Control of VOCs from printing press air emissions by anaerobic bioscrubber: performance and microbial community of an on-site pilot unit

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Abstract

A novel process consisted of an anaerobic bioscrubber was studied at the field scale for the removal of volatile organic compounds (VOCs) emitted from a printing press facility. The pilot unit worked under high fluctuating waste gas emissions containing ethanol, ethyl acetate, and 1-ethoxy-2-propanol as main pollutants, with airflows ranging between 184 and 1253 $m^3\ h^{-1}$ and an average concentration of 1126 ± 470 mg-C Nm^{-3}. Three scrubber configurations (cross-flow and vertical-flow packings and spray tower) were tested, and cross-flow packing was found to be the best one. For this packing, daily average values of VOC removal efficiency ranged between 83% and 93% for liquid to air volume ratios between $3.5 \cdot 10^{-3}$ and $9.1 \cdot 10^{-3}$. Biomass growth was prevented by periodical chemical cleaning; the average pressure drop was 165 Pa m^{-1}. Rapid initiation of anaerobic degradation was achieved by using granular sludge from a brewery wastewater treatment plant. Despite the intermittent and fluctuating organic load, the expanded granular sludge bed reactor showed an excellent level of performance, reaching removal efficiencies of 93±5% at 25.1±3.2ºC, with biogas methane content of 94±3% in volume. Volatile fatty acid concentration was as low as 200 mg acetic acid L^{-1} by treating daily average organic loads up to 3.0 kg COD h^{-1}, equivalent to 24 kg COD m^{-3} bed d^{-1}. The denaturing gradient gel electrophoresis (DGGE) results revealed the initial shift of the domains Archaea and Bacteria associated with the limitation of the carbon source to a few organic solvents. The Archaea domain was more sensitive, resulting in a drop of the Shannon index from 1.07 to 0.41 in the first 123 days. Among Archaea, the predominance of *Methanosaeta* persisted throughout the experimental period. The increase in the proportion of *Methanospirillum* and *Methanobacterium* sp. was linked to the spontaneous variations of operating

temperature and load, respectively. Among Bacteria, high levels of ethanol degraders (*Geobacter* and *Pelobacter* sp.) were observed during the trial.

Keywords

Air emissions, Anaerobic bioscrubber, Denaturing gradient gel electrophoresis, Expanded granular sludge bed reactor, Volatile organic compounds.

1. Introduction

The flexographic sector represents 17% of the European printing sector, contributing around 1.7% of the total turnover in 2003 (Ernst & Young, 2007). The consumed solvents are mainly oxygenated compounds, such as ethanol, ethyl acetate, 1-propanol, 2-propanol, 1-methoxy-2-propanol, n-propyl acetate, 1-methoxy-2-propyl acetate, acetone, and 1-butanol (Granström et al., 2002). Flexographic air emissions are characterized by high flow rates and low volatile organic compound (VOC) concentrations (Sempere et al., 2012), with temperatures ranging from 40 to 70°C and relative humidity varying from 5 to 15% (Rothenbuhler et al., 1995). According to the European Directive on Industrial Emissions (Council Directive 2010/75/EC), these air emissions must be controlled.

Biotreatments represent well-developed air pollution control techniques for removing VOCs in these conditions (Deshusses, 1997). Among biotreatments, bioscrubbers can handle higher gas loads than biotrickling filters and biofilters, and their capacity is up to 3000–4000 $m^3\ m^{-2}\ h^{-1}$ (Kennes et al., 2009). However, there are few available studies on aerobic bioscrubbers. Le Cloriec et al. (2001) reported removal efficiencies of 90.1–100% in a laboratory-scale bioscrubber, with liquid to air ratios ranging between $0.6 \cdot 10^{-3}$ and $2 \cdot 10^{-3}$, and with ethanol concentration in waste gas from 18.8 to 291.7 mg-C m^{-3}. Granström et al. (2002) investigated an onsite pilot-scale system for the treatment of waste gas from printing processes. In this study, the major VOC of the waste air was ethanol, with smaller amounts of ethyl acetate, 1-propanol, 2-propanol, 1 methoxy-2-propanol, and 3-ethoxy-1-propanol. The flow of the waste gases varied from 1.68 to 3.73 $m^3\ h^{-1}$, with 99.6% VOC removal efficiency, excluding evaporation losses. Nevertheless, aerobic bioscrubbers are still not widespread within

the biotreatment market due to the high energy consumption of aerobic bioreactors. In contrast, anaerobic bioscrubber could be an alternative for recycling waste gases into bioenergy, thereby resulting in a positive net energy balance.

To the best of our knowledge, no previous literature exists on the use of anaerobic bioscrubbers for the treatment of VOC waste gases, although the anaerobic degradation of solvents, such as alcohols (Eichler and Schink, 1985; Widdel, 1986; Zellner and Winter, 1987) or esters (Oktem et al., 2008; Yanti et al., 2014) is well documented. Recently, Lafita et al. (2015) demonstrated that anaerobic degradation of glycol ethers is feasible by reporting the treatment of synthetic packaging wastewater, which contains a mixture of ethanol and 1-methoxy-2-propanol in a mass ratio of 4:1. These authors achieved removal efficiencies of up to 94% at 18ºC and 97% at 25ºC in an expanded granular sludge bed (EGSB) reactor, with organic loading rates of methoxy-2-propanol of 6.4 and 9.3 kg COD m^{-3} d^{-1}, respectively.

The anaerobic degradation of organic solvents in granular sludge reactors relies on the microbial population developed in the anaerobic granules, which should in turn maintain its physical integrity. Leclerc et al. (2004) studied the microbial populations of 44 anaerobic digesters treating effluents from several sectors. These authors indicated that the occurrence and prevalence of the different species are influenced by the running and environmental conditions. Anaerobic granulated sludge coming from breweries is a common source of biomass for other industrial sectors. In this sense, the study of the evolution of the microbial population is an interesting tool to investigate the effect that a change in the substrate composition could have on the feasibility and robustness of the anaerobic degradation of solvents.

The characterization of microbial populations can be carried out using molecular biology tools, such as denaturing gradient gel electrophoresis (DGGE). This is based on

the electrophoretic separation of polymerase chain reaction (PCR) products of the same length, but with different sequences, on a linear denaturing gradient polyacrylamide gel (Muyzer and Ramsing, 1995). DGGE has been applied to evaluate the microbial diversity of anaerobic reactors, such as an upflow anaerobic sludge blanket (UASB) reactor treating brewery wastewater; this study showed that the dominant archaeal bands were closely related to *Methanosaeta* and *Methanobacterium* (Chan et al., 2001). The DGGE technique has also shown that the microbial population of a UASB treating wastewater from an unbleached pulp plant persisted throughout the experimental period (Buzzini et al., 2006). DGGE studies can also demonstrate the importance of environmental conditions in the diversity of microbial populations; for example, LaPara et al. (2000) indicated that a thermophilic reactor showed less biodiversity than a mesophilic one by treating wastewater from a pharmaceutical facility.

The present study provides the first successful example of an on-site pilot plant of anaerobic bioscrubbers controlling VOC emissions from a flexographic printing facility (Waalkens et al., 2015). The purposes of our work were as follows: (1) to evaluate the best scrubber configuration to achieve high VOC removal efficiencies, and at same time, control pressure drop; (2) to determine the maximum organic load that the EGSB can handle under intermittent and variable waste gas emissions; and (3) to study the dynamics of the microbial community of the EGSB reactor inoculated with granular sludge from a brewery anaerobic reactor using the DGGE technique.

2. Material and methods

2.1 Anaerobic bioscrubber setup

The pilot plant was provided by Pure Air Solutions (Heerenveen, The Netherlands) and was operated on-site in Altacel Transparant Verpakkingsind (Weesp, The Netherlands) by treating a fraction of its air emissions. The flexographic site operates on a two-shift (16 h) basis from Monday to Friday and on a one-shift (8 h) basis on Saturday. The pilot plant comprises a variable-speed fan with a maximum flow of 1500 m^3 h^{-1}, as well as several centrifugal pumps. The two main units were the scrubber and the anaerobic reactor (see Graphical Abstract). The scrubber unit had a total height of 3.06 m and a diameter of 0.5 m. The available height for the packing material was 2.0 m. The scrubber unit was assembled onto a bottom tank of 2 m^3 in volume. The anaerobic reactor had a total height of 5.08 m and diameter of 1.59 m, with an effective water volume of 8.7 m^3. Two intermediate tanks completed the setup; resulting in 16 m^3 of total effective water volume.

The scrubber was operated in the countercurrent mode during the working hours of the facility; VOC-polluted air coming from the factory was introduced to the bottom by the blower, and the water was sprayed from the top and collected in the bottom tank. From there, it flowed to an intermediate tank for supplementation with macronutrients (N, P, S, K) and sodium carbonate for pH control prior to pumping it to the anaerobic reactor for solvent degradation. Ca, Mg, trace metals (B, Co, Cu, Fe, Mn, Mo, Ni, Se, Zn), and yeast extract were discontinuously supplemented. The anaerobic reactor consisted of an EGSB operated at 3 h of hydraulic residence time. The EGSB was filled with granular sludge from an internal circulation (IC) reactor treating brewery wastewater (Heineken, The Netherlands) without further acclimation to simulate

operational protocols at the industrial scale. The expansion of the granular bed to 3 m^3 was achieved by mixing the influent water with 50% of the effluent of the reactor; the upflow velocity was kept constant at 3 m h^{-1}. The pilot unit worked in water-closed recirculation, with <10% daily water renewal. The daily purge was done overnight when no biogas production occurred. The plant setup was equipped with a programmable logic controller (PLC) with Twinsoft software (Servelec Technologies, United Kingdom) to monitor and control the parameters, such as the air and liquid flowrates, water and air temperatures, pH, conductivity, and water level in the tanks.

A flame ionization detector (FID) analyzer (model RS 53-T, Ratfisch Analysensysteme, Germany) continuously monitored the VOC concentration in the inlet and outlet of the gas phase. The composition of the inlet and outlet gases was measured by carbon sorbent tubes and post Gas Chromatography analysis. Biogas production was continuously measured by a gas meter (Bellows-BG 4 Gasmeters, Ritter, Germany), and its composition was determined by a dual-wavelength optical infrared analyzer (Combimass GA-m, Binder, Germany). The main parameters of the liquid phase were monitored twice a week with photometric commercial kits as follows: chemical oxygen demand (COD); volatile fatty acids (VFAs); nutrients ($N\text{-}NH_4^+$ and $P\text{-}PO_4^3$) with LCK 014, LCK 365, LCK 303, and LCK 348 kits from HACH Lange GmbH (Germany); and alkalinity with a titrimetric kit (MColortest™, Merck Millipore, Germany).

The pilot unit was operated for 484 days. The experimentation was divided into five stages characterized by a change in the scrubber configuration with the aim to evaluate the best one in terms of VOC removal. Table 1 summarizes the main operational conditions. Two packing materials were used, as follows: a cross-flow packing material, Packing A, with a 150 m^2 m^{-3} specific surface area (cross-fluted flow fills, KFP 319/619, GEA, Germany); and a vertical-flow packing material, Packing B, with 125

m^2 m^{-3} specific surface area (vertical-flow fills, KVP 323/623, GEA, Germany). The scrubber unit was also tested as a spray column, removing the packing material and installing three nozzles (MP156N 60º, BETE, USA) spaced 55 cm apart. Packing material A was used in stages 1 and 5; packing material B was used in stages 2 and 4; and the spray column was tested in stage 3. Several liquid to air volume ratios in the range of $1.9 \cdot 10^{-3}$–$10.1 \cdot 10^{-3}$ were tested with the aim of minimizing the recirculated water flow to the anaerobic reactor. The organic load (OL) to the anaerobic reactor was set by the operation of the scrubber. Six biomass samples were taken from a port located 1.05 m from the bottom of the EGSB. Sampling events are shown in Table 1.

<< Table 1 >>

2.2 Microbial community analysis

DNA from each sample was extracted with a Power Soil Isolation Kit (Mo Bio Laboratories, USA) using the supplier's protocol. DNA concentration and purity were measured using NanoDrop® (Thermo Scientific, USA). Extracted DNA was stored at –20°C for the analysis. To amplify 16S rDNA, two universal primer sets were used, as follows: F357-GC and R518 for bacterial 16S and F787-GC and R1059 for archaeal 16S. The PCR amplification was conducted according to the following protocol: 20 cycles of 94°C for 1 min, 65°C for 1 min, 72°C for 0.5 min, 10 cycles of 94°C for 1 min, 55°C for 1 min, 72°C for 0.5 min, and a final extension at 72°C for 10 min. After amplification, the PCR products were electrophoresed in 0.5% (w/v) agarose gel to evaluate the extent of amplification. The PCR product generated from each sample was separated on an 8% acrylamide gel with a linear denaturant gradient increasing from 20% to 35% using the KuroGel Verti 2020 DGGE System (VWR International Eurolab, Spain). DGGE was performed using 20 µl of PCR product in 1× Tris-acetate-EDTA

buffer at 60 °C with a sequence of 50 V for 5 min, 150 V for 120 min, and 200 V for 60 min. The DGGE gels were visualized in the MiniBIS Pro System (DNR Bio-Imaging System Ltd., Spain). Predominant bands were excised and resuspended in 30 µl of sterilized Mili-Q water, and then bands were stored at 4°C, allowing DNA to migrate to the liquid. The eluted DNA was reamplified by PCR with the same conditions as the previous PCR to the DGGE. The PCR product was purified with a High Pure PCR Product Purification Kit (Roche, Spain). Successfully reamplified and purified PCR products were sequenced by using an automated DNA analyzer (3730 KL DNA Analyzer, Applied Biosystems, Spain). Sequences were analyzed with MEGA 5.0 and then compared with those available from the NCBI GenBank using BLAST software.

2.3 Granule size distribution

The particle size distribution of biomass samples was measured to monitor the evolution of the granule size and to check the granule integrity by a Malvern Mastersizer 2000 instrument (Worcestershire, UK) with a detection range of 0.02–2000 µm. Samples from days 0, 238, and 430 (S-0, S-3, and S-6, Table 1) were analyzed.

3. Results and discussion

3.1 Characterization of air emissions

The air emissions showed a high variability associated with the number of printing presses in operation (see Fig. 1a: Daily average for the whole trial and Fig. 5a: Instantaneous pattern). Airflow ranged between 184 and 1253 m^3 h^{-1}, and the average daily VOC concentration was 1129 mg-C Nm^{-3} with a standard deviation of 460 mg-C Nm^{-3}. The detected compounds in major proportions by weight were ethanol (60–65%),

ethyl acetate (20–25%) and ethoxy propanol (10–15%); other minor compounds were 2-propanol (0.5–1%), 2-propyl acetate (0–0.5%), 1-propyl acetate (0–3%), 4-hydroxy-4-methyl-2-pentanone (0.2–5%) and 1-propanol (0–2%).

3.2 The scrubber unit

Scrubber performance was evaluated in terms of VOC removal efficiency (RE_{VOC}). Results of the online monitoring of the scrubber are shown in Fig. 1a, where the daily average RE_{VOC} is plotted along with the daily averages of the inlet and outlet VOC concentrations in the gas phase. High efficiencies were reached in stage 1 with packing A, which was usually over 83%. A maximum RE_{VOC} of 97% was achieved on day 75, at the maximum tested liquid to air volume ratio ($9.1 \cdot 10^{-3}$), when the inlet concentration was 2000 mg-C Nm^{-3}. The change to packing B (stage 2) caused a decrease in RE_{VOC}; the maximum value was 88% (day 129), even though a higher liquid to air ratio ($10.1 \cdot 10^{-3}$) was applied than in stage 1. The outlet emissions with packing B (stages 2 and 4) reached values up to 516 mg-C Nm^{-3} (day 227), while the maximum leak in stage 1 was 310 mg-C Nm^{-3} (day 52). Regarding the removal of the main pollutants, ethanol and 1-ethoxy-2-propanol were almost fully removed with packing A, while with packing B, the removals decreased to values between 80% and 94% (higher values for higher tested liquid to air ratios). In both packings, ethyl acetate was removed to a lesser extent due to its lower water solubility.

By testing the spray tower configuration (stage 3), RE_{VOC} dropped to values of 49–65% with outlet concentrations higher than 135 mg-C Nm^{-3}, even for inlet emissions as low as 310 mg-C Nm^{-3} (day 157). In this case, no effective removal of ethyl acetate was observed (< 60%), with moderate transfer to water of ethanol and 1-ethoxy-2-propanol (70–88%).

The achieved RE_{VOC} at the maximum liquid to air ratio ($3.7 \cdot 10^{-3}$) in stage 3 was 40% lower than that obtained with packing A and 33% lower than that obtained with packing B at similar liquid to air volume ratio. The experimental results indicated that unfeasible high water flow rates would be required for the spray tower configuration to fulfill the compliance levels compared with the use of a packing bed. In the case of packing A, a drop of about 9% in RE_{VOC} was achieved in stage 5 in comparison with stage 1 at similar liquid to air ratios. This result was attributed to the creation of preferential pathways, probably due to the self-assembly of the packing on day 266. In stage 5, a periodical chemical cleaning of the packing material was set up, allowing the pressure drop to maintain at 165 Pa m^{-1} (average value). The long-term testing period of stage 5 demonstrated that it is feasible to work with the cross-flow packing material by avoiding the attachment and biomass growth on the packing surface.

<<Fig. 1>>

The average RE_{VOC} versus the applied liquid to air to ratio for packing A (stage 1) and packing B (stages 2 and 4) is plotted in Fig. 2. In the case of packing A, stage 5 was discarded due to the reinstallation problem. A positive effect of increasing the liquid to air ratio can be observed for both packings. In case of packing A, RE_{VOC} increased from 83 to 93% by increasing the ratio from $3.5 \cdot 10^{-3}$ to $9.1 \cdot 10^{-3}$. For packing B, RE_{VOC} increased from 75 to 85% as the ratio increased from $3.9 \cdot 10^{-3}$ to $10.1 \cdot 10^{-3}$. Comparing both packing materials, higher removals were achieved with packing A due to the higher specific surface and the more complex water path, which favored the contact between both phases. With packing B, a liquid to air ratio higher than $6 \cdot 10^{-3}$ was required to achieve RE_{VOC} over 80%, while this value could be reached by applying nearly half this ratio ($3.5 \cdot 10^{-3}$) in packing A. The results indicate that packing A is the

best alternative for industrial applications if wall biomass growth is prevented by active control of pressure drop.

<< Fig. 2>>

3.3 The EGSB reactor

The water with the solvents was pumped to the EGSB reactor for solvent degradation prior to recirculation to the scrubber. The daily average OL was derived from the difference between the inlet and outlet VOC concentrations in the gas phase during production time (continuously monitored) and expressed as kg COD h^{-1}. The removal efficiency of the soluble organic matter (RE_{COD}) was calculated on a weekly basis from the mass balance (expressed in COD units):

$$RE_{COD}\ (\%) = \frac{OL_W - ACUM - PURGE}{OL_W} \times 100, \qquad (1)$$

where OL_W is the cumulative organic load applied to the EGSB during a week, ACUM is the intra-week accumulated solvents in water, and PURGE is the total amount of purged solvents during a week. ACUM and PURGE were derived from COD water analysis. The OL (daily average) applied to the EGSB is shown in Fig. 1b along with the weekly RE_{COD}. The daily average organic load to EGSB fluctuated quite a bit due to modifications in the facility's production and the performance of the scrubber, with values ranging from 0.37 (day 262) to 6.96 kg h^{-1} (day 47). Despite the organic load fluctuations, the weekly COD removal efficiency was maintained at very high values for the whole experiment, with an average value of 93±5%, verifying the anaerobic biodegradation of a mixture of solvents containing mainly ethanol, 1-ethyl acetate, and 1-ethoxy-2-propanol. Methane content in the biogas was stable at 94±3% (n=18). The methane yield was 0.32 $Nm^3_{CH4}\ kg^{-1}_{\ COD\ removed}$, which was close to the stoichiometric

value (0.35 m^3_{CH4} kg^{-1} $_{COD\ removed}$; Grady et al., 1998). The growth yield coefficient derived from the methane yield resulted in 0.06 mg-VSS mg-COD^{-1}.

Table 2 summarizes the VFA concentration, temperature, and pH of the water effluent of the anaerobic reactor grouped between biomass sampling events. The average temperature for all intervals was in the mesophilic range, although it was below 20ºC (psychrophilic conditions) some days during the first 330 days of operation. Variations were associated with air emission temperature. With the aim of preventing any day from being below 20ºC, a temperature control system was installed on day 334. VFA concentration was normally kept at values lower than 300 mg acetic acid L^{-1} for organic loads lower than 3.0 kg COD h^{-1}, indicating a good balance between acidogenesis and methanogenesis, although some VFA accumulation occurred at high OL. The pH was chemically controlled by adding sodium carbonate, keeping the pH above the minimum value for optimal growth of methanogens (6.8; Leslie Grady et al., 1998). The minimum pH values were reached on days when organic loads were high and VFA accumulated in water; for example, a daily average pH of 6.83 was measured on day 199 after 3 days running with OL higher than 3.9 kg COD h^{-1} and resulting in the maximum VFA concentration (1143 mg acetic acid·L^{-1}).

<<Table 2>>

Fig. 3 shows the variation of VFA concentration with the organic load. An accumulation of VFA in water can be observed for an average daily OL higher than 3.0 kg COD h^{-1}. The accumulation of VFA indicates that the slowly growing methanogens cannot sufficiently and rapidly metabolize the intermediate products from VFA producers (acidogenic and acetogenic populations). If it continues over time, this imbalanced situation could result in the destabilization of the reactor. Considering the

biomass volume of the reactor, the design organic loading rate should be less than 24 kg COD m^{-3} bed d^{-1} to ensure stable removal over 94%.

<<Fig. 3>>

Fig. 4 shows the granule size distribution of the samples taken during the trial (S-1, S-3, and S-6) based on volume. A narrow range of size distribution was observed in all samples, showing large mean diameters (0.88 mm for S-1, 0.95 mm for S-3, and 1.03 mm for S-6). The results demonstrated that the shift of the substrate from ethanol (S-1) to a mixture of ethanol, ethyl acetate, and 1-ethoxy-2-propanol, in which ethanol was the major component, did not show a marked difference in the granule size; a small increase in particle size was observed. This result contrasts with that previously reported by Lafita et al. (2015). These authors indicated a progressive deterioration in methane production and granule disintegration by working at 35 kg COD $m^{-3}d^{-1}$ with a mixture of ethanol and 1-methoxy-2-propanol (4:1 in mass) applied intermittently (16 hours per day, 5 days a week) to a 4-L reactor. Although the carbon source and the type of sludge were similar for both studies, the fluffy granule formation reported by these authors could be related to an excessive granular growth, with abundant extracellular polymeric substance (EPS) production that inhibited the release of gases. This type of granule cannot accommodate extremely high OLs or variations of organic strength (Fukuzaki et al., 1995). In our study, the hydraulic conditions maintained a stable biomass bed volume for the whole trial without excessive biomass accumulation, and the EPS required for granulation was produced in sufficient amounts to handle interruptions and variations of high OLs.

<< Fig. 4 >>

To demonstrate the performance of the EGSB under fluctuating and oscillating feeding of substrate, continuous monitoring of a typical working day (day 481) is

depicted in Fig. 5. The inlet and outlet VOC concentrations at the gas phase (scrubber unit) are plotted in Fig. 5a, and the moving hourly average OL and cumulative biogas production (EGSB reactor) are shown in Fig. 5b. The gas emission pattern varied depending upon the printing orders being processed, with an inlet VOC concentration ranging between 430 and 1900 mg-C·Nm^{-3} during production time (from 6:30 to 22:30), with an average RE_{VOC} at the scrubber of 84%. The variations of the VOC air emissions changed the organic load fed to the reactor from 1.7 to 4.7 kg COD h^{-1}, but the biogas production was kept at a nearly constant rate (0.67 m^3-biogas h^{-1}), indicating the capacity of the reactor to absorb these instantaneous shock loads. Biogas production started 1.5 h after the facility production began and stopped 1.5 h after facility production ended. Both shifts indicated that no solvent was accumulated in the water, corroborating the robustness of the process to recycle VOC emissions into bioenergy.

<<Fig. 5>>

3.4 Microbial community analysis

The result of the DGGE for archaeal and bacterial population is presented in Fig. **Fig.** 6 for all biomass samples collected during the trial. The predominant bands of the samples, which are labelled in Fig. 6, were excised and sequenced. In addition, Shannon's index (H') for each sample was indicated. Table 3 summarizes the DGGE band designation, the level of similarity to related GenBank sequences, and the phylogenetic group of each strain.

<< Fig. 6 >>

<< Table 3 >>

Archaeal DGGE (Fig. 6a) showed a shift in population during the first 123 days, where the biodiversity decreased as the Shannon index decreased from 1.07 (S-1) to

0.41 (S-2). After this initial shift in the archaeal population, it remained stable, with no high variations for more than a year (S-2 to S-6). The developed archaeal population after the shift in the EGSB reactor presented low archaeal biodiversity; A2 was the predominant band. Bacterial DGGE (Fig. 6b) also showed a smooth shift in the bacterial diversity at the beginning of the trial, where the Shannon index revealed a drop from 2.14 (S-1) to 1.86 (S-2) in the diversity of the bacterial population. This decrease in the biodiversity from the initial sludge seems to have been due to differences in the operational and environmental conditions of the reactor. As the granular sludge came from a reactor treating complex brewery wastewater, the microorganisms had to adapt to a defined wastewater containing only organic solvents as carbon source, with few major compounds (ethanol, ethyl acetate, and 1-ethoxy-2-propanol). The increase in the Shannon index from 1.86 (S-2) to 2.00 (S-5) from day 123 to day 413 indicates that the biodiversity increased because new microorganisms were slowly becoming abundant, such as in bands B4, B6, and B7. At the end of the experiment, the Shannon index slightly decreased to 1.85 (S-6) because bands that initially were predominant, B1 and B2, progressively decreased in intensity and finally, in this sample, disappeared. As a conclusion, the use of granular sludge from a brewery wastewater treatment plant seemed to be a good choice for treating oxygenated solvents coming from VOC emissions of the flexographic industry. The predominant bands (A2, B5, B6, and B7) initially came with the sludge, and the change of the carbon source to pure solvents resulted in a population with less biodiversity in which some microorganisms prevailed.

The predominant band found in the archaeal DGGE in all samples along the operation period was band A2; this band was identified as *Methanosaeta concilii*. This is a well-known acetotrophic archaea, and it is the most abundant microorganism in anaerobic granular processes like EGSB and UASB (Díaz et al., 2006).

Methanospirillum species, band A1, are hydrogenotrophic archaea. Tsushima et al. (2010) found that *Methanospirillum* species were able to proliferate under psychrophilic conditions, in line with that *Methanospirillum* were found in some anaerobic reactors working at low temperatures (Xing et al., 2009). Initially, the intensity of this band was lower than in the rest of the samples; the development of *Methanospirillum* in the reactor can be attributed to the operational temperature of the reactor, especially from days 0 to 334. During the first year of the experiment, the temperature evolved spontaneously in association with the temperature of the air emissions in the facility, and the daily average temperature reached values as low as 18.5 ºC (Table 2), with 8 days lower than 20ºC; this favored the development of *Methanospirillum*. At any rate, the smooth variation of temperature during the whole trial (average of 25.1±3.2ºC) did not seem to effect the removal efficiency of the process, showing that microbial functionality was not adversely influenced.

Methanobacterium species, band A3, were hydrogenotrophic archaeas. Wang et al. (2015) found that *Methanobacterium* species became predominant in the reactor when a drastic increase in the organic load was applied. In our study, the intensity of this band was high in the brewery granular sludge, which was taken for a reactor working at high organic load; then, the intensity of *Methanobacterium* increased again in S-3 (day 238). This could be because working at average daily organic loads higher than 3.5 kg COD h^{-1} from day 195 to day 202 seemed to be an advantage to this species.

No *Methanosarcina* was found in the reactor; this could be explained by the competition with *Methanosaeta* for acetate. *Methanosaeta* has a higher affinity to acetate than *Methanosarcina* (Jetten et al., 1990); hence, *Methanosaeta* can be predominant against *Methanosarcina* in stable reactors with low acetate concentrations (McMahon et al., 2001). In our study, the EGSB reactor showed quite stable

performance, and the acetate concentrations were mostly lower than 200 mg L^{-1}. Furthermore, the archaeal diversity found in our study was extremely similar to that found by Xing et al. (2009), who ran an EGSB treating a synthetic brewery wastewater at 15°C. In their study, *Methanosaeta*, *Methanobacterium*, and *Methanospirillum* reached 95% of the archaeal population, and *Methanosaeta* was the most predominant archaea. Furthermore, *Methanosaeta* has been described as an important microorganism in anaerobic granulation processes, and it has a key role in granule integrity (Xing et al., 2009). Therefore, its dominance facilitated the maintenance of the granule integrity throughout the trial, as shown in Fig. 4. In conclusion, methane in this anaerobic reactor was produced by hydrogenotrophic and acetotrophic pathways, as the presence of hydrogenoclastic and acetoclastic species in the reactor revealed.

In terms of the bacterial community, B1 and B2 were identified as *Candidatus Cloacamonas acidaminovorans*. Previous studies suggested that these bacteria are probably syntrophic (Pelletier et al., 2008). *Sulfurovum agreggans*, band B3, was a strictly chemolithoautotrophic bacteria (Mino et al., 2014). This bacterium was previously found in an EGSB working with high sulfate concentration. In our reactor, the sulfate average was low (values < 10 mg L^{-1}), so this bacterium probably came in the brewery sludge and was able to survive under low sulfate concentrations. *Pelobacter propionicus*, band B4, produce acetate and propionate from ethanol with sulfate presence (Schink et al., 1987), so it is involved in VFA production. It was found in anaerobic reactors treating winery wastewater (Cresson et al., 2009). Furthermore, it is remarkable that this bacterium can be associated with *Methanospirillum species*, to which it transfers H_2, to degrade primary alcohols and diols (Eichler and Schink, 1985). Yanti et al. (2014) proposed that the mechanism of ethyl ester degradation is the same as the mechanism for methyl ester degradation, so ethyl acetate is probably transformed

into acetate and ethanol. In the case of 1-ethoxy-2-propanol, its degradation mechanism is not yet known, but Lafita et al. (2015) proposed that after enzymatic ether cleavage, 1-methoxy-2-propanol decomposes to acetone and methanol. By analogy, 1-ethoxy-2-propanol should also be transformed into acetone and ethanol. Considering that the main intermediate to be degraded was ethanol, a primary alcohol, these two microorganisms—*Methanospirillum* and *Pelobacter*—probably played an important role in the solvent degradation of this study, a claim that is corroborated by their progressive increase in abundance during the trial.

B5, B6, and B7 were identified as species belonging to the *Geobacter* genus. These organisms have been identified in different anaerobic reactors treating brewery wastewater (Shrestha et al., 2014); they can use different substrates as ethanol or acetate. Lovley (2011) demonstrated that *Geobacter* species can use direct interspecies electron transfer (DIET). This electron exchange between *Geobacter* and syntrophic partners seems to be an important process in anaerobic wastewater treatment (Commault et al., 2015). It has been demonstrated in laboratory-scale digesters that *Methanosaeta* is one of these syntrophic partners, and one-third of the methane production in an UASB is produced due to DIET between these two species (Rotaru et al., 2014). In our study, *Methanosaeta* and *Geobacter* were the predominant microorganisms in archaeal and bacterial populations, respectively, indicating that these types of interactions occur in the reactor treating a mixture of alcohols and ethers.

4. Conclusions

The anaerobic bioscrubber was shown to be an effective solution for VOC control emission coming from the flexographic sector. The optimization of a pilot unit composed of a packed scrubber and an expanded granular sludge bed reactor ensured

high VOC elimination removal and efficient control of pressure drop in the scrubber. Despite the high fluctuations in the waste gas emissions, with interruptions during nights and weekends and temperature oscillations, stable conversion of alcohols, esters, and glycol ethers to enriched methane biogas was demonstrated. The use of granular sludge from a brewery wastewater treatment plant has been proven to be an adequate strategy to achieve consistently high efficiencies since startup. The limitation of carbon sources to a few organic solvents caused an initial decrease in biodiversity, especially in the domain Archaea, and then the predominant population persisted over time. The predominant Archaea and Bacteria species can be associated with the carbon source and operational parameters, such as temperature and organic load.

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Table 1
Experimental set-up of anaerobic bioscrubber pilot unit.

Scrubber unit					
Stage	1	2	3	4	5
Days of operation	0 - 95	96 - 130	131 - 180	181 - 265	266 - 484
Configuration	Packing A	Packing B	Spray	Packing B	Packing A
Specific surface area (m^2 m^{-3})	150	125	---	125	150
Liquid/air volume ratio · 10^3	3.5 - 9.1	7.6 - 10.1	1.9 – 3.7	3.8- 8.0	4.3 – 7.9
EGSB reactor					
Biomass sampling event (day)	S-1 (0)	S-2 (122)		S-3 (237)	S-4 (333) S-5 (412) S-6 (429)

Table 2
Daily average parameters of the water effluent of the EGSB reactor.

Biomass sampling events	**Days of operation**	**VFA concentration, mg-acetic acid L^{-1}**			**Temperature, °C**			**pH**		
		Average	**Min**	**Max**	**Average**	**Min**	**Max**	**Average**	**Min**	**Max**
S-1	0 - 122	176 ± 183	43	934	22.8 ± 2.0	18.5	26.4	7.50 ± 0.21	7.09	8.41
S-2	123 - 237	365 ± 327	58	1154	24.9 ± 2.8	19.7	30.6	7.38 ± 0.20	6.83	7.96
S-3	238 - 333	156 ± 175	44	615	23.0 ± 1.6	18.8	26.7	7.36 ± 0.35	6.85	8.75
S-4	334 - 412	133 ± 102	49	393	27.2 ± 1.7	23.8	30.8	7.55 ± 0.44	6.88	8.63
S-5	413 - 429	218 ± 199	78	359	29.9 ± 0.7	28.9	30.9	7.32 ± 0.27	7.10	7.74
S-6	430 - 484	122 ± 53	80	200	27.5 ± 1.1	25.5	29.3	7.38 ± 0.31	6.85	8.45

Table 3
DGGE band designation, accession numbers in GenBank and levels of similarity to related organisms according to Fig. 6.

DGGE band	**Closest organism in the GenBank (accession number)**	**Similarity**	**Phylogenetic group**
A1	*Methanospirillum lacunae* (NR_112981.1)	99%	Methanospirillaceae [a]
A2	*Methanosaeta concilii* (NR_102903.1)	100%	Methanosaetaceae [a]
A3	*Methanobacterium formicicum* (NR_115168.1)	99%	Methanobacteriaceae [a]
B1	*Candidatus Cloacamonas acidaminovorans* (NR_102986.1)	89%	Cloacimonetes [b]
B2	*Candidatus Cloacamonas acidaminovorans* (NR_102986.1)	87%	Cloacimonetes [b]
B3	*Sulfurovum sp* (NR_074503.1)	97%	Epsilonproteobacteria [c]
B4	*Pelobacter propionicus* (NR_074975.1)	100%	Pelobacteraceae [a]
B5	*Geobacter argillaceus* (043575.1)	92%	Geobacteraceae [a]
B6	*Geobacter psychrophilus* (043075.1)	97%	Geobacteraceae [a]
B7	*Geobacter toluenoxydans* (NR_116428.1)	83%	Geobacteraceae [a]

[a] Family, [b] Phylum, [c] Class

Figure Captions

Fig. 1. Performance of the pilot unit. (a) Daily averages of (⊟) RE_{VOC}, (○) Inlet VOC concentration and (●) Outlet VOC concentration in the gas phase of the scrubber. Broken lines represent the days when the configuration of the scrubber was changed. (b) (●) Daily average OL and (⊟) weekly RE_{COD} of the EGSB. Broken lines indicate biomass sampling event.

Fig. 2. Influence of the liquid to air volume ratio on the VOC removal efficiency of the scrubber unit. (●) Packing A, stage 1, (○) Packing B, stages 2 and 4.

Fig. 3. Effect of the organic load on the water effluent VFA concentration of the EGSB.

Fig. 4. Variation of the granule size distribution of biomass over time.

Fig. 5. Plant monitoring data, day 481. a) (**—**) Inlet VOC concentration and (**▪▪▪**) Outlet VOC concentration in the gas phase in the scrubber, b) (**▪▪▪**) Cumulative biogas production and (**—**) Moving hourly average OL in the EGSB.

Fig. 6. DGGE profiles of biomass samples from the EGSB reactor including their Shannon index (H'). (a) Archaeal DGGE profiles, (b) Bacterial DGGE profiles.

Fig 1. 1.5 column

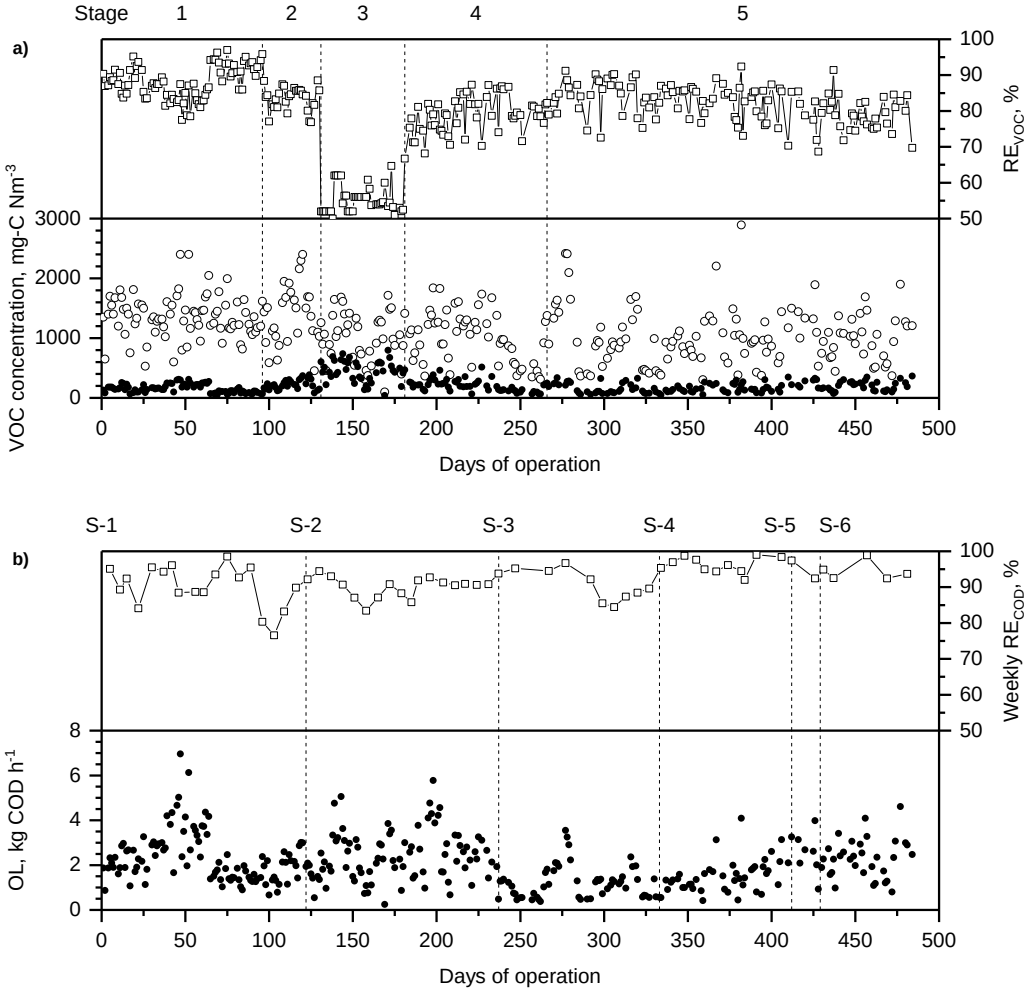
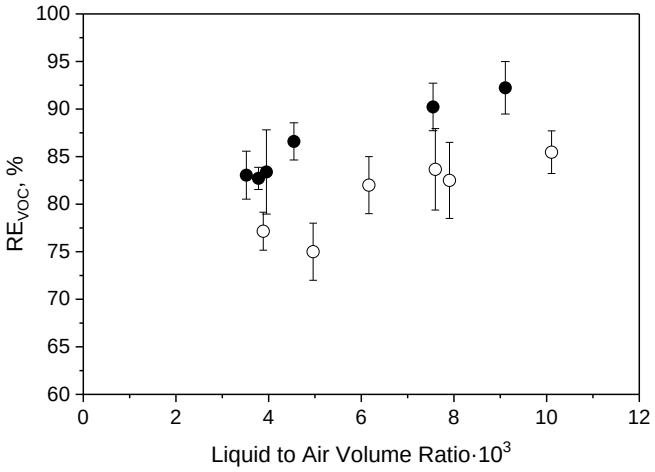


Fig 2. 1 column



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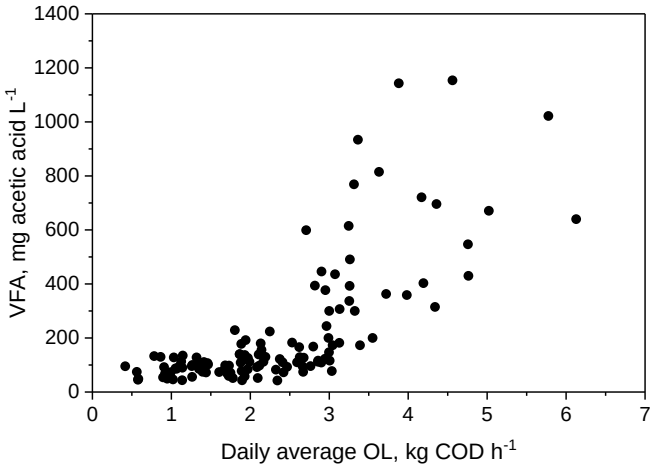

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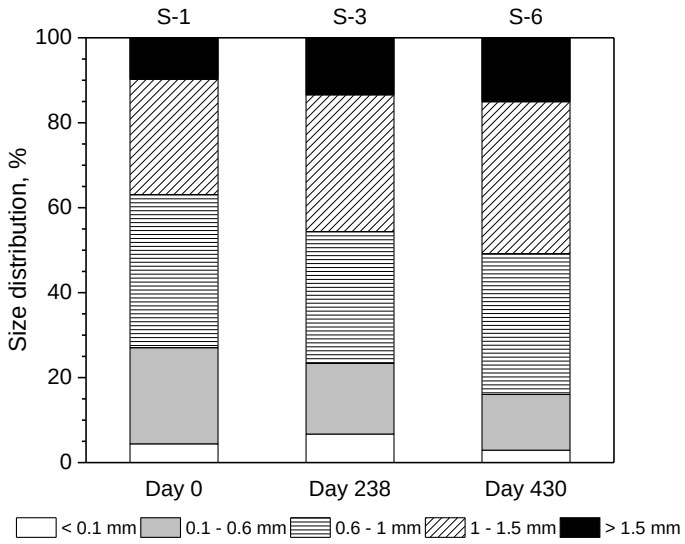

Fig 5. 1 column

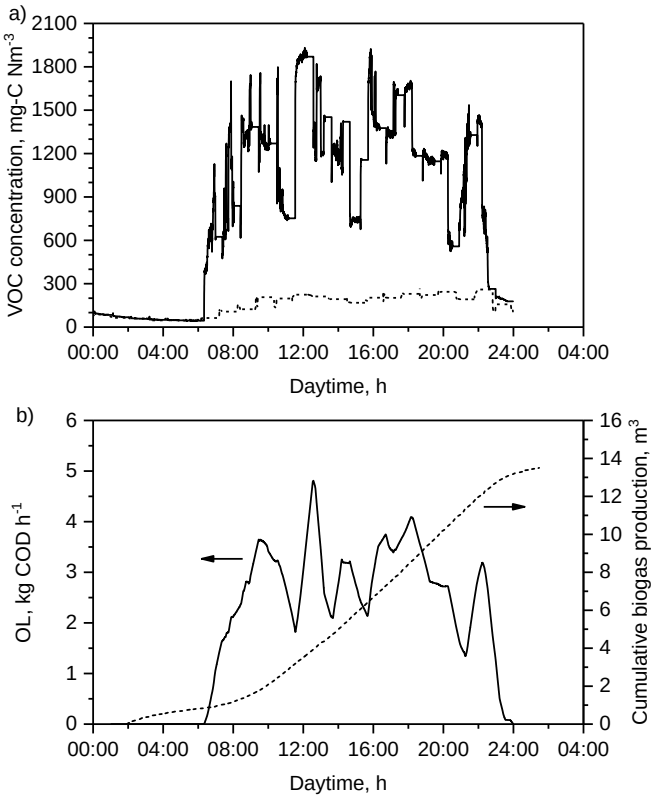

Fig 6. 1.5 column