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TEMPORAL AND SPATIAL EPS ANALYSIS OF A BIOFILTER TREATING ETHYL ACETATE DURING OZONATION

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ABSTRACT

The present paper focuses on the biofilm composition and pattern of biomass in gas biofiltration of ethyl acetate working under continuous addition of ozone (O_3). Two biofilters were operated for 230 days, one under continuous addition of O_3 (90 ppb_v) and another one without. Throughout the operation time, the exopolysaccharides (EPS) extracted from the biofilm were characterized, qualitatively using Fourier Transform Infrared Spectroscopy with attenuated total reflectance (FTIR-ATR), and quantitatively by analyzing its main constituents: carbohydrates, proteins and glucuronic acid. EPS characterization has been attempted mainly with biofilm aggregates related to water treatment, not air biofiltration. Since EPS are the main constituents in the biofilm, the results of this study may be helpful and provide more information about EPS structure when O_3 was added. O_3 addition only affected the amount of EPS, and not its composition. The greater effect was observed on carbohydrate content, since it is the main component in EPS. The EPS/biomass ratio measured was twice lower with O_3 addition. Higher RE and mineralization rates were obtained with the biofilter subjected to O_3 addition, and a smaller volume of reactor would be necessary to treat all contaminant under this condition. All about suggest that EPS are only being reduced by O_3 addition and that the low concentration of applied O_3, did not affect the composition of the EPS.

Keywords: exopolysaccharides; ozone; biomass composition; ethyl acetate, biofiltration, longitudinal behavior.

1. Introduction

Biofilms are the place where microorganisms live, multiply, grow, and interact in aggregated forms. In most biofilms, the microorganism account for less than 10% of the dry mass, whereas the matrix can account for over 90%. The matrix is the extracellular material, mostly produced by the organism themselves, in which the biofilm cells are embedded. It consists of a conglomeration of different types of biopolymers (extracellular polymeric substances "EPS"). The EPS determine the immediate conditions of life of biofilm cells living in this microenvironment by affecting porosity, density, water content, charge sorption properties, hydrophobicity, and mechanical stability (Flemming and Wingender 2010). The production of EPS is a general property of microorganism in natural environments and has been shown to occur both in prokaryotic (bacteria, archaea) and in eukaryotic (algae, fungi) cells (Wingender et al. 2001). Furthermore biofilms can exist in technical systems such as heat exchangers, plumbing systems and reactors. Particularly, biofilms in packed bed bioreactors named as biofilters offer a cost-effective and eco-friendly alternative to control air pollution, since biofiltration is based on the ability of microorganism to convert, under aerobic conditions, organic pollutants to water, carbon dioxide and biomass. Further, the biofilm is where the microorganisms are harbored. Biofiltration offers a promising solution to remove volatile organic compounds (VOCs) from airstreams. However, this technology must address some challenges such as "clogging", which appears when an excess of biomass is produced. Numerous methods have been developed to reduce the excess of biomass, chemical, physical and biological (Cox and Deshusses 1999; Mendoza et al. 2004; Soria et al. 1997; Wang et al. 2009; Xi et al. 2014; Zhou et al. 2016; Dorado et al. 2012; Cox and Deshusses 2012; Woertz et al. 2002). Recently, O_3 has been added to biofilters at low concentrations in order to study its effects on biofilter clogging and for VOC removal; O_3 addition has been reported as an effective biocide that can remove exopolysaccharides in the biofilm matrices (Tachikawa et al. 2009; García-Pérez et al. 2013; Maldonado and Arriaga 2015; Zhou et al. 2016). Ozone has been used to disintegrate excess sludge, pathogen inactivation, as a pretreatment or hybrid process for the removal of pollutants in wastewater treatment plants and for prevention of biofilms formation during wastewater treatment at concentrations ranged from 3.2 mg L^{-1}_{water} to 540 mg L^{-1}_{water} (Tachikawa et al. 2009; Cheng et al. 2012; Lotito et al. 2014). However, in the case of gas phase bioreactors for emission treatment, the O_3 concentrations that have been used are very low, in the range of 0.18 mg m^{-3}_{air} to 120 mg m^{-3}_{air} (García-Pérez et al. 2013; Xi et al. 2014; Zhou et al. 2016). García-Pérez et

al. (2013) indicated that O_3 concentrations of 90ppb$_v$ (0.18 mg m$^{-3}_{air}$) only affected extracellular components of the biofilm and not the cells directly; however the effect of O_3 on the EPS detachment from the biofilm could not be confirmed due to the too low biomass content obtained by these authors for formaldehyde biofiltration. At the same time, the EPS composition was evaluated, analyzing proteins, carbohydrates and glucuronic acids in the same study. A major effect was found for proteins. Wang et al. (2009) reported that O_3 (40-120 mg m$^{-3}_{air}$) could lower the EPS content in a biofilter treating gaseous chlorobenzene. Recently, Zhou et al. (2016) reported the effect of O_3 addition on biomass and EPS contents of a biofilter treating toluene; they found a high effect of O_3 at concentrations above 10 mg m$^{-3}_{air}$. Also, they reported that the hydrophobicity of the biofilm decreased when the O_3 injections increased. The doses of O_3 used in biofilters are very wide. Zhou et al. (2016) indicate that O_3 doses under 10 mg m$^{-3}_{air}$ can induce higher removal efficiencies and good biomass detachment but higher O_3 concentrations affect the microbial activity of microorganisms. Even if Zhou et al. (2016) also reported the profile of EPS content under O_3 additions, the number of samples was limited, with 10 samples for 360 days of operation, giving some uncertainty in the real evolution of EPS content. In addition, several studies have focused on the characteristics of EPS and the influence of thermochemical and oxidation mechanisms on degradation and flocculation of EPS in wastewater treatment systems but not in gas phase biofiltration systems with O_3 addition. Therefore, very studies have been carried out to provide a better understanding of the effects of O_3 addition on EPS, its production and composition. Thus, it is necessary to disclose more tangible evidence of the effects of O_3 on the EPS matrix, since such production is a limitation in the previous biofiltration experiments. In a earlier study of biofiltration of ethyl acetate (EA), a readily degradable molecule, the effectiveness of ozone in clogging prevention was proved due to the high biomass content that the system produced (Covarrubias-García et al. 2017). In that study, neither the quantity of EPS produced nor the longitudinal effect of O_3 on biofilter performance were evaluated. Thus, the main purpose of the present study was to evaluate in detail the effect of O_3 addition at very low concentration (90ppb$_v$) in a biofilter treating EA on the EPS content, its composition and the spatial and temporal performance.

2. Materials and methods

2.1 Biofilter System

EA (J.T. Baker, 99%) biodegradation was carried out in two identical laboratory biofilters of 3.3 L divided into three identical modules (1.1 L) (M1: upper, M2: middle and M3: lower). Fig 1 show the scheme of the biofilter used. The biofilter has two sampling points between each module, one for biomass in the middle of each module filter bed and another after the end of the module for gas sampling. Each biofilter module was made of glass, with a total length of 0.45 m and an internal diameter of 0.097 m. The packing material was perlite with an average diameter of 3.3 mm inoculated with activated sludge obtained from a wastewater treatment plant (Tangamanga Park, San Luis Potosi, Mexico). Biofilters were fed in downward mode with an empty bed retention time of 60 s. One biofilter operated without O_3 addition and the other operated under the same conditions but with a fixed addition of O_3 of 90 ppb_v. For the biofilter with O_3 addition, a controlled airstream with a mass flow controller (GFC17; Aalborg, Orangeburg NY) passed through an EA solution contained in a stripping reactor and was mixed with an airstream coming from an humidifier and with O_3 airstream to have a total inlet flow of 3.3 L min^{-1}. Generation of O_3 was produced with a technology called "Corona Discharge Technology", which consists in the use of a high frequency generator that causes the breaking of oxygen molecule due to the electrical field (OZONE GENERATOR, A2ZS-3GLAB), for instance air from the compressor at a flow of 100 mL min^{-1} was fed into the equipment. O_3 concentration was estimated with the yodimetric method of Rakness et al. (1996) at the inlet and the outlet of the biofilter. The operational stages for both biofilters were divided according to Table 1. The stages of operation were applied according to Covarrubias-García et al. (2017).

2.2 Gas phase analyses

EA concentration in gas phase was measured with a gas chromatograph Thermo Scientific Trace 1300 equipped with a flame ionization detector and a DB-624 capillary column. The operation temperatures were 230, 100, and 230 °C for the injector, column and detector, respectively. CO_2 concentration was measured with a gas chromatograph GC-6850 (Agilent Technologies, CA USA) equipped with a thermal conductivity detector and an HP-PLOT Q capillary column. Helium was used as a carrier gas at a flow rate of 10.1 mL

min^{-1}. The temperatures of the injector, column, and detector were 200, 50 and 250 °C, respectively. Inlet, outlet and middle concentrations of EA and CO_2 were measured.

2.3 Biomass analyses

The biomass content was measured in terms of the volatile solids content by standard methods (APHA 2005), then, biomass concentration was determined by dry weight (mg biomass per g of perlite).

2.4 EPS extraction and its characterization

EPS content was extracted by the NaOH-formaldehyde method reported by Liu and Fang (2002) from a representative sample from each module of the biofilter consisting in perlite and biomass. EPS were characterized in terms of proteins (Lowry et al. 1951), carbohydrates (DuBois et al. 1956) and glucuronic acid (Blumenkrantz and Asboe-Hansen 1973), standard substances used were bovine serum albumin, glucose and glucuronic acid, respectively.

2.5 EPS analysis by FTIR (Fourier Transformation Infrared Spectroscopy)

EPS lyophilized samples from different days of operation, loads and modules were analyzed. About 5-10 mg of powder was obtained in a mortar. Scans were performed by ATR-FTIR (Attenuated Total Reflection-Fourier Transform Infrared) in the Thermo-Nicolet brand equipment (Nexus 470 FT-IR E.S.P.) with a resolution of 4 cm^{-1} for 120 cycles. In every case, the spectra of lyophilized sample were recorded and divided by the background single beam spectrum before converting to absorbance spectra.

3. Results and discussion

3.1 Longitudinal removal of ethyl acetate by modules

The performance of the biofilter was evaluated in terms of EC (elimination capacity) and RE (removal efficiency), from the top of the biofilter to each sampling point (M1, M2 and M3), the values were related to the volume of each section. The performance of the biofilter without O_3 and with O_3 is shown in Fig. 2.

M2 of the biofilter with O_3 addition presented the higher RE and EC along the operation time, whereas removal was more uniform in all modules in the biofilter without O_3 addition. M3 in the biofilter with O_3

addition was useless, since in M2 the pollutant was removed almost completely from stage D. At the final stages (F and G), M3 presented a small contribution in the RE and EC. O_3 addition allows a better RE and it EA treatment over a shorter stretch of the biofilter (two modules), which could represent an advantage over the biofilter without O_3, in which the three modules were functional to remove EA with a lower performance along time. Thus, the addition of O_3 to a biofilter not only helps to increase the RE and life time of the filter bed, but also impacts the costs allowing the use of a smaller reactor volume. Only Maldonado-Diaz and Arriaga (2015), Xi et al. (2014), Zhou et al. (2016) have studied biofiltration systems with modules and O_3 additions, considering three, two and four modules, respectively. However no information of EC or RE by modules has been reported. Overall, biofilter performance with O_3 addition achieved higher EC and RE by modules comparing with the biofilter without, except for M3 in the stages D and E of the biofilter with O_3. This O_3 concentration (90pp_v) was enough to degrade EA in two modules, although, it could be interesting to try a lower concentration of O_3 in order to minimize the operation cost of using O_3 and save space when this type of systems are installed. It can be thought that if O_3 concentrations were increased, degradation of the same inlet load of EA would be possible in only one module, which would maintain the microbial activity. Xu et al. (2016) operated six biofilters in parallel packed with perlite treating gaseous toluene with different inlet O_3 concentrations ranging from 0-300 mg m^{-3}, they indicated that different O_3 concentration affected the microbial community and that the microorganism exposure to O_3 showed higher metabolic activities. Thus, it is possible that O_3 concentration of 90 pp_v could be enhancing the microbial activity of some microorganism present in the biofilter.

3.2 CO_2 production profile The plots of CO_2 production throughout the modules and for the complete reactor are showed in Fig. 3. The highest CO_2 production in the biofilter without O_3 addition was presented in M1 (400 g m^{-3} h^{-1}) and the lowest in M3. The biofilter with O_3 addition presented similar CO_2 values in M1 at stages A, B and C, then the CO_2 increased as high as 350 g m^{-3} h^{-1} in the next stages. CO_2 in M1 and M2 without O_3 addition were almost similar and presented the highest values at stages E and forward. The higher production of CO_2 in the biofilter with O_3 could be related to O_3 reacting with some organic matter, including EA, byproducts, extracellular polymeric substances (EPS), dead cells and cell debris, converting them to more readily biodegradable matter and finally CO_2 (Xi et al. 2006). Estimating the ratio by module along the

operation time, dividing the CO_2 produced by the EA fed (ignoring the biomass, all EA is converted to CO_2), the calculated values were: without O_3, M1= 0.51, M2= 0.35 and M3= 0.21 ($g_{CO2}\ g^{-1}_{EA\ fed}$); with O_3, M1= 0.84, M2= 1.0 and M3= 0.35 ($g_{CO2}\ g^{-1}_{EA\ fed}$). According to this, the mineralization ratios were lower in M1, M2 and M3 in the biofilter without O_3 than with O_3. These results agree with the CO_2 production along the operation time, where the CO_2 production in the biofilter without O_3 was quite similar, and in the biofilter with O_3 were higher than without. CO_2 production in M2 in the biofilter with O_3 was higher than in M1 and M3 along all the stages of operation, this was due to the fact that M2 in this biofilter removed the higher amount of EA as was discussed previously (see Fig. 2). CO_2 production can be an indicator of the intensity of the microbial activity in the biofilters, and data indicated that the biofilter with O_3 presented better removal efficiency, since more EA was mineralized. The profile of total CO_2 confirmed the above statement, as an increase on the production of CO_2 along time for the biofilter with O_3 addition was attained which contrast with the biofilter without O_3 addition, in which CO_2 production remained stable during almost all the stages of operation (A, B, C, F and G). Álvarez-Hornos et al. (2007a) reported similar values of CO_2 production in a biofilter treating EA than the obtained in the present study for the biofilter working without O_3 addition (550 $g\ m^{-3}\ h^{-1}$). However, the total CO_2 production in the biofilter with O_3 was more than the double of the CO_2 production reported for EA (Alvarez-Hornos et al. 2007a). Also, in another study, Álvarez-Hornos et al. (2007b) reported values as high as 150 $g\ m^{-3}\ h^{-1}$ for a mixture of EA and toluene in the first quarter, half and three quarter of the biofilter.

3.3 Biomass content

Table 2 summarizes the biomass content by modules (M1, M2 and M3) along time, in the biofilter with O_3, M1 presented the lower biomass amount compared with the other modules, this could be due to the fact that it was the module more directly exposed to O_3. Although O_3 concentration was only measured in the entrance and the outlet of the biofilter (not between the modules), it is probable that O_3 concentration was decreasing along the biofilter. For instance O_3 is a strong oxidant and disinfectant, the higher concentration in the first module was responsible for inactivating some of microorganisms present there, inducing a lower amount of biomass in M1. Some authors (Chang 1971; Khadre et al. 2001) concluded that molecular O_3 is the main inactivating agent of microorganisms, being powerfully active against bacteria, fungi, viruses, protozoa, and

bacterial and fungal spores. M2 in the same biofilter (with O_3) presented the higher values of amount of biomass. Such biomass in M2 increased with time and then dropped in stage G. This could be due to the fact that this module received more degradable substrate than EA, which allowed the faster microbial growth. This happened with M1 in the biofilter without O_3, the entrance in this biofilter is the place where the gradient of substrate concentration was higher, thus, being the rate of microbial growth proportional to the gradient of substrate concentration, the biomass concentration there was high. On the other hand, biomass content in M3 for the biofilter without O_3 addition increased with the time then dropped at stage D. In general, the biofilter without O_3 addition clearly increased its biomass content along the operation time. Previous studies have reported lower biomass amount with O_3 addition with more recalcitrant pollutants (García-Pérez et al. 2013; Maldonado-Diaz and Arriaga 2014; Xi et al. 2014). García-Pérez et al. (2013) concluded that there was not enough biomass quantity to prove the effect of O_3 on the biomass, the presented range of biomass concentration was ~7.5-24 mg $_{biomass}$ $g^{-1}_{perlite}$ for one biofilter with O_3 pulses for formaldehyde degradation. In the present study, the range of biomass concentration for both biofilters was between 19.9 and 302.3 mg $_{biomass}$ $g^{-1}_{perlite}$, which are far higher than the previous reports. Recently, Zhou et al. (2016) reported the effect of various concentrations of O_3 (5 mg m^{-3} - 30 mg m^{-3}) on biomass content in a biofilter treating toluene, biofilters subjected to O_3 had a biomass content between 25 g_{VSS} g^{-1}_{pellet} and 10 g_{VSS} g^{-1}_{pellet} against the biofilter without O_3 which had 30 g_{VSS} g^{-1}_{pellet}. In that study, O_3 concentrations above 10 mg m^{-3} had a strong effect on biomass content and in the microbial activity. Also, the biomass concentration was higher in the bottom section than in the upper section as toluene was fed upward. The biomass content reported in that study was at least 30 times higher than the attained in the present study for the biofiltration of EA, also, the biomass content along the biofilter had not the same profile than the study of Zhou et al. (2016), the highest biomass concentration prevailed in M2 of the biofilter not in the upper zone which was subjected to a major concentration of EA. The results by module in this study allowed to analyze better the effects of O_3 over the biomass production. Comparing both biofilters, the greater difference is between modules 1. Also, as it can be seen (Table 2), along time the biomass amount in the biofilter with O_3 increased at some modules, which could still lead to a clogging problem.

3.4 EPS content and its characterization

Fig. 4 shows the EPS content and its characterization in composition by modules. According to the results, EPS production (gray area) decreased in M1 since stage E (day 159), the same happened in M2 but in M3 EPS decreased until stage D (day 79) in the biofilter with O_3 addition. In general, M1, M2 and M3 of the biofilter without O_3 addition did not present a decrease in the amount of EPS. Comparing both biofilters, O_3 addition affected the EPS production, presenting a lower amount of EPS in each module along the operation time. Maldonado-Diaz and Arriaga (2015) indicated that in a biofilter treating formaldehyde, the highest concentration of EPS was detected in periods without O_3 addition. These results suggest, as O_3 is a high reactive molecule, that it could oxidize the EPS, or the radicals of O_3 could react with EPS during O_3 addition (Boncz 2002). Fig. 4, also showed an increase in EPS contents along time. Similar to the present study, Zhou et al. (2016) showed that EPS increased with time in a biofilter treating toluene that operated for 300 days under O_3 addition. This indicated that EPS could continue increasing in biofilters subjected under O_3 addition and then the clogging of the biofilter could be attained but in longer periods of operation.

With respect to the characterization of EPS, a greater effect on carbohydrates, which is the main component of EPS matrix (Wingender et al. 1999), can be seen in the stacked column. This result contrasts with Zhou et al. (2016) in which the effect of O_3 addition on carbohydrates was insignificant. Secondly, glucuronic acid significantly increased in biofilter without O_3 on stages F (day 189) and G (day 230) in the three modules, and proteins increased on stage G (day 230) also in the three modules. M2 in the biofilter with O_3 presented the higher EPS, proteins, carbohydrates and glucuronic acid contents, whereas this behavior was presented in M1 of the biofilter working without O_3 addition. This behavior could be related to the fact that M2 of the biofilter with O_3 addition received more degradable substrate than EA, and in the biofilter without O_3 in M1 more substrate was fed, which allowed more microbial growth and thus more biomass and EPS content. The effect of O_3 addition in biomass composition for biofilters treating toluene was already reported, with an EPS protein content which increased with time (Zhou et al. 2016). Analyzing the results presented in this study the greater effect when O_3 was added was with carbohydrates content, it can be assumed that carbohydrates were in the outer layer of the biofilm, as EPS are distributed in layers though the biofilm depth and their yield varies along the biofilm depth (Zhang et al. 1998), thus O_3 addition reacted first with these, then with proteins. McSwain et al. (2005) reported that the cells and carbohydrates were present in the outer layer of

aerobic granular sludge and most proteins were found in the inner layer. Other studies have reported by confocal scanning microscopy (CLSM) or fluorescent microscopy that the spatial distribution of EPS components is heterogeneous in biological wastewater treatment systems (Sheng et al. 2010). Saingam et al. (2016) used CLSM in a biofilter treating toluene with O_3 addition to observe cell viability and the thickness of the biofilm but they did not study EPS and their components.

In order to better analyze the results of this study, the ratio between EPS amount and biomass production was calculated (Table 3). To our knowledge, no information has been reported with this relation in biofilters with O_3 addition. The biofilter without O_3 addition presented quite stable relations in all modules along the operational stages (0.1), whereas the biofilter with O_3 decreased in most points until the half or lower (0.05, 0.005). The increments at some points could be due to the fact that more EPS are being secreted by microorganisms to protect themselves and it was function of the operational conditions. Thus, the addition of O_3 reduced the biomass amount and then prevented the biomass clogging but at the same time helped to oxidize more EA and to maintain higher RE.

3.5 EPS analysis by FTIR

Extracted FTIR spectra of EPS are showed in Fig. 5 a and b. In this study, the whole spectra are presented. The peaks in the range of 3800-3100 cm^{-1} correspond to H-O stretching (Alvarez and Vazquez 2006), amides I to 1600-1700 cm^{-1}, amides II to 1500-1600 cm^{-1}, and polysaccharides to the region 1200-900 cm^{-1}. In both biofilters in the polysaccharides region only a peak at 1030 cm^{-1} on day 10 and 1010 cm^{-1} on day 108 was found, the signal corresponded to C-O bond (Borchani et al. 2015). This particular region presented a difference in absorbance intensity, which indicates that there was a variation in the quantity not in the composition as it was previously confirmed in Fig. 3, in which carbohydrates were more affected along time in the biofilter with O_3. For EPS samples taken on day 10, a difference can be seen in absorbance intensity, where it decreased in the biofilter without O_3 from M1 (upper module), then M2 and M3. In the biofilter with O_3 the lowest absorbance intensity was in M1 and then increased in M2; absorbance intensity in M3 was higher than M1 but lower than M2 which is accordance with the quantitative analysis presented in Fig. 3. On the other hand, on day 108 of operation, biofilter without O_3 presented a quite stable absorbance in the three modules, whereas M1 and M3 of the biofilter with O_3 addition were higher than in day 10.

Regarding the other wavelengths a signal at 1400 cm^{-1} was found, which did not change and appeared in both operation days of both biofilters, this signal corresponded to the stretching C-O of carboxylic groups overlapped with amide III band. Also, the peak at 1600 cm^{-1} did not change, which correspond to Amides II and it is associated with proteins, on the other hand the peaks on 1650 cm^{-1} correspond to the group of amides I also associated with proteins. Previous studies of EPS analysis with FTIR have been reported (Görner et al. 2003; Eboigbodin and Biggs 2008; Wang et al. 2012) . The reported bands assignment were 1645 cm^{-1} (amine I), 1450 cm^{-1} (CH_3), 1400 cm^{-1} (C-O), 1260 cm^{-1} and 1080 cm^{-1} (P=O) (Eboigbodin and Biggs 2008) of *Escherichia coli*. Görner et al. (2003) analyzed the EPS composition from activated sludge by the same technique; they reported 1647 cm^{-1} (Amide I), 1550 and 1540 cm^{-1} (Amide II), 1410 and 1388 cm^{-1} (Amide III), 2970-2850 cm^{-1} (CH2 vibrations) and 1733 cm^{-1} (C=O). Comparing this study with the previous one, it is clear that not all the peaks appeared, since this system is different to the others. Moreover, no FTIR analysis has been attempted with EPS and O_3 addition. With these results, it can be concluded that there is no significant difference between the modules and the biofilters, so their compositions were unchanged.

4. Conclusion

This work provides further information into EPS identification when O_3 is added in a biofilter. The results of FTIR analysis suggested that O_3 addition did not significantly affect the type of functional groups identified in the EPS. The quantification of each component of EPS analyzed indicated that O_3 addition made greater effect on carbohydrate amount. Thus, O_3 is affecting only the amount of EPS, and not its composition. The longitudinal EPS/biomass ratio in the biofilter working under O_3 addition was the half than without, thus biomass clogging could be prevented when O_3 is added. Overall, biofilter with O_3 addition presented higher EC and CO_2 production by modules and globally. A small volume of reactor would be necessary when O_3 is added, then operational and investment costs of biofilters would be reduced. However, for practical applications O_3 cost also needs to be considered. Regarding the biofilters performances, the biofilter with O_3 in this study presented a more stable tendency, globally and by modules, but it would be interesting to find the limiting inlet load that the system with O_3 could withstand without affecting the system stability. Finally, it is highlighted that more research is still needed on EPS component distribution taking in consideration that the

variation in the composition of the extracted EPS depends on many factors, such as bioreactor type, process operational conditions and analytical tool used among others.

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Figure Captions

Fig. 1 Experimental setup for the biofiltration process with and without ozone. Line ---- in the biofilter with ozone addition; M1: upper module, M2: middle module, M3: lower module; A: biomass sampling points, B: gas phase sampling points; U: union between ozone and EA gas flow.

Fig. 2 Longitudinal behavior of elimination capacity and removal efficiencies along stages of operation for the biofiltration of ethyl acetate without (a) and with O_3 (b). *EC M1; EC M2; EC M3; RE M1; RE M2; RE M3. Lapse days; A=0-10, B=11-38, C=39-78, D=79-108, E=109-159, F=160-189, and G=190-230*

Fig. 3 Carbon dioxide production during the biofiltration of EA under several stages of operation. **a)** Without O_3; **b)** with O_3. Average CO_2 production g m^{-3} h^{-1}; CO_2 M1; CO_2 M2; CO_2 M3; —Total. Days; A=0-10, B=11-38, C=39-78, D=79-108, E=109-159, F=160-189, and G=190-230

Fig. 4 EPS content and its characterization, by modules and stages of operation in biofilters treating EA. (■) EPS content; (■) Proteins; (□) Glucuronic acid; (▨) Carbohydrates; Days; A=10, B=38, C=78, D=108, E=159, F=189, and G=230

Fig. 5 FTIR spectra of EPS samples at day 10 (inlet load 60 g $m^{-3}h^{-1}$, a) and 108 (inlet load 180 g $m^{-3}h^{-1}$, b) of operation

Figure

Fig. 1

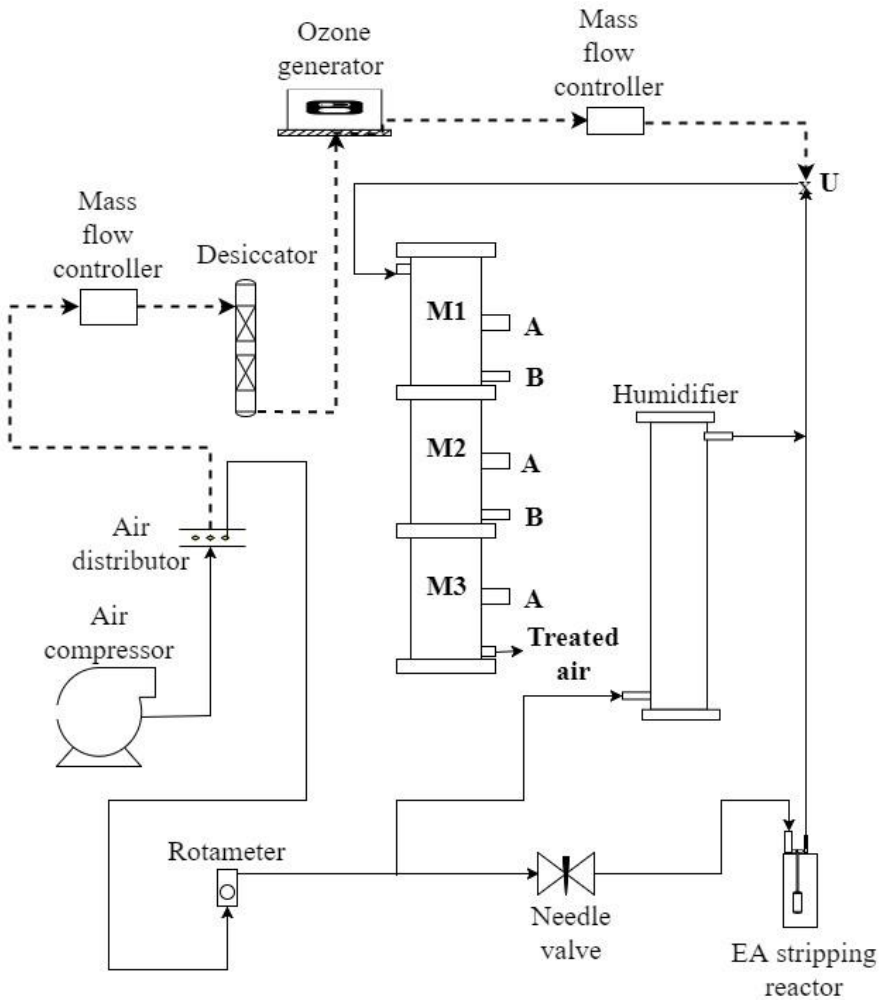

Ozone generator
Mass flow controller
U
Mass flow controller
Desiccator
M1
A
B
M2
A
B
M3
A
Treated air
Humidifier
Air distributor
Air compressor
Rotameter
Needle valve
EA stripping reactor

Fig. 2

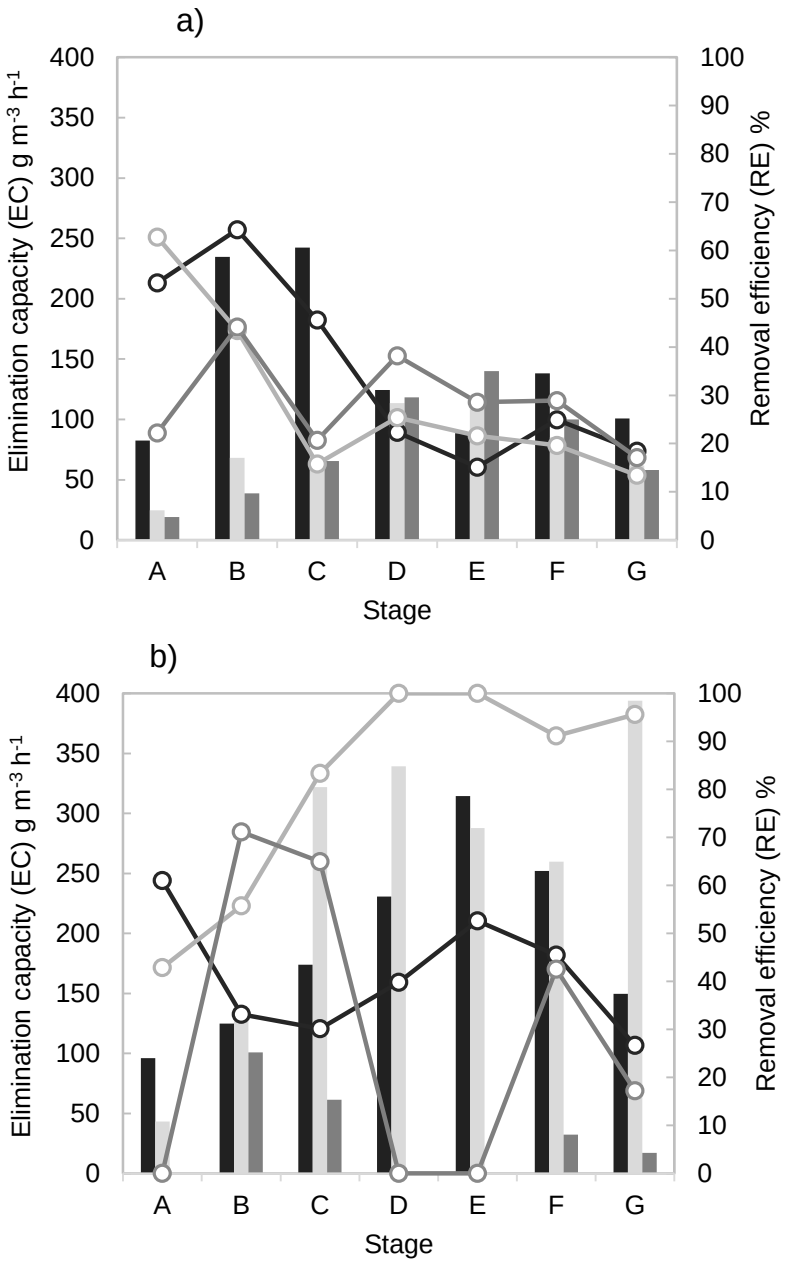
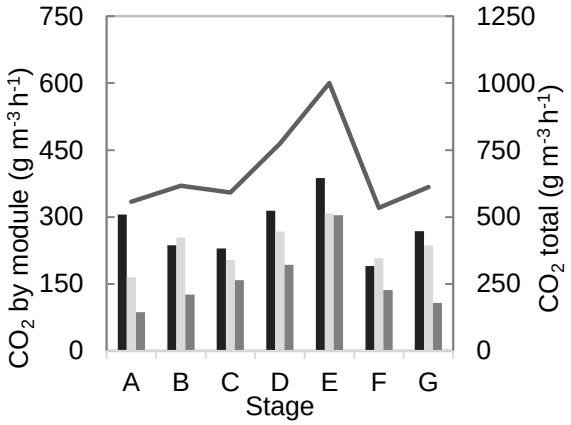

a)
Elimination capacity (EC) g m-3 h-1
Removal efficiency (RE) %
Stage
A
B
C
D
E
F
G
b)
Elimination capacity (EC) g m-3 h-1
Removal efficiency (RE) %
Stage

Fig. 3

a)


750
600
450
300
150
0
1250
1000
750
500
250
0
CO2 by module (g m-3 h-1)
CO2 total (g m-3 h-1)
A B C D E F G
Stage

b)

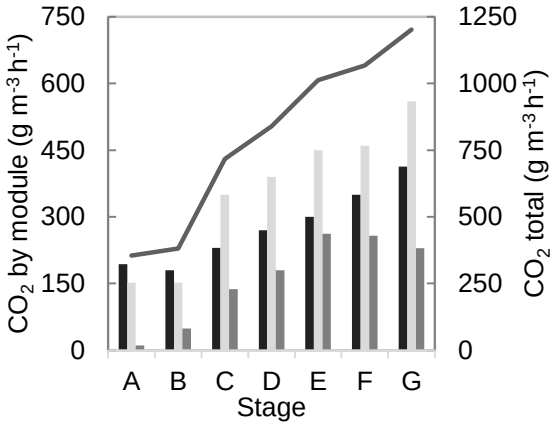

750
600
450
300
150
0
1250
1000
750
500
250
0
CO2 by module (g m-3 h-1)
CO2 total (g m-3 h-1)
A B C D E F G
Stage

Fig. 4

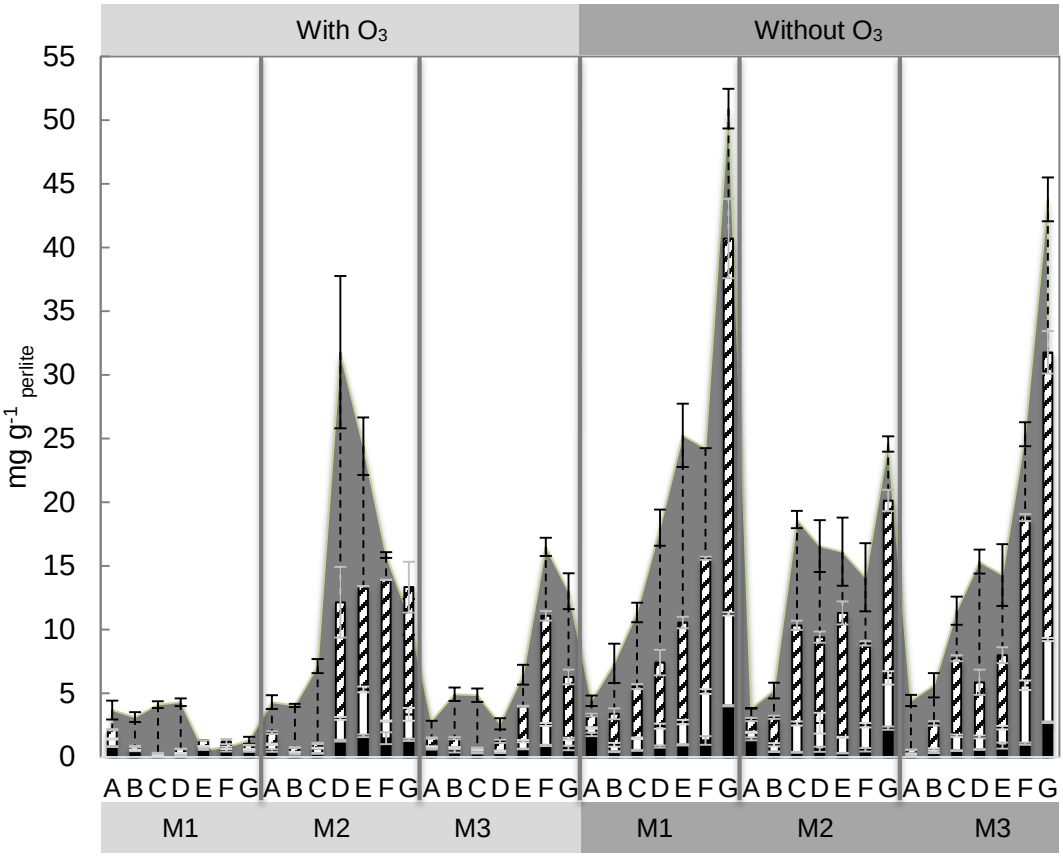
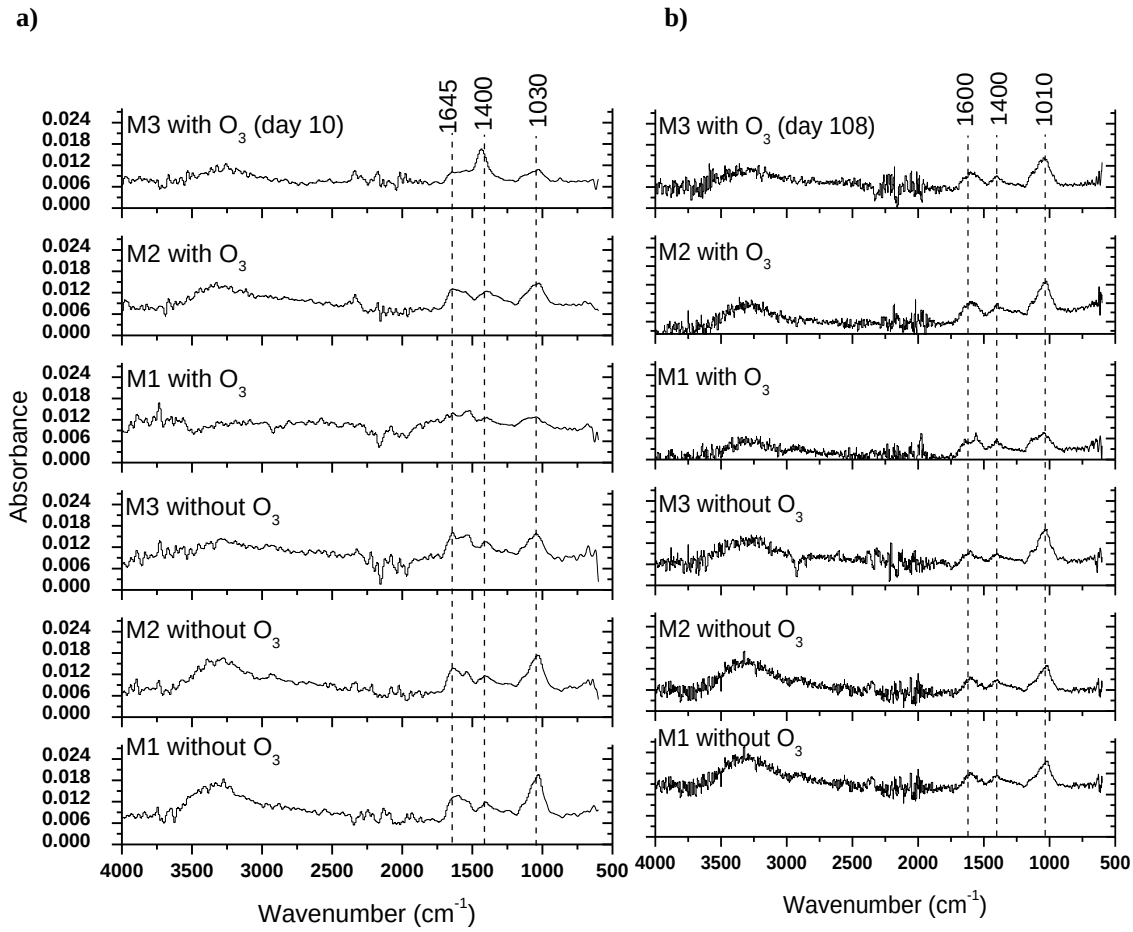

With O3
Without O3
55
50
45
40
35
30
25
20
15
10
5
0
mg g-1 perlite
A B C D E F G A B C D E F G A B C D E F G A B C D E F G A B C D E F G A B C D E F G
M1
M2
M3
M1
M2
M3

Fig. 5


a)
b)
M3 with O3 (day 10)
M2 with O3
M1 with O3
M3 without O3
M2 without O3
M1 without O3
M3 with O3 (day 108)
1645
1400
1030
1600
1400
1010
Absorbance
0.024
0.018
0.012
0.006
0.000
4000
3500
3000
2500
2000
1500
1000
500
Wavenumber (cm-1)

Table

Table 1. Stages of operation for the biofiltration of ethyl acetate with and without O_3 addition

Stage of operation	**A**	**B**	**C**	**D**	**E**	**F**	**G**
Inlet Load **IL (g m^{-3} h^{-1})**	60	120	180	180	180	180	180
Lapsed days	0-10	11-38	39-78	79-108	109-159	160-189	190-230

Table 2. Average biomass content by modules and stages of operation during the biofiltration of EA with and without O_3 additions.

With O_3	A	B	C	D	E	F	G
M1	19.9 ± 3.8	34.7 ± 5.0	42.3 ± 6.8	53.2 ± 6.8	110.8 ±25.0	125.9 ± 4.3	91.9± 19.0
M2	24.9 ± 1.7	68.1 ± 2.6	153.8 ± 10.0	228.7 ± 17.8	315.2 ± 15.2	290.7± 48.9	231.9± 16.1
M3	25.1 ± 1.4	47.2± 1.9	100.3 ± 9.5	79.2 ± 16.3	139.7± 5.2	161.1 ± 15.0	218.3± 20.2
Without O_3	A	B	C	D	E	F	G
M1	56.8 ± 2.9	66.4 ± 4.0	129.9 ± 10.6	267.6± 36.4	259.1± 10.6	354.7± 21.3	334.6 ± 44.9
M2	38.7 ± 4.1	51.2 ± 1.8	120.7 ± 23.4	247.1 ± 34.7	196.7± 6.7	149.9 ± 24.4	228.5± 7.6
M3	34.6 ± 1.3	58.5 ± 4.7	119.1 ± 1.6	136.6 ± 2.9	197.9± 7.9	219.9± 6.1	302.3± 12.3

Average biomass content (mg $_{biomass}$ $g^{-1}{}_{dry\ perlite}$) ± Standard deviation. Days; A=0-10, B=11-38, C=39-78, D=79-108, E=109-159, F=160-189, and G=230.M1, M2 and M3: modules of the biofilter.

Table 3. Ratios of EPS and biomass amount produced by modules and stages of operation. Lapsed days; A=0-10, B=11-38, C=39-78, D=79-108, E=109-159, F=160-189, and G=190-230.

Module **with O_3**	A	B	C	D	E	F	G
M1	0.18 ± 0.001	0.09 ± 0.002	0.10 ± 0.008	0.08 ± 0.004	0.005 ± 0.001	0.006 ± 0.0005	0.01 ± 0.0001
M2	0.17 ± 0.009	0.06 ± 0.0003	0.05 ± 0.001	0.14 ± 0.014	0.08 ± 0.003	0.05 ± 0.007	0.05 ± 0.003
M2	0.11 ± 0.006	0.10 ± 0.007	0.05 ± 0.001	0.03 ± 0.001	0.05 ± 0.004	0.10 ± 0.005	0.06 ± 0.001
Module **without O_3**	A	B	C	D	E	F	G
M1	0.08 ± 0.003	0.11 ± 0.016	0.09 ± 0.001	0.07 ± 0.003	0.10 ± 0.005	0.07 ± 0.004	0.15 ± 0.014
M2	0.10 ± 0.009	0.10 ± 0.008	0.15 ± 0.020	0.07 ± 0.001	0.08 ± 0.010	0.09 ± 0.002	0.11 ± 0.001
M3	0.13 ± 0.008	0.10 ±0.008	0.10 ± 0.008	0.11 ± 0.004	0.07 ± 0.009	0.12 ± 0.001	0.14 ± 0.0002

Average ratio EPS/Biomass ($mg_{EPS}\, mg^{-1}_{biomass}$) ± Standard deviation.