Anaerobic degradation of glycol ether-ethanol mixtures using EGSB and hybrid reactors: performance comparison and ether cleavage pathway

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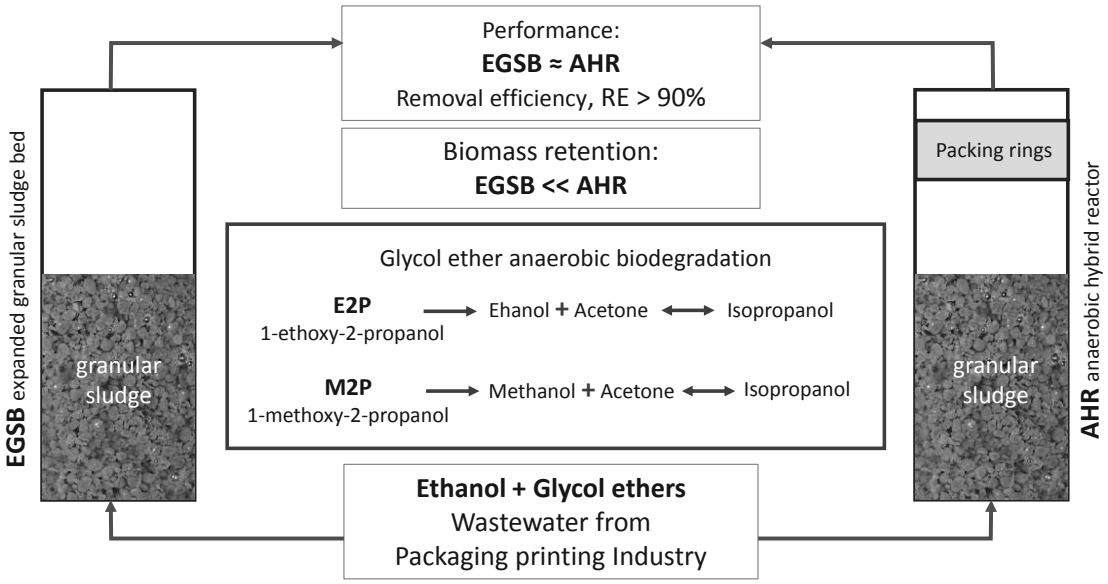
Graphical Abstracts



Highlights

- Anaerobic treatment is feasible for the removal of 1-ethoxy-2-propanol from water
- The hybrid reactor showed a solid retention capacity of 92% higher than control
- Hybrid and control reactors showed similar performances with global efficiencies >90%
- The detected byproducts clarified the degradation pathway of studied glycol ethers

Abstract

The anaerobic biodegradation of ethanol-glycol ether mixtures as 1-ethoxy-2-propanol (E2P) and 1-methoxy-2-propanol (M2P), widely used in printing facilities, was investigated by means of two laboratory-scale anaerobic bioreactors at 25^{o}C-an expanded granular sludge bed (EGSB) reactor and an anaerobic hybrid reactor (AHR) which incorporated a packed bed to improve biomass retention. Despite AHR showed almost half of solid leakages compared to EGSB, both reactors obtained practically the same performance for the operating conditions studied with global removal efficiencies (REs) higher than 92% for organic loading rates (OLRs) as high as 54 kg of chemical oxygen demand (COD) m^{-3} d^{-1} (REs of 70% and 100% for OLRs of 10.6 and 8.3 kg COD m^{-3} d^{-1} for E2P and M2P, respectively). Identified byproducts allowed clarifying the anaerobic degradation pathways of these glycol ethers. Thus, this study shows that anaerobic scrubber can be a feasible treatment for printing emissions.

Keywords: 1-ethoxy-2-propanol degradation; 1-methoxy-2-propanol degradation; anaerobic biotreatment; biomass retention capacity; printing industry wastewater

1. Introduction

Solvents are commonly used in packaging printing industries as ink components that are dried through evaporation, which are the sources of volatile organic compounds (VOCs) in air emissions throughout the printing process. These emissions show ethanol as the main solvent, which is used in many solvent-based inks, but other solvents such as glycol ethers are also predominantly identified (Bravo et al., 2017; Sempere et al., 2012). The glycol ethers that are present in these emissions are 1-ethoxy-2-propanol (E2P) and/or 1-methoxy-2-propanol (M2P) that are frequently used as retarders in the printing process (Cheremisinoff, 2003). Several regulations and guidelines have been established in many countries for the control of the emissions of VOCs in printing industry. In the European Union, VOC concentrations of these emissions must comply with limit values ranging from 20 to 100 mg C Nm^{-3} (Council Directive 2010/75/EU), depending on printing activity (offset, rotogravure, flexography, etc.) and solvent consumption threshold (>15 or >25 tonnes per year). USA Environmental Protection Agency also recommends levels of control for these emissions ranging from 65 to 80% for overall control efficiency and from 90 to 95 % for control device efficiency, depending on installation date of equipment and printing activity. Many states and local agencies have adopted regulations for controlling emissions from printing industry consistent with these control levels (USEPA, 1978; USEPA, 2006). To fulfill these regulations and guidelines, the printing industry frequently needs end-of-pipe treatments of these emissions. In this regard, biological treatments such as biofiltration have been studied to remove volatile organic compounds from air emissions (Malhautier et al., 2005). A new alternative treatment based on an anaerobic bioscrubber has recently been described (Bravo et al., 2017; Waalkens et al., 2015). In this process, solvents are initially transferred from the gas phase to the liquid phase and subsequently degraded in

an anaerobic reactor, where solvents are transformed into bioenergy in the form of biogas. The anaerobic biodegradability of M2P was demonstrated by Lafita et al. (2015) when working with an expanded granular sludge bed (EGSB) reactor at 25°C. The removal efficiency (RE) of M2P reached up to 87% and showed a long lag phase that took 34 days to remove more than 50% of M2P. A first evidence of anaerobic degradation of M2P was reported by the European Chemicals Bureau (2006). In this study, a batch test using inoculum from a municipal digester, only a removal efficiency of 38% was observed after 81 days of operation.

Regarding the mechanism for the ether cleavage, it is not yet well understood, but the most accepted mechanism for glycol ether cleavage includes a double H/OH interchange (*trans*hydroxylation), resulting in the *gem*-diol intermediate (Speranza et al., 2002; White et al., 1996), which rapidly collapses to the carbonyl or the keto group. In the anaerobic degradation of polyethylene glycol (PEG), migration of hydroxyl group generates an intermediate hemiacetal leading to acetaldehyde. Thus, degradation of the polymer occurs by successive eliminations of acetaldehyde units catalyzed by a cobalamin-dependent intracellular enzyme (PEG acetaldehyde lyase), according to the following reaction (Frings et al., 1992):

$$R\text{-}O\text{-}CH_2\text{-}CH_2\text{-}OH \rightarrow R\text{-}O\text{-}CH(OH)\text{-}CH_3 \rightarrow R\text{-}OH + CH_3\text{-}CHO$$

Kawai (2002) hypothesized that the metabolism of polypropylene glycol (with identical terminal OH group as M2P and E2P) is the same as that of PEG. By analogy, Lafita et al. (2015) proposed the same mechanism for the degradation of M2P, in this case leading to methanol and acetone or propionaldehyde.

Biological treatments have been shown to be capable to remove slowly biodegradable industrial compounds (Zhang et al., 2016: T., Huang, 2017). Among

these, anaerobic reactors based on granular sludge technology have become interesting options for the cost-effective and sustainable treatment of industrial wastewaters (Petta et al., 2017; Delforno et al., 2016). In this sense, the high contact between wastewater and biofilm that is promoted by expanding the sludge bed makes the EGSB a suitable technology to treat high stress, less biodegradable or toxic components in wastewater. Expanding the granular sludge requires high upflow velocity (v_{up}) that can produce the wash-out of granular biomass from the reactor (Dries et al., 1998). This wash-out phenomenon can also be caused by a low-concentrate substrate (Puñal et al., 2003); wastewaters containing fat, oil, and grease (Jeganathan et al., 2006); or the combination of organic overload and daily interruption in the substrate supply (Lafita et al., 2015).

To improve biomass retention, Guiot and van den Berg (1985) used an anaerobic hybrid reactor (AHR) in treating synthetic soluble sugar wastewater, where an upflow anaerobic sludge bed (UASB) reactor was modified by adding an anaerobic filter in the upper zone to replace the gas-liquid-solid (GLS) separator. The results showed the filter as an inexpensive method to enhance biomass retention. This type of AHR offers different advantages, such as higher biomass retention capacity, a polishing effect to the effluent due to the biomass accumulated in the filter (Shivayogimath and Ramanujam, 1999), or a higher buffering effect against shock loading (McHugh et al., 2006). Because of these characteristics, the AHR seems a promising and emerging alternative that can be considered feasible for the treatment of (especially high-strength) wastewaters from different industries. Thus, for wastewater from a distillery spent wash, Shivayogimath and Ramanujam (1999) demonstrated that a hybrid reactor combining a UASB reactor in the lower part with polypropylene pall rings filter media in the upper part could treat high organic loading, such as 36 kg of chemical oxygen demand (COD) $m^{-3}d^{-1}$, which was a very efficient GLS separator. The AHR also

showed high REs (up to 90.3%) in the treatment of a dairy industry effluent (Belançon et al., 2010). Treating a chemical synthesis-based pharmaceutical wastewater, Oktem et al. (2008) revealed that the AHR presented strong stability to changes in organic loadings. McKeown et al. (2009) showed that the AHR had a suitable configuration for long-term low-temperature treatment of acidified wastewaters.

The main purposes of this work were (i) to evaluate the feasibility of the anaerobic biodegradation of wastewater containing solvents from a flexographic printing industry by working with an AHR, including common glycol ethers such as M2P and E2P, and (ii) to compare the operation performance and biomass retention capacity between the AHR and the EGSB reactor. Furthermore, (iii) the study of the degradation of two different glycol ethers such as E2P and M2P might be useful for an in-depth understanding of the mechanisms involved in the anaerobic ether cleavage. Additionally and to the best of the authors' knowledge, this research is the first study about E2P degradation in an anaerobic reactor.

2. Materials and Methods

2.1. Reactors' setup

Most of the experiments were performed in two anaerobic bioreactors, an EGSB reactor (R1) and an AHR (R2). Fig. 1 shows a schematic diagram of both systems. The EGSB diagram reactor was equivalent to the AHR but without the presence of the packing material (Fig. 1, part 4 in R2). The upper part of the AHR, from 1.40 m to 1.57 m in height, was randomly packed with polyethylene rings (0.0254 m in diameter). Both reactors had the same dimensions, with a sludge bed (Fig. 1, part 3) of 4 L (height of 1.205 m and internal diameter of 0.065 m) and a total volume of 19 L. In this paper,

organic load rate is calculated on the basis of this sludge bed effective volume (4L). The supernatant reactor samples were taken at the recirculation port installed at a 1.60-m height. The gas was conducted to a gas seal (Fig. 1, part 8) with a solution of NaOH to remove H_2S and CO_2. The reactors' temperature was maintained at 25°C, with an external jacket with water recirculation connected to a thermostat system (Polyscience SD15R-30, USA). Each anaerobic system was equipped with two peristaltic pumps that provided feeding and recirculation flows (Fig. 1, part 7) through the reactor. The feeding (Fig. 1, part 1) and the recirculation were maintained at constant flows of 10 L day^{-1} and 32 L h^{-1}, respectively, to provide a v_{up} of 9.80 m h^{-1} in the sludge bed and a v_{up} of 0.98 m h^{-1} in the fixed film section. The increment in the organic loading rate (OLR) was carried out by increasing the COD concentration, keeping the feeding flow constant. A second EGSB (R3), identical to R1, was also utilized in the last part of this study to elucidate the reaction mechanism. For this purpose, an ethanol-acetone mixture was used as the substrate.

2.2. Reactor operation

The reactors were started up by using anaerobic granular sludge (4 L) taken from a local brewery wastewater treatment plant (Font Salem S. L., El Puig, Spain). Both R1 and R2 were operated for more than 300 days (Table 1), with influent buffered with 5 g of $NaHCO_3$ L^{-1}; magnesium and calcium were maintained at 40 mg L^{-1}, and macronutrients and micronutrients were added in proportion to the COD (Table 2). All chemicals used were of analytical grade. The startup (stage S-I) of the reactors was carried out in the first 90 days of the experimental period (Table 1). At this stage, the OLR was increased from 9.1 to 47.0 kg COD $m^{-3}d^{-1}$, with a readily biodegradable

substrate such as ethanol. From day 91 to day 202 (stage S-II), the influent composition was changed to a binary mixture of ethanol and E2P with a mass ratio of 9:1, and the average OLR was kept at 47.4 kg COD $m^{-3}d^{-1}$. At stage S-III, from day 203 to day 221, the OLR of E2P was increased to an ethanol-E2P mass ratio of 4:1, resulting in an OLR of 54.1 kg COD $m^{-3}d^{-1}$. At stage S-IV, from day 222 to day 230, the composition of the influent was changed to a ternary mixture of ethanol, E2P, and M2P with a mass ratio of 8:1:1 to check if there would be no need for a period of adaptation for M2P degradation. At this stage, the average OLR was 48.8 kg COD $m^{-3}d^{-1}$. At stage S-V, from day 231 to day 271, no feeding and no recirculation were applied to evaluate the effect of a long starvation period that typically occurs in industrial facilities. At stage S-VI, from day 272 to day 305, a restartup was carried out with the same ternary mixture used at stage S-IV and with the OLR range from 15.9 to 40.7 kg COD $m^{-3}d^{-1}$. Finally, at stage S-VII, from day 306 to day 335, the average OLR was 46.2 kg COD $m^{-3}d^{-1}$, and the mass ratio of the mixture of ethanol, E2P, and M2P was changed to 7:1:2.

The influent of the R3 reactor was buffered, and the micronutrients and the macronutrients were added in the same way as in the previously described reactors. The startup was carried out with ethanol until it reached almost complete substrate degradation for an OLR of 25.0 kg COD $m^{-3}d^{-1}$. Then, maintaining the OLR, the feeding composition was changed to an ethanol-acetone mixture with a mass ratio of 1:1, and the reactor was operated for 27 days under these conditions.

2.3. Effluent and biogas analyses

The COD, as well as volatile fatty acid (VFA), total suspended solid (TSS), and volatile suspended solid (VSS) concentrations were analyzed according to Standard

Methods (American Public Health Association, 1999). The NH_4^+, Ca^{2+}, Mg^{2+}, K^+, PO_4^{3-}, and SO_4^{2-} concentrations were measured by ionic chromatography (883 Basic IC Plus, Metrohm, Switzerland). The effluent's byproducts and solvents were identified by gas chromatography-mass spectrometry (5973 N MS/GC Agilent Technologies, Spain). The solvents in the effluent were determined by gas chromatography (Agilent GC 7890A, Spain) equipped with flame ionization detector; the solvents and the byproducts of the solvent degradation were separated on a Restek Rtx-VMS (USA) column (30 m long x 0.25 mm i.d. x 1.4 μm particle size), and helium was used as the carrier gas. The biogas composition before the gas seal was determined by using a portable biogas analyzer (COMBIMASS® GA-m, Binder, Germany). The methane flow rates of both reactors were measured after H_2S and CO_2 in the gas seal were removed by a gas meter (MGC-10 PMMA, Ritter, Germany). Conductivity and pH were determined by a precision handheld meter (Multi 340i, WTW, Germany).

The monitoring of bioreactors has been carried out from individual determinations, average values and standard deviation have been considered for overall operation conditions for each stage (Table 1).

3. Results and Discussion

3.1. Reactor performance

The evolution of the global performance of both reactors, R1 and R2, is plotted in Fig. 2, where stages S-I to S-VII are indicated. The detailed evolution of the main parameters of the system (pH, conductivity, COD inlet concentration, etc.) can be found as supplementary material (Fig. S.1 to Fig. S.6). REs higher than 95% were achieved during all startup periods (S-I), including when the OLR reached the maximum value of

47.0 kg of COD $m^{-3}d^{-1}$, as can be observed in Fig. 2. This was a predictable behavior since the organic substrate was ethanol, and the sludge came from a brewery wastewater treatment plant, so the biomass was well adapted to this substrate. The VFA concentrations were below 70 mg L^{-1} in the first 50 days, but when the OLR was increased to 47.0 kg COD $m^{-3}d^{-1}$, the VFA concentrations reached values of 221.9 and 172.0 mg CH_3COOH L^{-1} for R1 and R2, respectively. The minimal accumulation of VFA can be attributed to the faster metabolism of acidogenic bacteria compared to methanogenic archaea, but the VFA concentrations decreased when the high OLR was maintained over time, reflecting the methanogenic population's adaptation to these operational conditions. In any case, the VFA concentrations were always relatively low (< 250 mg CH_3COOH L^{-1}) in both reactors, indicating no significant kinetic decoupling between the acidogenic and the methanogenic communities.

When E2P was included as a component of the influent at stage S-II (Table 1), the total RE in both reactors dropped from 99% to 85%, but the RE was progressively recovered to more than 95% (Fig. 2). This behavior can be explained by assuming that the biomass was not adapted to E2P, so the biomass needed an acclimatization period to be able to metabolize this substrate. This period could be related to the development of ether-cleavage enzymes to degrade this substrate as suggested by Lafita et al. (2015). This approach is shared by Chen et al. (2008) who point the induction of specific enzymes as one of the mechanisms through which adaptation can occur. Various anaerobic bacteria yielding rearrangements of carbon-carbon bonds via transhidroxylation and depending on cobalamin were described for the degradation of polyethylene glycol (Schink et al., 1992; Frings et al., 1992). It would seem plausible that this same catalytic system governs the degradation of ether glycols as E2P, M2P, but no empirical evidences have been reported so far.

Fig. 3a illustrates the evolution of the OLR and the RE of E2P in both reactors. The RE of E2P was very low in the beginning, at 21% and 16% for R1 and R2, respectively, but gradually increased to around 80% for both reactors after 40-50 days of exposure to E2P. Generally, longer adaptation periods had been reported for similar solvents. Traverso-Soto et al. (2016) needed 169 days to reach 99.7% of anaerobic biodegradation of alcohol ethoxylates in anaerobic degradation assays with marine sediments. A longer adaptation period for another glycol ether such as M2P was also observed by Lafita et al. (2015), who reported a 34-day acclimatization period for degrading more than 50% of M2P. In a recent study (Lafita et al., 2017), the time required to obtain 80% of removal of M2P was shortened to 22 days when chitosan was supplemented. In the present study, the acclimatization period for removing over 50% of E2P lasted only 11 and 16 days for R1 and R2, respectively. This shorter acclimatization period could be explained by taking into account the different substrate and the slightly lower OLR of the glycol ether used in comparison with previous studies. Besides, during these first weeks of exposure to E2P, acetone and isopropanol were transitorily observed in the effluents of both reactors. The appearance of these byproducts indicates the amenability of E2P to anaerobic treatment and sheds some light on the mechanism of degradation as discussed further below.

The OLR of E2P was doubled to 10.6 kg COD $m^{-3}d^{-1}$ at stage S-III (Table 1), reaching an average total load of 54.1 kg COD $m^{-3}d^{-1}$, which resulted in a decrease of the global RE to around 92% for both reactors (Fig. 2). This decline can be essentially attributed to the decrease in the RE of E2P down to 70% , as shown in Fig. 3a, indicating that the presence of E2P in the system has no observable effect on the ethanol's RE.

At stage S-IV, the average of the total OLR was maintained at around 50 kg COD $m^{-3}d^{-1}$ (similar to stages S-II and S-III), but a ternary mixture of ethanol, E2P, and M2P, with a mass ratio of 8:1:1, was used as the organic substrate. At this stage, the performance of both reactors barely changed in terms of the global RE (Fig. 2), as well as the RE of E2P in comparison to the previous stage (S-III). The RE of M2P at the end of S-IV was practically complete as shown in Fig. 3b. Even from the first day of exposure to this compound, the RE of M2P was very high (> 85%) in both reactors, indicating no significant adaptation period for metabolizing this compound. This result could be attributed to the fact that both glycol ethers are degraded through the same mechanism; therefore, the adaptation period for M2P degradation is negligible for a system degrading E2P. As in the case of E2P, degradation byproducts were detected in the effluent during the first days of exposure to M2P (acetone and methanol in this case). Regarding the biodegradability of E2P and M2P, the RE is greater for M2P (almost 100%), which could be related to the substrate's accessibility to the active center of the enzyme, as an M2P molecule is smaller than an E2P molecule. Therefore, the results suggest that the use of M2P would be preferable to E2P as a retarder additive if an anaerobic system is considered for the effluent treatment configuration of the packaging printing facility.

A noticeable conductivity increase to 7.48 mS cm^{-1} and 7.65 mS cm^{-1} for R1 and R2, respectively, was observed at the end of S-V, the stage in which no feeding and no recirculation were applied to the system. This conductivity increase above the typical value in both reactors, around 5.50 mS cm^{-1}, was caused by the increase in ammonium and phosphate concentrations as a consequence of a lysis phenomenon during the starvation period. From a mass balance and considering the cell content of nitrogen and phosphorus, the total biomass decay during this period can be estimated: 52.2 g and

66.0 g for R1 and R2, respectively. This higher increase in nitrogen and phosphorus concentration in R2 than in R1 reflects the AHR system's (R2) greater biomass retention capacity. Because during this period (S-V), no feeding was applied, the cell growth can be neglected, and the concentration of microorganisms (X) can be related to the microorganism concentration before the starvation period (X_0) from a mass balance, as shown in the following equation:

$$X = X_0 \exp(-bt)$$

where b is the decay coefficient of biomass, and t denotes the duration of the starvation period. The calculated decay coefficients were 0.0024 d^{-1} and 0.0022 d^{-1} for R1 and R2, respectively. These values are quite similar to the decay coefficients obtained by Wu et al. (1995) with methanogenic granules that previously degraded VFA at 22ºC (between 0.0015 and 0.0028 d^{-1}).

During the restartup, at stage S-VI, conductivity decreased after 4 days to the habitual values, and the OLR was gradually increased from 15.9 to 40.7 kg COD $m^{-3}d^{-1}$, with the same feeding composition as in stage S-IV. Both reactors showed a high total RE, 95% for R1 (EGSB) and 94% for R2 (AHR), indicating that a typical industrial long starvation period (> 30 days) does not negatively affect the biomass activity. The glycol ethers' REs were high since the beginning of stage S-VI, 100% for M2P (Fig. 3b) and 77% for E2P (Fig. 3a) in both reactors, so no adaptation period was observed despite the biomass not being exposed to these compounds for more than 40 days.

At stage S-VII, the average total OLR was 45.9 kg COD $m^{-3}d^{-1}$ (Table 1), but the feeding composition was changed to a ternary mixture of ethanol, E2P, and M2P with a mass ratio of 7:1:2. In both reactors, the total RE was maintained at a high value, similar to the previous stage (Fig. 2). Moreover, M2P was completely removed despite its

increase in OLR, indicating that the levels of M2P concentration used in the experiment did not affect the performance of the reactors. Regarding the REs of E2P (Fig. 3a), these reached 76% and 80% for R1 and R2, respectively, until day 315 when the pall rings in the hybrid reactor (R2) were replaced by clean ones to avoid clogging problems in the filter media. After the replacement, the RE of E2P for R2 dropped to 65%, and the average concentration of VFA in the effluent increased from negligible values to 68.5 mg $CH_3COOH\ L^{-1}$, while in the EGSB reactor, it only amounted to 14.6 mg $CH_3COOH\ L^{-1}$. The decrease in the E2P's RE and the increase in VFA after the replacement of the packaging material showed that a not quite significant part of the R2 activity was related to the active biomass associated with the packed fraction of the reactor.

The methane average and standard deviation (SD) yield in R1 was $0.324 \pm 0.051\ m^3\ CH_4\ kg\ COD_{degraded}^{-1}$ and in R2, it was $0.318 \pm 0.049\ m^3\ CH_4\ kg\ COD_{degraded}^{-1}$, indicating no significant difference between both reactors in the methane production. The evolution of methane production can be found in the supplementary material (Fig. S.7). The average and SD proportion of methane in the biogas was 82.4 ± 2.6 % and 83.0 ± 2.9 % for R1 and R2, respectively, with a presence of H_2S below 130 ppm_v. Narra et al. (2014) reported a methane yield between 0.12 and 0.16 $m^3\ CH_4\ kg\ COD_{degraded}^{-1}$ when working with anaerobic hybrid reactors that treated the wastewater of mild alkali-treated rice straw in ethanol fermentation process. This low yield could be due to the different substrate used. Treating more similar substrates such as solvents that included ethanol, acetone, propanol, and methanol, Enright et al. (2009) obtained methane yields between 0.11 and 0.35 $m^3\ CH_4\ kg\ COD_{degraded}^{-1}$, within the range of those obtained in the present study for both reactors.

Since acetone was detected as a key intermediate product of anaerobic biodegradation of glycol ethers, an EGSB reactor was fed with this solvent and ethanol

(1:1 mass ratio) to shed light on the mechanism. Fig. 4 shows the evolution of the organic compounds detected in the effluent of R3. The acetone concentration was quite high in the beginning of the trial, but since day 9, the concentration gradually decreased, and the system practically reached complete degradation (98%) of this compound on day 21. These results showed that the biomass needed an adaptation period to remove acetone, as what occurred with E2P, but in this case, the adaptation period was shorter than in the case of E2P, indicating that acetone could be more readily biodegradable than E2P. Thus, experimental evidence seems to point out that the first step of the cleavage to alcohol and acetone could be considered the controlling step for the biodegradation of glycol ethers. This hypothesis would be reinforced, considering the detection of negligible amounts of acetone and/or isopropanol in the effluent found in the experimental series with R1 and R2 (these compounds were only briefly detected when the glycol ethers were first fed into the bioreactors). Furthermore, isopropanol was identified in the effluent of bioreactor R3, as shown in Fig. 4. The appearance of isopropanol in a system treating acetone was explained by Tonouchi (2004) and Zellner and Winter (1987), who concluded that isopropanol could be produced from acetone in the presence of hydrogen, with a reversible reaction. Additionally, Vermorel et al. (2017) observed a significant presence of acetone, besides isopropanol, in the effluent of a laboratory-scale anaerobic CSTR that treated synthetic wastewater with ethanol and isopropanol as organic substrates. This observation would support the hypothesis of the reversibility of the transformation between isopropanol and acetone.

3.2. Anaerobic mechanism of ether cleavage

The mechanism of the anaerobic ether cleavage is not well established yet, although it is suggested that intracellular enzymes depending on cobalamin are involved in this process (Frings et al., 1992). The most accepted mechanism implies a double H/OH

interchange (hydroxyl shift) resulting in the *gem*-diol that rapidly collapses to a carbonyl or a keto group (Speranza et al., 2002). As previously described, the identified byproducts during the first days of the biomass exposure to E2P were acetone and isopropanol, while acetone and methanol were identified in the case of M2P. By analogy with the hydroxyl-shift mechanism of ether excision for anaerobic ether cleavage (White et al., 1996), the theoretical byproducts should be ethanol and acetone from E2P degradation and methanol and acetone from M2P degradation (Lafita et al., 2015). In the case of E2P, no ethanol was observed in the effluent as a readily biodegradable substrate. In any case, as a main substrate, ethanol could not have been attributed as an intermediate product of degradation. On the other hand, the presence of isopropanol could be related to the formation of acetone as it was corroborated with the R3 reactor performance, as previously described. Thus, acetone together with the hydrogen can make the conversion to isopropanol possible, explaining the appearance of this compound in the effluent.

In the case of M2P, the presence of methanol was briefly detected in the effluent of both bioreactors (R1 and R2) after M2P was added to the influent. However, isopropanol was not observed because at this phase of the study, the biomass was already well adapted after more than 100 days of degrading E2P; thus, the formed isopropanol should be undetectable by the equipment. Therefore, the hypothetical mechanism schematized in Fig. 5 would explain the presence of the detected organics in the effluent of the reactors. Regarding acetone, its degradation to methane and CO_2 was reported to be the first case in which acetate is the only intermediate transferred between a fermenting bacterium and a methanogen (Platen and Shink, 1987). According to these authors, acetone is first carboxylated to acetoacetate by condensation with CO_2, from which acetate is formed and then transferred to *Methanosatea sp.* (formerly

Methanothrix sp.). Methanol can be directly utilized as a carbon and energy source by several species of methanogens and acetogens.

3.3.Biomass retention capacity of AHR and EGSB reactor

Granular and non-granular accumulated solids of each stage collected from the effluent of reactors R1 and R2 can be found in Table 3, and the evolution of total accumulated solids of both reactors are plotted in Fig. 6, which shows that during stage S-I, the solids in the effluent of R1 were considerably higher than in R2, reaching 73.2 g and 29.0 g of accumulated solids, respectively. In the beginning of S-II, the solids collected from both effluents were similar, but from day 133 to day 161, a granular sludge flotation occurred in R1. Therefore, the accumulated solids in the effluent increased considerably with respect to R2, where no granular sludge flotation was registered. Thus, during this stage, 255.0 g of solids were collected from R1 and only 109.0 g from R2. During stage S-III, the solids in the effluent were also slightly higher in the EGSB reactor (28.3 g) than in the AHR (20.1 g). Anaerobic granular sludge flotation was observed in the systems (stage S-IV), reaching 45.9 g and 36.5 g of accumulated solids for R1 and R2, respectively. It is important to clarify that the sludge flotation observed has not been a total breakdown that had led to a complete wash out as those described extensively in literature (Macarie et al., 2017; Yoda and Nishimura, 1997; Lafita et al., 2015). In fact, if the leakage of solids is quantified in reference to the effluent flow, the average outlet concentration of reactor R1 in stage S-II (stage and reactor most unfavorable in terms of leakage of solids) is 228 g SST m^{-3} which can be considered as a normal value for this kind of anaerobic systems.

After the shut-down period (S-V), when feeding was resumed, 20.0 g of solids in total were collected from R2, while only 13.8 g were washed out from R1 during the

first week of stage S-VI. This unusually larger amount of biomass washed out from R2 could be explained by the lysis that occurred during the starvation period; as more biomass was retained by R2 than by R1, more granules were proportionally broken in R2, yielding greater biomass washout. The last two stages also showed greater washout from R1, in accordance with the global experimental trend. At the end of the experiment, the accumulated solids collected in the effluent were 563.2 g and 293.7 g for R1 and R2, respectively. This difference shows the AHR's (R2) higher capacity to retain biomass, supporting the study of Borja et al. (1995), who reported that the packing of a hybrid anaerobic reactor significantly enhanced the retention of active biomass treating slaughterhouse wastewater. Furthermore, Guiot and van den Berg (1985) demonstrated that the AHR's biomass retention capacity was between 1.5 and 2.8 times higher than those of other bioreactor configurations such as the UASB or the anaerobic baffled reactor.

The significant improvement in the biomass retention capacity of the hybrid system (R2) was not reflected in its performance since no significant differences in the REs of the different compounds were observed over the experimental period. Only slightly more stable and lower concentrations of VFA were observed in the effluent of R2.

In previous research, episodes of biomass flotation in the AHR had been attributed to lipid adsorption in granules (Belançon et al., 2010), but as no lipids were present in the feeding of the present study, the biomass flotation produced during stages S-II and S-IV had to be caused by other factors. Furthermore, in the AHR, no biomass flotation was observed during stage S-II, probably due to the higher capacity to retain biomass. However, the episode of anaerobic granular sludge flotation observed in the AHR at stage S-IV indicated that the filter was highly clogged and close to its maximum retention capacity. This fact can also be observed in Table 3 where, in addition to the

absolute values of the leakage of solids from each reactor, the ratios of the solids in the effluent between control EGSB (R1) and AHR (R2) have been included (granular, non-granular, and total solids). The ratio of the total solids (R1/R2) decreased over the experimental period from 2.5 at stage S-I to 1.1 at stage S-VI, and then, at stage S-VII, it increased again to 2.11 when the packing material was replaced by new and clean polyethylene rings. The decrease in the ratio at every stage was even higher if only granular solids were considered—from 5.6 at stage S-II to 1.1 at stage S-VI, and then, after the replacement of the packing material, the ratio increased again to 4.0 at stage S-VII. In contrast, the non-granular solids (suspended solids) collected from both systems were not significantly different at each of the stages studied (the ratio varied from 1.2 to 0.6). These results indicated that the packing material selectively retained the granular biomass versus the solids in suspension. Furthermore, the recovered capacity to retain the solids after the replacement of the packing material suggested that the filter was clogged; thus, to maintain proper performance, it should be periodically cleaned or replaced. It seems that the leakage of granules would be related with regular discharge of surplus biomass in normal operation as reported by Pereboom et al (1994). Likewise, Hulsshoff Pol et al. (2014) affirmed that light sludge is separated from mature granules using the rapid upward velocity of EGSB reactor. This selective wash-out, resulting in an increased growth of retained (heavier) sludge agglomerates would be crucial for the granulation process (Lim and Kim, 2004). Thus, it is interesting to highlight that the observed leakage throughout the experimental series has not had significant influence with the performance of the EGSB reactor. Even more interesting is that no difference has been observed between the performance of both systems (control EGSB and AHR) for any stage, which would indicate that the activity of the excess sludge (that was

retained in the case of the AHR system) was practically negligible and no comparable to the activity of retained granules.

As a resume, the main advantages and disadvantages of the AHR in comparison with the EGSB observed in this long-term study have been synthesized in Table 4.

4. Practical applications and future perspective

The present work is part of a broader research related to the industrial implementation of anaerobic bioscrubber, an innovative system for the control of VOC emissions that is capable to transform solvents into bioenergy (Waalkens et al., 2015). A pilot-scale study of this technology, composed of a packed scrubber and an EGSB reactor of 8.7 m^3 treating on-site emissions from a packaging printing facility, showed to be an effective solution for VOC emission control (Bravo et al., 2017). In this context, the results obtained from the present work, based on the acquired knowledge about the retention of the biomass and the anaerobic degradation of glycol-ethers, will allow extending the horizons of practical application of the anaerobic bioscrubber.

With regard to the packing printing sector, given the general tendency of packaging manufacturing firms to move nearer to their customers, investment potentials for developing countries abound in this sector. The appeal of products from the developing Southern economies to their Northern and Western markets will have to be extended to their packaging (FAO, 2014). As a result of this sector's trend to install the facilities at point of origin, the demand for these VOCs control systems is expected to be on the rise.

The current research lines are focused in the study of the dynamics of the microbial communities and their interrelation with the bioreactor operation, including its response to starvation periods. On the other hand, the increase of the feasibility of this technology by using a more available and economic source of anaerobic sludge (e.g. from municipal waste water treatment plant) is being investigated.

5. Conclusions

Granular anaerobic treatment was shown to be a feasible technology for the biodegradation of ethanol-glycol ether mixtures such as E2P and M2P after a short period of acclimatization. Of the two systems tested, the AHR showed a higher solid retention capacity than the EGSB but did not result in the AHR's improved performance. Both systems could completely remove M2P (M2P OLR of 8.3 kg COD m^{-3} d^{-1}) and 70% of the E2P (E2P OLR of 10.6 kg COD m^{-3} d^{-1}). The detected byproducts allowed clarifying the degradation pathways of the studied glycol ethers.

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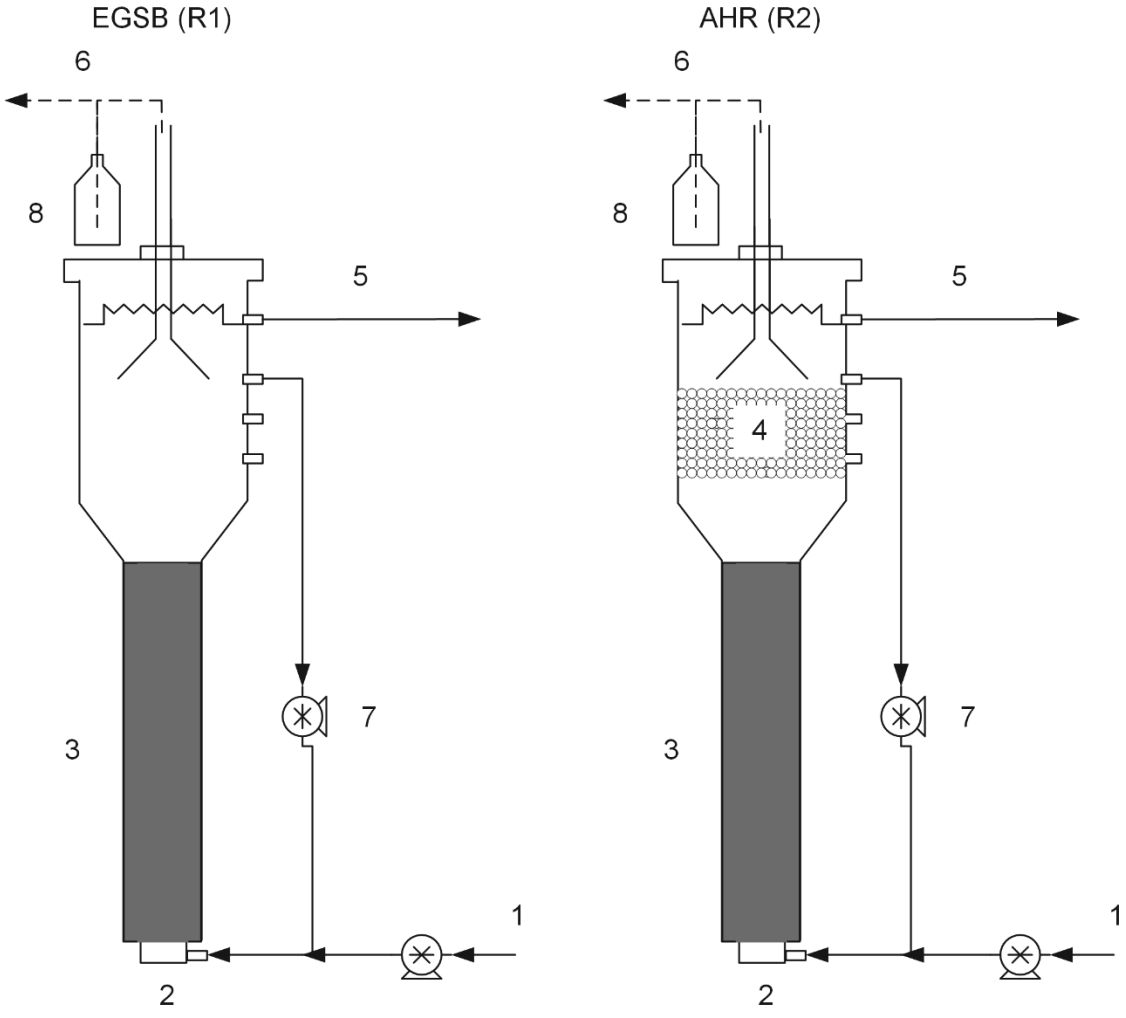

Fig. 1. Diagram of the EGSB reactor (R1) and AHR (R2) with numbered parts: 1) Feed, 2) Glass balls, 3) Effective volume, 4) Packing material, 5) Effluent, 6) Biogas, 7) Recirculation, 8) Gas seal.

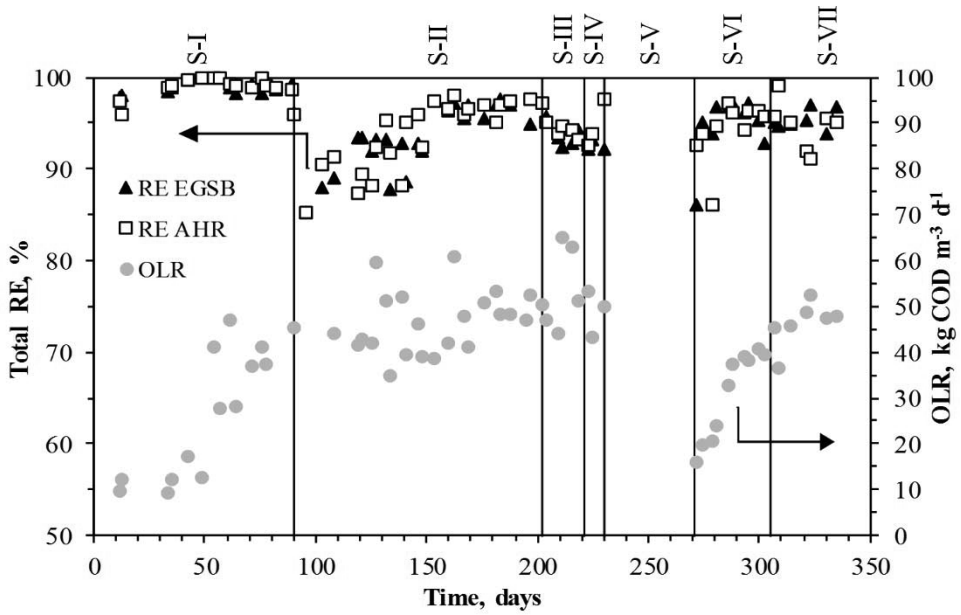

Fig. 2. Evolution of total OLR and COD removal efficiency of EGSB reactor (R1) and AHR (R2).

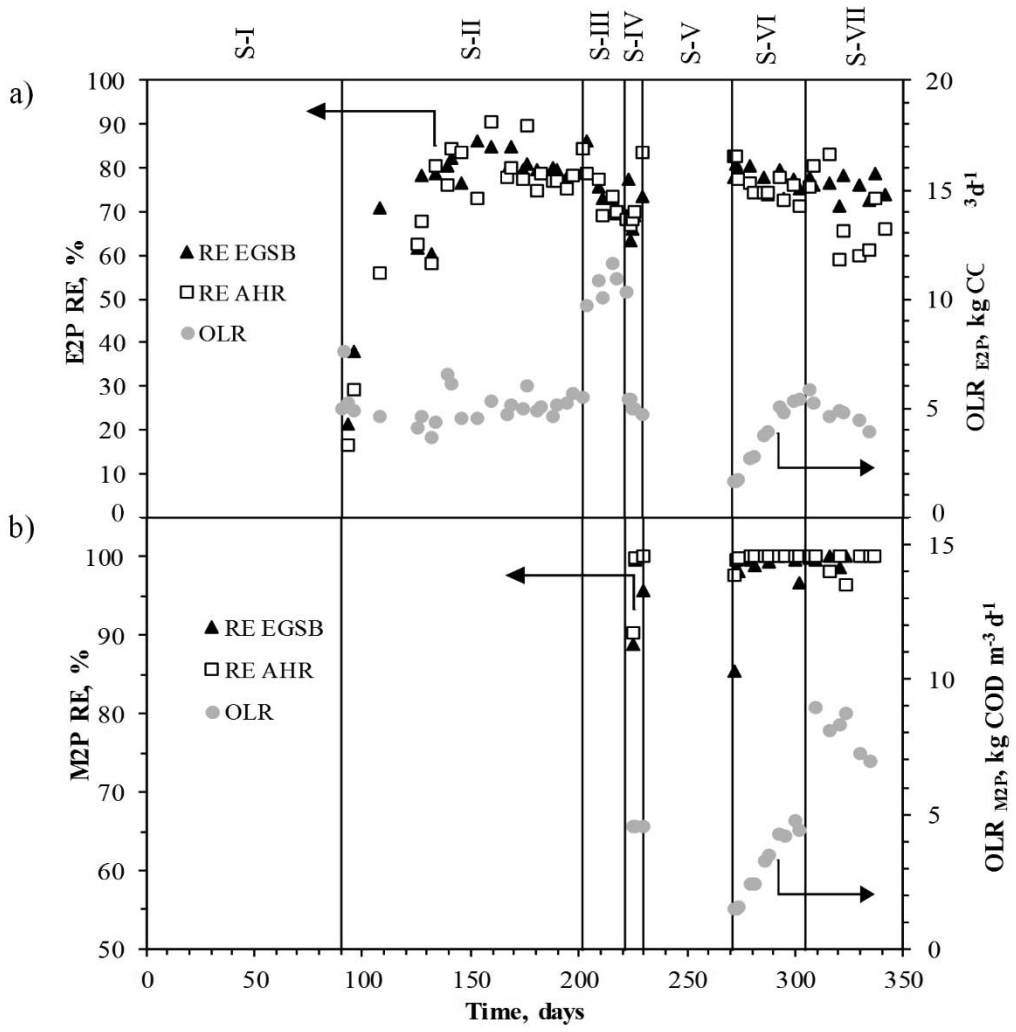

Fig. 3. Evolution of glycol ethers OLR and COD removal efficiency of EGSB reactor (R1) and AHR (R2), a) E2P OLR and E2P removal efficiency, b) M2P OLR and M2P removal efficiency.

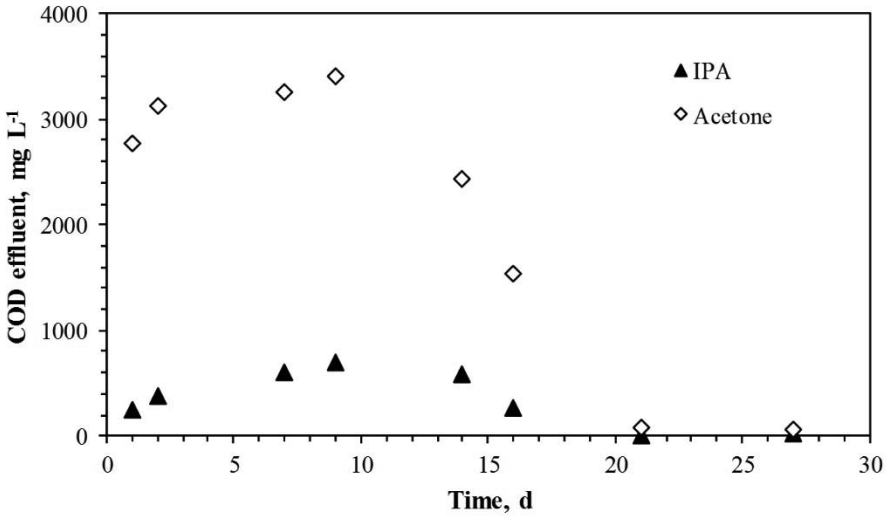

Fig. 4. Evolution of intermediate compounds' concentration found in the effluent in the reactor R3 treating the ethanol-acetone mixture with an average inlet COD concentration of 11.2 ± 1.5 g L^{-1}.

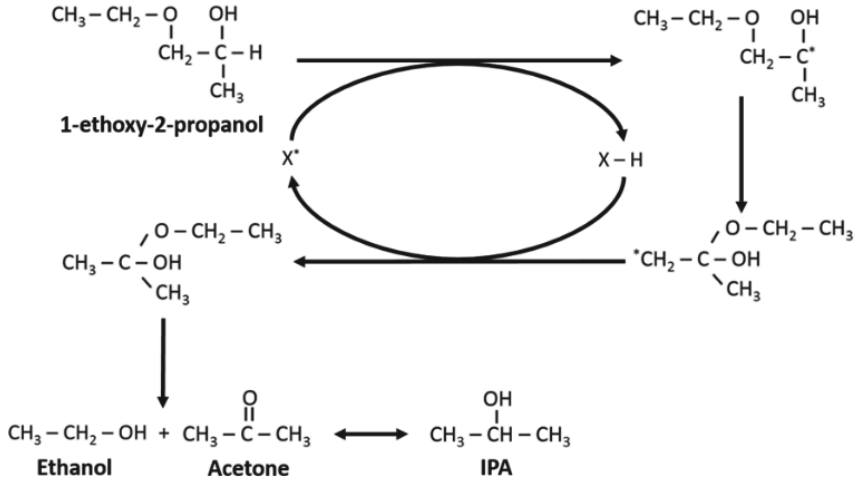

Fig. 5. Proposed mechanism for the anaerobic degradation of 1-ethoxy-2-propanol, including isopropanol (IPA) formation from acetone.

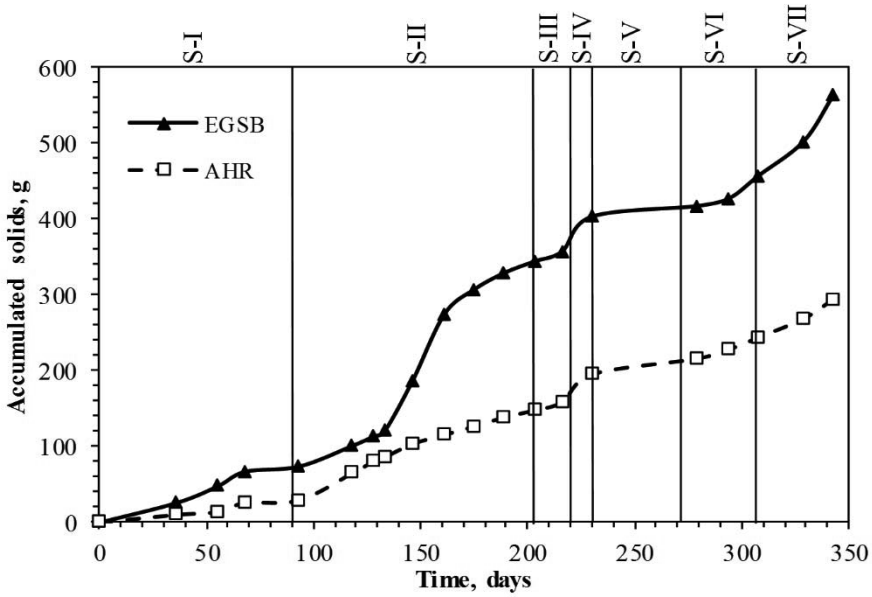

Fig. 6. Accumulated suspended solids in the effluent of EGSB reactor (R1) and AHR (R2).

Table 1. Operation conditions for the EGSB reactor (R1) and AHR (R2). Average values and Standard deviation.

Stage	S-I	S-II	S-III	S-IV	S-V	S-VI	S-VII
Time, d	0-90	91-202	203-221	222-230	231-271	272-305	306-335
COD, g L^{-1}	3.9 to 18.8	18.9 ± 3.0	21.6 ± 3.8	19.5 ± 2.1	-	6.3 to 16.3	18.5 ± 1.9
OLR, kg COD m^{-3} d^{-1}	9.1 to 47.0	47.4 ± 7.4	54.1 ± 9.5	48.8 ± 5.1	-	15.9 to 40.7	46.2 ± 4.8
E2P, g COD L^{-1}	0.0	2.0 ± 0.3	4.2 ± 0.3	2.4 ± 0.9	-	0.7 to 2.1	1.9 ± 0.2
E2P OLR, kg COD m^{-3} d^{-1}	0.0	5.1 ± 0.8	10.6 ± 0.7	6.1 ± 2.3	-	1.7 to 5.3	4.8 ± 0.6
M2P, g COD L^{-1}	0.0	0.0	0.0	1.9 ± 0.1	-	0.6 to 1.9	3.3 ± 0.4
M2P OLR, kg COD m^{-3} d^{-1}	0.0	0.0	0.0	4.8 ± 0.2	-	1.5 to 4.7	8.3 ± 1.1

Table 2. Macronutrient and micronutrient supplementation influent.

Compound	Dose, mg g^{-1} COD	Compound	Dose, mg g^{-1} COD
NH_4Cl	15.7	H_3BO_3	0.1143
Yeast extract	7.5	EDTANa	0.100
KCl	6.1	$(NH_4)_2Mo_7O_{24}·4H_2O$	0.0625
$(NH_4)_2HPO_4$	5.6	Al_2O_3	0.0595
$FeCl_3·6H_2O$	0.4208	$NiSO_4·6H_2O$	0.0447
$CoCl_2·6H_2O$	0.1615	$CuCl_2·2H_2O$	0.0134
$MnCl_2·4H_2O$	0.1441	$ZnSO_4·7H_2O$	0.132

Table 3. Accumulated suspended solids (SS) in the effluent from each reactor in the different stages

	Stage:	S-I	S-II	S-III	S-IV	S-VI	S-VII
Non-granular solids (g)	EGSB	73,1	101,2	17,3	6,7	15,0	15,4
	AHR	29,0	81,5	16,6	7,1	12,2	27,5
Granular solids (g)	EGSB	< 0,1	153,8	11,0	39,2	39,1	91,3
	AHR	< 0,1	27,5	3,5	29,4	36,5	22,9
Ratio SS_{EGSB}/SS_{AHR}	Non-granular	2,52	1,24	1,04	0,94	1,24	0,56
	Granular	-	5,59	3,14	1,34	1,07	3,98
	Total	2,52	2,34	1,40	1,26	1,11	2,12

Table 4. Advantages/disadvantages of the AHR in comparison with the EGSB system.

Advantages
- Higher solid retention capacity
Disadvantages
- AHR increases to some extent the operational complexity of the treatment
- Increasing biomass retention in the upper packed filter can lead to clogging
- Slight increase in the cost of capital of the system

Supplementary Material:

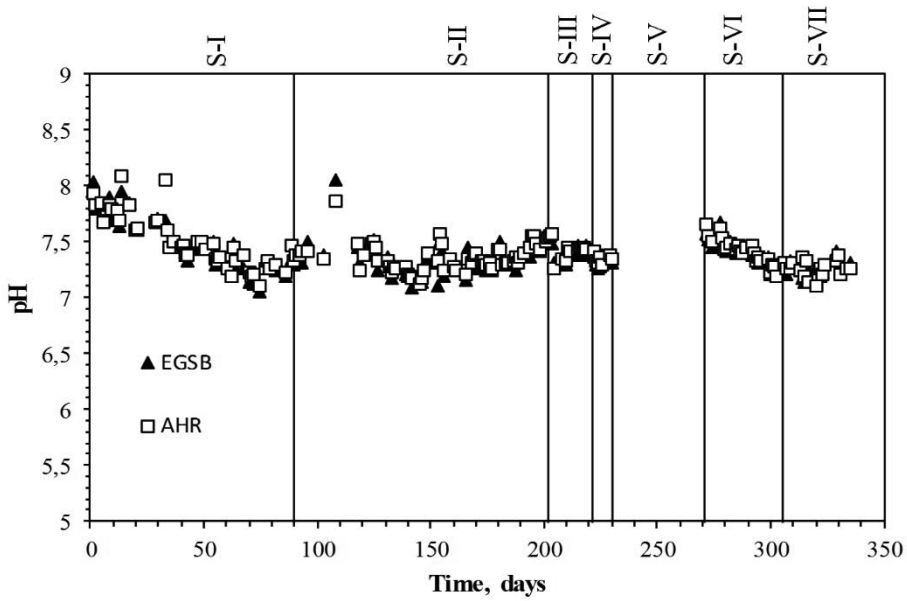

Fig. S.1. Evolution of pH in the effluent of the EGSB reactor (R1) and AHR (R2).

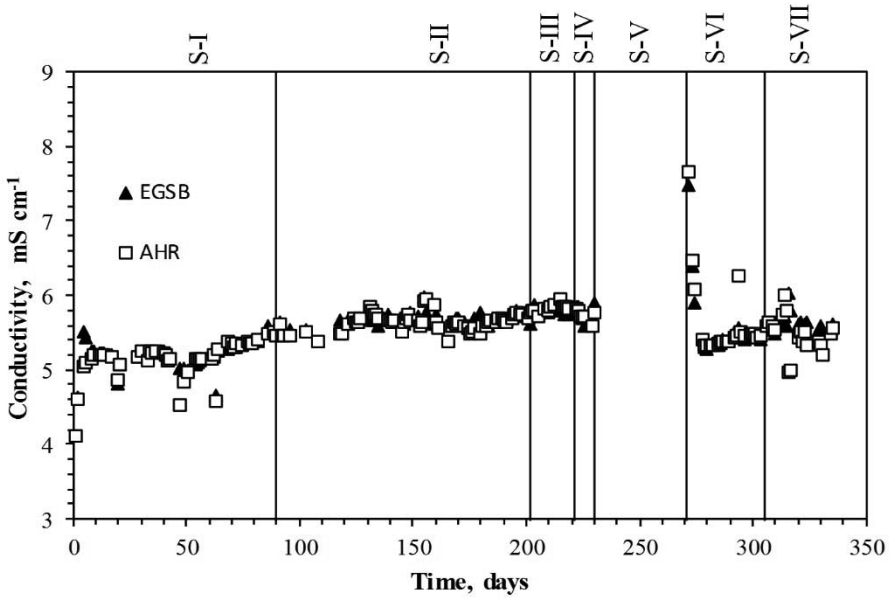

Fig. S.2. Evolution of contuctivity in the effluent of the EGSB reactor (R1) and AHR (R2).

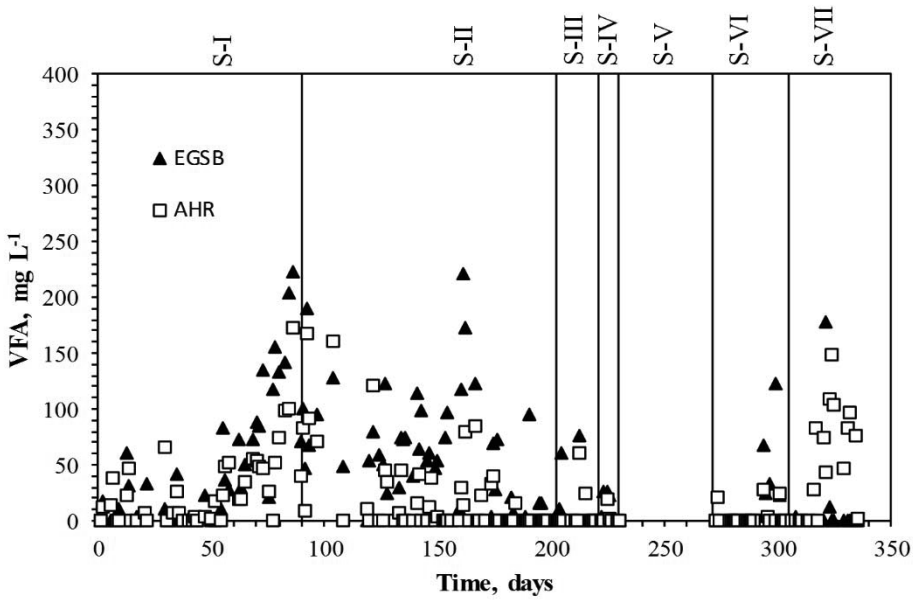

Fig. S.3. Evolution of volatile fatty acid (VFA) concentration in the effluent of the EGSB reactor (R1) and AHR (R2).

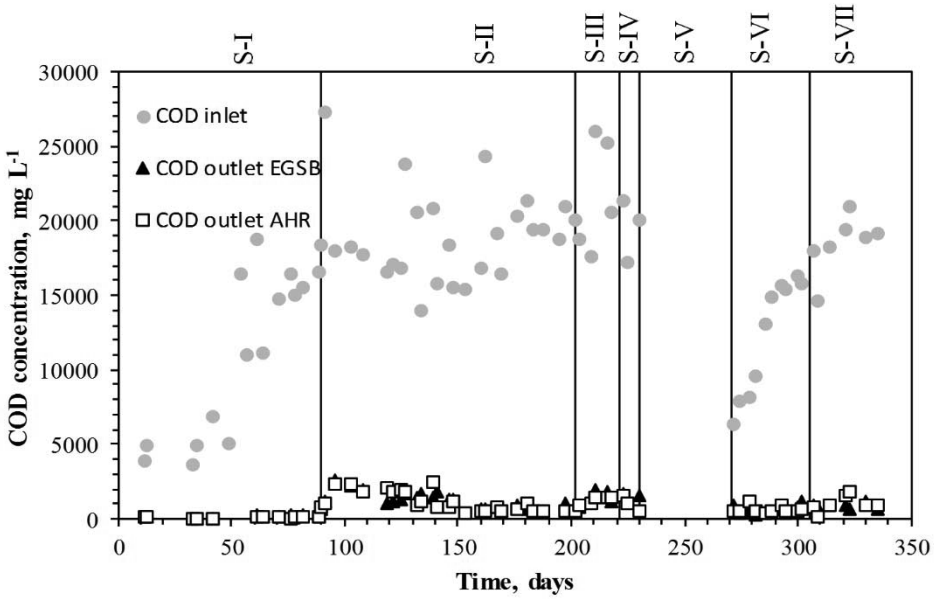

Fig. S.4. Evolution of COD inlet concentration for both reactors and the COD outlet concentration in the effluent of the EGSB reactor (R1) and AHR (R2).

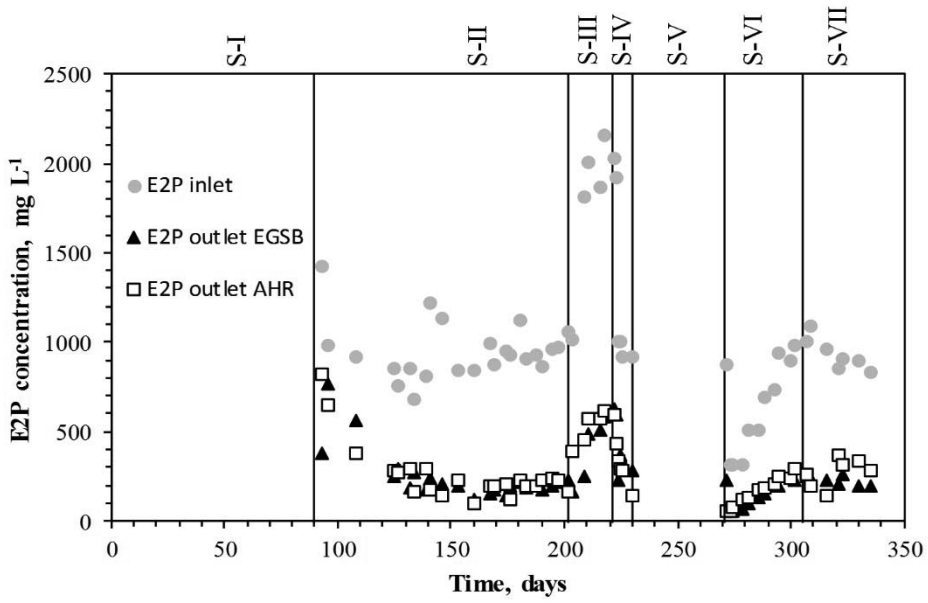

Fig. S.5. Evolution of E2P inlet concentration for both reactors and the E2P outlet concentration in the effluent of the EGSB (R1) and AHR (R2).

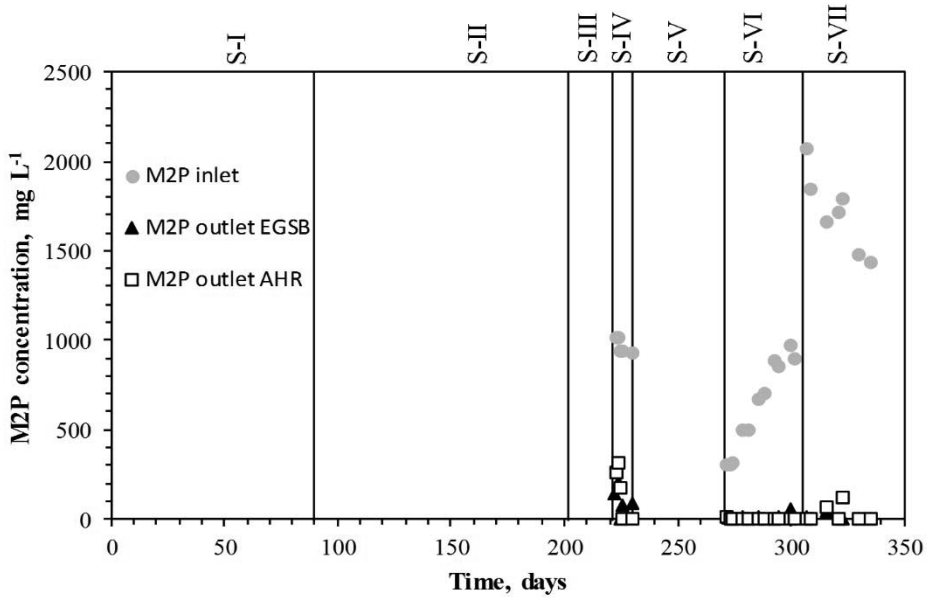

Fig. S.6. Evolution of M2P inlet concentration for both reactors and the M2P outlet concentration in the effluent of the EGSB reactor (R1) and AHR (R2).

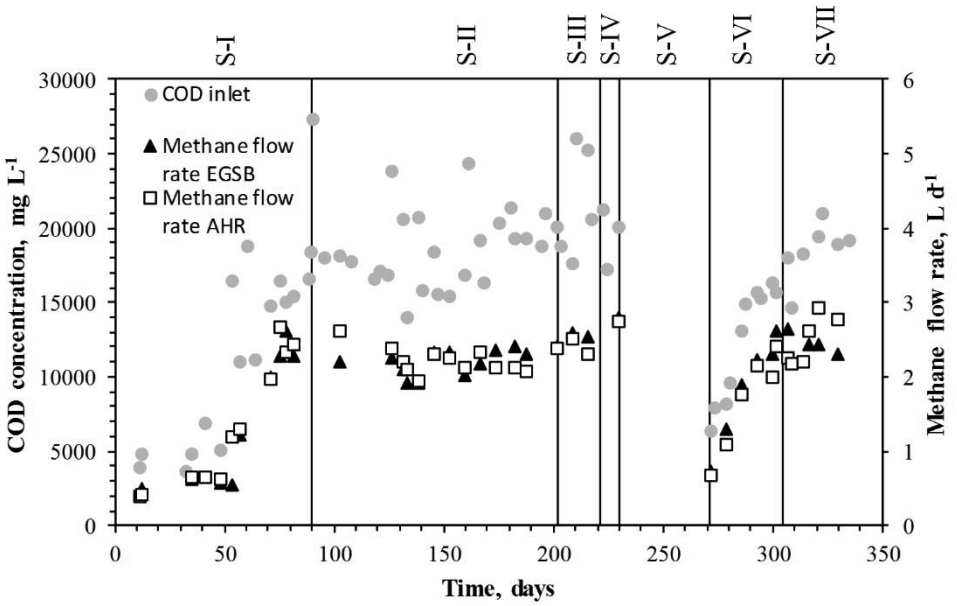

Fig. S.7. Evolution of the methane flow rate of the EGSB reactor (R1) and AHR (R2) and COD inlet concentration for both reactors.