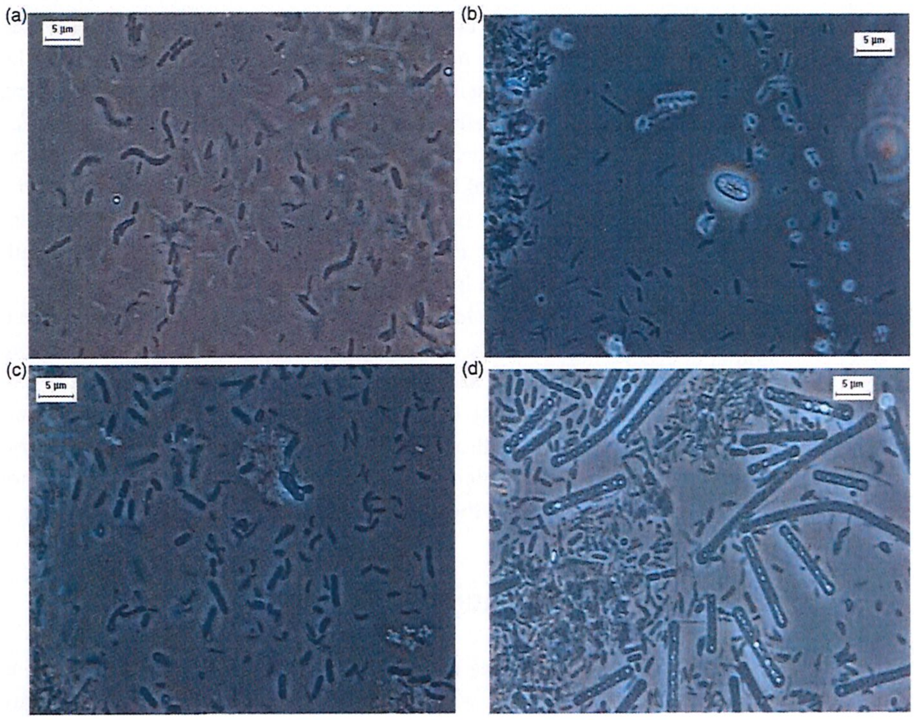

Figure 9 | Microscopy images for R1 and R2 before re-inoculation. (a) R1 Point 2, (b) R2 Point 2, (c) R1 effluent, (d) R2 effluent.

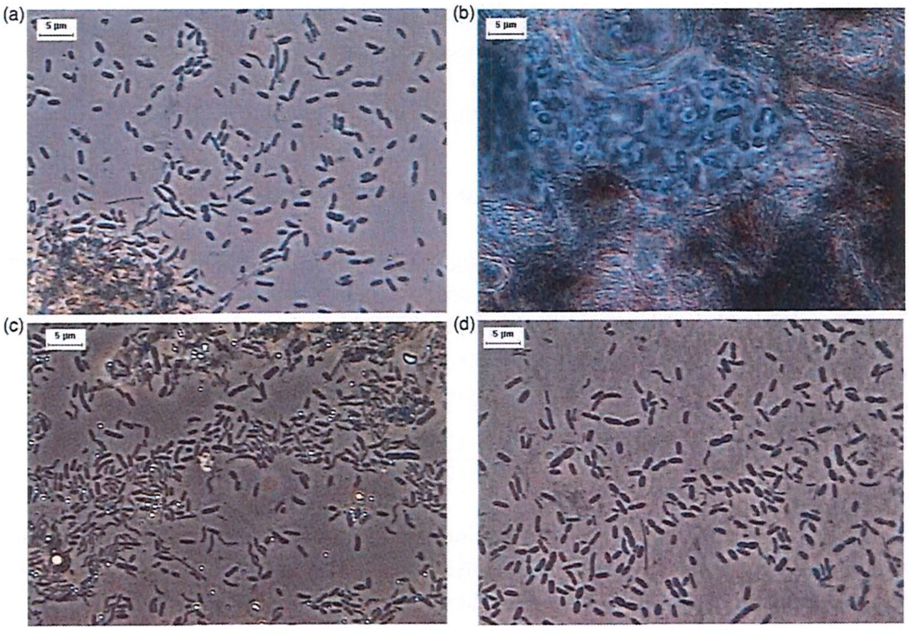

Figure 10 | Microscopy images for R1 and R2 after re-inoculation. (a) R1 Point 2, (b) R2 Point 2, (c) R1 effluent, (d) R2 effluent.

if comparing prior to and after the re-inoculation. In addition, the re-inoculation improved the kinetics by more than twice the kinetic parameter value for each reactor. After the OSL, with an OLR of 32 kgCOD/m^3d, it was found that the apparent kinetic constant was also 20% higher in R2 with a significant difference relative to that of R1 (p value = 0.0208).

Several authors have demonstrated the possibility of increasing the robustness of anaerobic processes with the addition of zeolite. The results obtained in the present study are in agreement with those reports. Borja et al. (1993) used 20 g/L of zeolite to treat cow manure, with increases in the kinetic constant of 35% and 54%. Kotsopoulos et al. (2008) used zeolite concentrations from 4 to 12 g/L in pig waste, favoring the methane production rate under thermophilic conditions at 8 and 12 g/L of zeolite. However, Zheng et al. (2015) proposed a novel configuration using zeolite for ammonium-rich wastewater at 10 g/L. The biodegradation was increased by a factor of 96 in comparison with the control reactor. Liu et al. (2015) used from 1.4 to 7.1 g/L of zeolite in the anaerobic co-digestion of animal manure with wheat for an increase in the methane yield of 50%.

Nevertheless, Milán et al. (2001) used zeolite at concentrations from 0.2 to 10.0 g/L for the treatment of waste piggery, but at values higher than 6.0 g/L of zeolite the kinetic constant decreased. This result was attributed to an increase of the IA/PA ratio and pH value upon the addition of more zeolite to the batch system. This behavior was not observed in the present research.

Table 3 | Mean values of the apparent first-order constant for different OLRs in reactors R1 and R2

	kapp (h^{-1})	
OLR (kgCOD/m^3d)	**R1**	**R2**
1	0.28 ± 0.11	0.32 ± 0.14
4	0.15 ± 0.03	0.17 ± 0.04
6[1]*	0.19 ± 0.03	0.21 ± 0.02
6[2]*	0.38 ± 0.05	0.51 ± 0.04
8	0.35 ± 0.04	0.36 ± 0.04
12	0.23 ± 0.04	0.27 ± 0.04
24	0.25 ± 0.02	0.28 ± 0.04
32*	0.25 ± 0.02	0.30 ± 0.03

[1]Before re-inoculation.
[2]After re-inoculation.
*Kinetic values with significant differences (p values <0.01).

CONCLUSIONS

The presence of zeolite in the EGSB reactor was positive. The reactor with zeolite at 40 g/L showed higher stability of the anaerobic process at high organic loads and after

OSLs. Although the OSLs provoked an increase in effluent COD and VFA, the reactor recovered its operational performance. At an OLR higher than 30 kg COD/m^{3}d, the COD removal efficiency in the presence of zeolite was 78% with significant differences in comparison with the control reactor. The sludge quality was observed to influence significantly the microbiology and the COD removal efficiency of the AD process. Slight differences in TKN were found between reactors after increasing the OLR to values of 12 kg COD/m^{3}d and greater due to the presence of zeolite. The kinetic behavior demonstrated the robustness of the anaerobic process with the addition of 40 g/L of zeolite at higher OLR values and with a *k*app value of 0.3 h^{-1} based on significant differences.

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