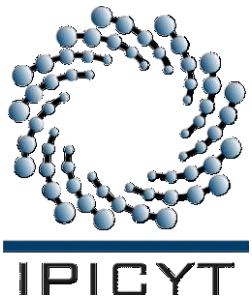

INSTITUTO POTOSINO DE INVESTIGACIÓN CIENTÍFICA Y TECNOLÓGICA, A.C.

POSGRADO EN CIENCIAS AMBIENTALES

Effect of the continuous addition of ozone in a biofilter treating ethyl acetate vapors

Tesis que presenta

Itzel Covarrubias García

Para obtener el grado de

Maestra en Ciencias Ambientales

Directora de la Tesis:
Dra. Sonia Lorena Arriaga García

San Luis Potosí, S.L.P., septiembre de 2016

IPICYT

Constancia de aprobación de la tesis

La tesis ***"Effect of the continuous addition of ozone in a biofilter treating ethyl acetate vapors"*** presentada para obtener el Grado de Maestra en Ciencias Ambientales fue elaborada por **Itzel Covarrubias García** y aprobada el **veintiséis de septiembre del dos mil dieciséis** por los suscritos, designados por el Colegio de Profesores de la División de Ciencias Ambientales del Instituto Potosino de Investigación Científica y Tecnológica, A.C.

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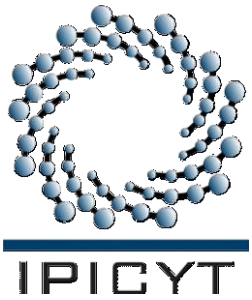

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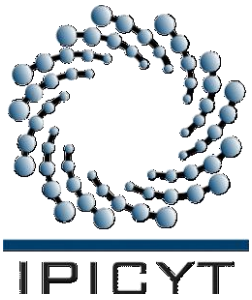

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Dra. Sonia Lorena Arriaga García	**Secretaria**	**IPICYT**

a fin de efectuar el examen, que para obtener el Grado de:

MAESTRA EN CIENCIAS AMBIENTALES

sustentó la C.

Itzel Covarrubias García

sobre la Tesis intitulada:

Effect of the continuous addition of ozone in a biofilter treating ethyl acetate vapors

que se desarrolló bajo la dirección de

Dra. Sonia Lorena Arriaga García

El Jurado, después de deliberar, determinó

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SECRETARIA ACADEMICA

Para mis padres por todo su amor, apoyo y aliento, a los que debo todo lo que soy.

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Abbreviations

µL	microliter
µm	micrometer
atm	atmospheres
ATR	Attenuated Total Reflectance
C	Celsius
cP	centipoise
DRE	Destruction and Removal Efficiency
EA	Ethyl Acetate
EBRT	Empty Bed Residence Time
EC	Elimination Capacity
EPS	Exopolysaccharides
F	Fahrenheit
FID	Flame Ionization Detector
FTIR	Fourier transform infrared spectroscopy
g	gram
GC	Gas Chromatography
h	hour
Hg	Mercury
IL	Inlet Load
kPa	kilopascals
L	liter
m	meter
M1	Upper module
M2	Middle module
M3	Lower module
mg	milligrams
min	minute
mL	milliliter
mm	millimeters
O_3	Ozone
ppb_v	Part Per Billion by Volume
RE	Removal Efficiency
rpm	Revolution per minute
SEMARNAT	Secretaria de Medio Ambiente y Recursos Naturales
SINEA	Sub-sistema del Inventario Nacional de Emisiones a la Atmósfera de México
SPME	Solid Phase Microextraction
TCD	Thermal Conductivity Detector
TOC	Total Organic Carbon
VICs	Volatile Inorganic compounds
VOCs	Volatile Organic Compounds
ΔP	Pressure drop

Resumen
Efectos de la adición continúa de Ozono en un biofiltro para el tratamiento de vapor de acetato de etilo

En este trabajo se analizaron los efectos de la adición continua de ozono (O_3) en un biofiltro para el tratamiento de Acetato de Etilo (EA). Se operaron tres filtros, un control abiótico para estimar oxidación química, un biofiltro sin adición de O_3 y uno bajo la adición continúa de O_3. El trabajo de tesis se dividió en 4 capítulos. El capítulo I presenta una visión general sobre la contaminación del aire, datos estadísticos de los Compuestos Orgánicos Volátiles (COVs) en México y el problema del taponamiento en biofiltros; el capítulo II evalúa la adición continua de O_3 como un método para el control la biomasa; el capítulo III se enfoca a los efectos que tuvo la adición continua de O_3 sobre los componentes de la biopelícula microbiana a lo largo de los sistemas de biofiltración; finalmente, el capítulo IV presenta los efectos de la adición continua de O_3 sobre la composición de la comunidad microbiana. Con el control abiótico se comprobó que más del 94% de la eliminación del contaminante en los biofiltros fue llevada a cabo por los microorganismos. La adición continua de O_3 ayudó a mantener estable la eficiencia de remoción en un 100%, y además tuvo un claro efecto sobre la concentración de la biomasa y la caída de presión. Sin embargo, los resultados de fracción vacía del lecho indicaron que eventualmente el biofiltro con O_3 se obstruirá cuando se alcancen periodos de operación más largos. Con los análisis de FTIR y sus componentes, se concluyó, que la adición continúa de O_3 solo tiene un efecto sobre la cantidad de EPS y no en sus componentes. Los análisis de la comunidad microbiana mostraron, que ambos biofiltros con/sin O_3 presentaron una composición similar. Las principales bacterias identificadas fueron *Beijerinckia, Gluconacetobacter* y *Acidocella*. Los principales hongos identificados fueron *Rhinocladiella similis, Trichosporon veenhuissi, Exophilia oligosperma,* entre otros. El estudio mostro la diversidad y la evolución de comunidad bacteriana y fúngica y como estas fueron afectadas por la continua adición de O_3.

PALABRAS CLAVE. Biofiltro; ozono; acetato de etilo; taponamiento; biomasa; comunidad microbiana

Abstract

Effects of the continuous addition of ozone in a biofilter treating ethyl acetate vapors

In the present Master thesis, it was analyzed the effects of O_3 addition on a biofilter treating EA (Ethyl Acetate). Three filters were operated, an abiotic control merely to estimate the chemical oxidation, a biofilter without O_3 addition and a biofilter working under O_3 addition. The thesis work was divided into 4 chapters. Chapter I is an overview of air pollution, statistical data on of VOCs in México and clogging issues, in Chapter II it was evaluated the biofilter performance with and without continuous addition of O_3 in order to assess the continuous addition of O_3 as a biomass control method, in Chapter III the effects of the continuous O_3 addition on the main components of the biofilm along biofilter systems were discussed, and finally in Chapter IV microorganisms in biofilters that work with and without O_3 addition were identified in order to study the effect of the O_3 treatment on the microbial community composition by culture-independent molecular approaches. With the abiotic control was ensured that the removal of the pollutant in the biofilters was merely by microbial activity. In general, O_3 addition helped to keep stable the 100% removal efficiency of the system with O_3 addition, with a clear effect over the biomass concentration and the pressure drop. However, the results of void fraction indicated that eventually the biofilter will clog. On the other hand, O_3 addition was affecting only the amount of EPS not the composition with a greater effect on carbohydrate. Regarding the microbial community of the biofilter investigated, its composition was similar in the biofilter working with O_3 and without, main genus of bacteria identified were *Beijerinckia, Gluconacetobacter* and *Acidocella*, principally; the gene fungal presented in the biofilters were, *Rhinocladiella similis*, *Trichosporon veenhuissi*, *Exophilia oligosperma* and others. Thus the study showed the diversity and development of bacterial and fungal communities and how they were affected by continuous treatment of O_3.

KEY WORDS. Biofilter; ozone; ethyl acetate; clogging; biomass; microbial communi

Chapter I. INTRODUCTION

1. Air Pollution

Contamination is simply the presence of a substance where it should not be or at concentrations above background. Pollution is contamination that results in adverse biological effects to resident communities. All pollutants are contaminants, but not all contaminants are pollutants. Differentiating pollution from contamination cannot be done solely on the basis of chemical analyses because such analysis do not provide information on bioavailabilty or toxicity (Chapman, 2006). Environmental pollution is the presence of an agent potentially damaging to either the environment or human health. If such agent is present in air then it is considered as an air pollutant. Polluting agents are called pollutants which include particles, chemicals, and microorganisms. Air pollutants can be classified by their source, chemical composition, size, and mode of release into indoor or outdoor environments*, **Table 1*** distinguishes between primary versus secondary, indoor versus outdoor, and gaseous versus particulate matter pollutants.

Table 1. *Classification of air pollutants (Bernstein et al., 2004).*

A. Primary-secondary pollutants
(i) Primary: pollutants emitted directly into the atmosphere (SO_2, some NOx species, CO, PM)
(ii) Secondary: pollutants formed in the air as a result of chemical reactions with other pollutants and gases (ozone, NOx, and some particulates)
B. Indoor-outdoor pollutants
(i) Indoor pollutants
(a) Sources: cooking and combustion, particle resuspension, building materials, air conditioning, consumer products, smoking, heating, biologic agents
(b) Products: Combustion products (tobacco and wood smoke), CO, CO_2, VOCs (aldehydes, alcohols, alkanes, and ketones), microbial agents and organic dusts, radon, manmade vitreous fibers
(ii) Outdoor pollutants
(a) Sources: industrial, commercial, mobile, urban, regional, agricultural, natural
(b) Products: SO_2, ozone, NOx, CO, PM, VOCs
C. Gaseous-particulate pollutants
(i) Gaseous: SO_2, NOx, ozone, CO, VOCs (Dioxins, benzene, aldehydes, 1,3-butadiene)
(ii) Particulate: coarse PM (2.5-10 μm; regulatory standard = PM10), fine PM (0.1-2.5 μm; regulatory standard = PM2.5); ultrafine PM (<0.1 μm; not regulated)

NOx, Nitrogen oxides; VOCs, volatile organic compounds; PM, particulate matter; SO_2, Sulfur dioxide; CO, carbon monoxide; CO_2, carbon dioxide.

Gaseous pollutants may be divided into volatile organic compounds (VOCs), volatile inorganic compounds (VICs) and greenhouse gases such as carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O), hydrofluorocarbons (HFCs), perfluorocarbons (PFCs), sulfur hexafluoride (SF_6) and others.

1.2 Volatile organic compounds (VOCs)

Volatile organic compounds (VOCs) are among the most common air pollutants emitted from chemical, petrochemical, and allied industries. VOCs are the main sources of photochemical reaction in the atmosphere leading to various environmental hazards. On the other hand, these VOCs have good commercial value (Khan & Kr. Ghoshal, 2000). VOCs are defined as organic compounds or, in other words, carbon containing molecules that also contain other species, such as H or O (excluding carbon monoxide, carbon dioxide, carbonates, carbides and cyanides). Vapor pressure at room temperature or, otherwise but less frequently, the boiling point at atmospheric pressure is often considered to decide whether a compound is volatile or not. VOCs have a vapor pressure of 10^{-2} kPa or more at 20 °C. A brief list of common VOCs are presented in ***Table 2***.

Table 2. *Common VOCs* (Khan & Kr. Ghoshal, 2000)

Volatile Organic Compounds
Acetaldehyde
Acetone
Benzene
Carbon tetrachloride
Ethyl acetate
Ethyl glycol
Formaldehyde
Heptane
Hexane
Isopropyl alcohol
Methyl chloride
Naphthalene
Styrene
Toluene
Xylene

On the other hand, VOCs are also frequently found in indoor air. The quality of air inside homes, office and other work place has become a matter of growing concern

as homes and work places are not fully safe (Chandrappa et al; 2016). This has become an important issue the last 10 years because the number and amounts of organic compounds have increased with greater use of chemicals and synthetic building materials, which decrease the quality of indoor air. VOCs may exert highly different and variable effects on human health depending on their nature, they may irritate the eyes, nose and throat, or act as central nervous system depressants. Some VOCs are carcinogenic and mutagenic (Kostiainen,1995). From an environmental point of view, it is necessary to limit and control vapor emissions, since they affect the change of climate, the growth and decay of plants, and the health of human beings (Khan & Kr. Ghoshal, 2000).

1.3 Emissions of Volatile Organic Compounds (VOCs) in Mexico

Nowadays air pollution causes thousands of deaths around the world. In Mexico more than 10,800 people died each year due to the bad air quality. The Air pollution in Mexico is one of the 10th main cause of death (Instituto Nacional de Salud Pública, 2013). According to the last national emission report in 2008, 57, 556, 755 tons correspond to gaseous pollutants, of this, 29% corresponds to VOCs and 36% of the total comes from anthropogenic source (***Figure 1a, b***).(SINEA;SEMARNAT, 2008)

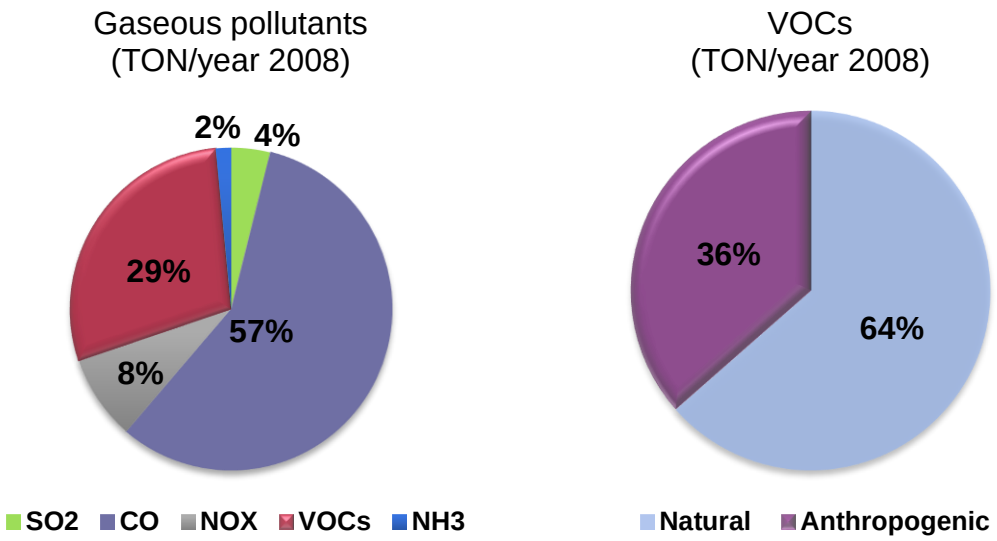

Figure 1. *Percentage of National gaseous emissions of Mexico 2008.* **a)** *Total percentages of gaseous emissions,* **b)** *Percentages of VOCs from anthropogenic and natural sources.* (http://sinea.semarnat.gob.mx) 2008.

1.4 Ethyl Acetate as VOC

Ethyl Acetate (EA) and toluene are among the key pollutants in the exhaust air from printing and coating facilities, and paint manufacturing (Liu et al., 2002), also they are used as a solvent in pharmaceutical applications, in artificial fruit essences, and in the manufacture of smokeless powder, artificial leather, photographic films and cleaning textiles, resulting in its release to the environment through various waste streams. EA is a kind of irritating and explosive compound with fragrant odor, which is harmful to the respiratory system.

EA is 10 % soluble in water but soluble in most inorganic solvents. It is colorless and has a characteristic fruity smell. ***Table 3*** present the main physicochemical characteristics of EA.

Table 3. *Main physicochemical properties of Ethyl Acetate.*
(Agency United States Environmental Protection, 2006)

Physicochemical properties of Ethyl Acetate		
Property	**Magnitude**	**Units**
Density	900	Kg m^{-3}
Molecular Weight	88.11	g mol^{-1}
Melting point	-83	° C
Boiling point	77	° C
Viscosity	0.45 a 20 ° C	cP
Water Solubility	10	%
Vapor pressure	74.4 (20 ° C)	mm Hg
Henry constant	1.34 x 10^{-4}	atm m^3 mol^{-1}
Relative density (water=1)	0.902 (20 ° C)	
Relative vapor density (air=1)	3.04	
Specific gravity	0.8945	
Values under standard conditions 25 ° C y 1 atm, unless otherwise stated value.		

1.5 Toxicity and risk of EA in health.

Exposure of EA in short time with high concentrations results first in irritation of eyes, nose and throat, followed by headache, nauseous, vomits, somnolence and loss of consciousness. Long exposure can cause eye opacity, damage in the lungs and hearth, kidney and liver problems. EA contact is possible if people breathe contaminated air or if people drink or eat contaminated food, also it can be adsorbed by the skin. Consumers may also be exposed when using products, such as, thinners for lacquers, enamels for painting, etc. According to Worksafe Australia

(2011), workers can be exposed to concentrations of 200 parts per million for an eight hour shift.

1.6 VOCs Treatment technologies

Nowadays emission and control of VOCs have become an important issue, due to their harmful effects in long or short term over human, organisms and environment. In general the available technologies that can treat these atmospheric pollutants are divided in two main groups, destruction and recovery systems (***Figure 2***).

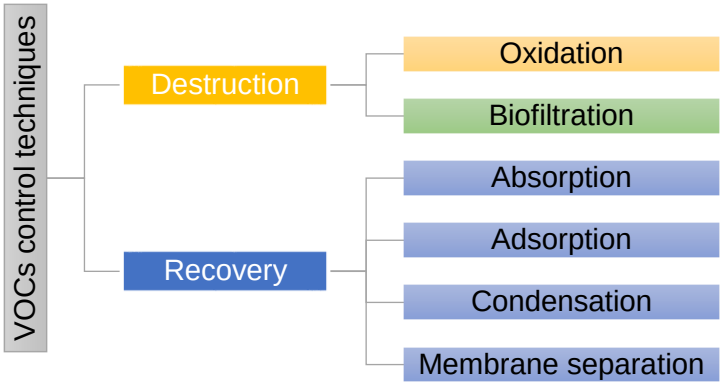

Figure 2. *VOC removal techniques.*

In destructive techniques, VOCs are destroyed by different types of oxidation such as thermal and catalytic, and degradation of VOCs under aerobic condition by microbes (biofiltration). Thermal oxidation systems combust VOCs at temperatures of 1300-1800 °F. The operating temperature is a function of the type and the desired DRE (Destruction and Removal Efficiency). High DRE requirements will require higher temperatures and longer retention times in the combustion zone. Operating temperatures near 1800 °F can produce elevates levels of nitrogen oxides (from nitrogen in the air), a secondary pollutant that may, in turn, require further treatment. Catalytic oxidation systems combust VOCs in a manner similar to thermal oxidizers. The main difference is that the catalytic system operates at lower temperature (typically 700-900 °F). This is made possible by the use of catalysts that reduce the

combustion energy requirements. Catalytic systems have been installed, but are not as popular as direct thermal oxidation systems, mainly due to the high costs of catalyst replacement (Khan & Kr. Ghoshal, 2000). Catalyst systems can produce secondary combustion wastes such as acidic species. Also, the spent catalyst materials can require disposal as a hazardous waste if they are not recyclable. On the other hand, biofiltration is a process in which contaminated air is passed through a porous medium that supports a thriving population of microorganisms. This process is carried out at ambient conditions of pressure and temperatures, which represents lower energy requirement.

Several techniques for recovery of VOCs such as condensation, absorption, adsorption and membrane separation are used. The driving force for condensation is over-saturation by chilling or pressurisation (or both) of the waste was stream. Condensation is most efficient for VOCs with boiling points above 100 °F. Low boiling points of VOCs can require extensive cooling or pressurisation, which sharply increases operation costs. Absorption is used to remove VOCs from gas streams by contacting the contaminated air with a liquid solvent. This take place in an absorber tower designed to provide the liquid vapour contact area necessary to facility mass transfer (Berenjian et al; 2012). Membrane separation has been classified in many groups: gas permeation, reverse osmosis, dialysis, electro-dialysis, gel permeation and pre-evaporation. Gas permeation and reverse osmosis are used in recovery of VOCs from air. Although this technique promises good results, its drawbacks are important, such as, the cost of the membrane, its maintenance, if the membrane is rechargeable or reusable and process rate (Khan & Kr. Ghoshal, 2000). The last recovery technique is adsorption, which is based in the interaction between adsorbate and adsorbent. The main disadvantage in the use of this technique is the cost of the packing material.

1.7 Biofiltration

Biofilters are a cost-effective and eco-friendly alternative to physicochemical air pollution control methods. Biofiltration technology can treat a large variety of VOCs and inorganic gases, with innocuous by-products. This technique is based on the

ability of microorganisms (generally bacteria) to convert, under aerobic conditions, organic pollutants to water, carbon dioxide and biomass (cellular synthesis). Biofilters are reactors in which a humid polluted air stream is passed through a bed containing a porous support matrix harboring the active pollutant degrading microorganisms, the filter bed has to have an adequate moisture content, nutrients and oxygen supply in order to support microbial activity.

Biofilters are systems which combine several phenomena, such as adsorption, absorption, biodegradation and desorption. Basically, biofiltration is a two-step process consisting of the transfer of the compounds from the gas phase to the biofilm (diffusion) and the subsequent biodegradation (oxidation) of the absorbed compound by the microorganisms present in the filter bed. ***Figure 3***.

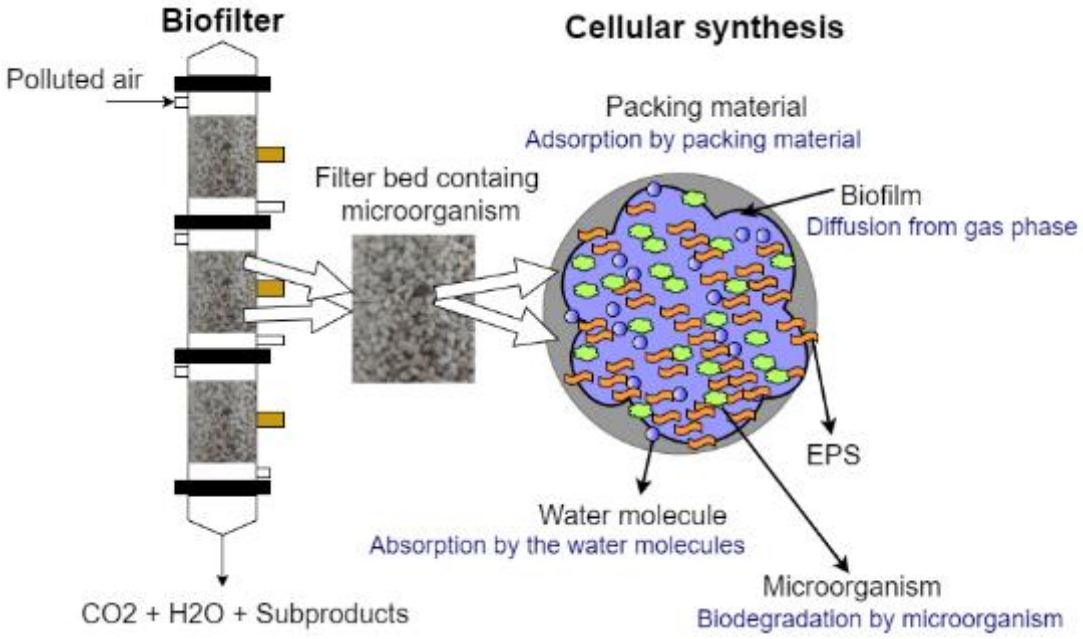

Figure 3. *Phenomena involved in biofiltration process.*

1.8 Clogging in biofilters

In gas biofiltration processes, pollutants from waste phase are degraded into environmentally benign end-products when gases pass through biologically active media on which biofilms attached (Zhu et al; 1996), which as mentioned above, makes biofiltration process be considered a cost-effective, reliable and environmental friendly technology. Although, biofiltration has many advantages,

some problems remain. One of the most serious problems consists in the excess accumulation of biomass within biofilter beds. Biomass is a critical factor in gas biofiltration, which often result in clogging, generating operational problems such as dead zones, channeling, uneven biomass distribution and excessive head loss within biofilter beds, and consequently, the deterioration of performance. Under these conditions the biofilter must be washed or eventually replaced to remove the excess of biomass in order to maintain the operating cycles. In ***Table 4*** are presented several studies which reported "clogging" in biofiltration systems for VOCs removal. The main pollutant studied is toluene and the first parameter that is described when clogging appears, is an increment in the pressure drop and the decrease of the removal efficiency.

Table 4. *Relevant studies reported clogging in biofilters*

System	Pollutant	Findings Comments	Parameter	Reference
Biofilter	Styrene	In achieving the high removal rates in the ammonia supplied biofilter, the excess of biomass accumulates on the filtering pellets and causes progressive clogging.	Maximum pressure drop 14 cm H_2O by day 50	Jorio et al; 2000
	Toluene	Decrease in removal efficiency and increase in the pressure drop due to excess of biomass. More biomass content was accumulated in the inlet section.	After day 50, the biomass amount reached above 10,000 g C m^{-3} and the performance declined.	Xi, Hu, & Qian, 2006
	Toluene	Conditions more favorable for toluene biodegradation are equally those that contribute strongly to excess biomass and biofilter clogging.	Optimum elimination capacities [90-95 g $m^{-3}h^{-1}$]	Delhoménie et al; 2003
	Benzene	Benzene removal efficiency was decreased to 75% after 27 days, and pressure drop in the biofilter was increased to 100 mm H_2O m-1 due to clogging.	Maximum pressure drop for maintaining stability of the biofilter 30-33 mm H_2O	Ryu, Cho, & Chung, 2010
Trickle bed biofilter	Toluene	Excessive accumulation of biomass in the reactor has a negative effect on the contaminant removal efficiency.	Decrease in the removal efficiency to 78%. Pressure drop increased rapidly.	Alonso et al., 1997

Several methods have been developed in order to reduce the biomass accumulation, these methods are classified as biological, chemical and physical. ***Table 5***.

Table 5. *Methods for clogging prevention in biofiltration systems*

	Method	System	Pollutant	Findings Comments	Advantages	Disadvantages	Reference
Biological	Starvation	Biofilter packed hybrid organic/ inorganic	Toluene	The success in the operation was partially explained by the suitability of the clay pellets.	Recover rapidly the activity after several weeks of starvation.	Watering and properly air velocity is necessary in order to wash the excess of biomass.	Dorado, et al; 2012
	Protozoan Predation	Biotrickling filter	Toluene	Toluene degradation rates increased and were slightly higher in the filter enricher with protozoa.	Little reactor maintenance.	Challenge to maintain long-term stable performance.	Cox & Deshusses, 1999
	Fungal predation	Bench Bioreactor	Toluene	Mites as fungal predators improved the performance.	Mites promote complete mineralization. Improving the excess of biomass.	Mites are mobile and sensitive to changes in the packing medium.	Woertz et al., 2002
Chemical	Chemical washing H_2O, NaOH, NaClO, H_2O_2,	Biotrickling filters	Toluene	NaOH, NaClO, H_2O_2 showed a significantly higher removal of biomass that the control (pure water) NaClO was found to be the most promising reagents; mass removal efficiency, was high at relatively low concentration.	NaClO remove large amounts of biomass, it is cheaper than the others. NaOH low residual biological activity was observed.	In most cases, efficient biomass removal resulted in a complete loss of biological activity. NaOH resulted in generation of relatively large amounts of suspended solids.	Cox & Deshusses, 1999

	Backwashing H_2O, NaOH, NaClO	Biofilter bed	Toluene	The efficiency of backwashing and air sparging was relatively similar, and more effective when chemicals were added.	Addition of chemicals allowed the elimination of significantly more biomass.	Addition of chemicals resulted in a significant decrease of bio filter's performance (Inhibitory effects).	Mendoza, Prado, Veiga, & Kennes, 2004
	Backwashing H_2O Nitrate instead of Ammonia	Trickle bed air biofilter	Toluene	The use of nitrate was very effective in reducing the biomass yield.	Cost advantages of using nitrate instead ammonia.	Recovery time after backwashing Still needed backwashing to maintain the performance.	Smith et al., 1996
	Frequency and duration of Backwashing Nutrient control P	Trickle-bed biofilter	Styrene	Backwashing necessary to maintain biofilter performance. Nutrient P failed as a biomass control.	Periodic backwashing attained stable, long-term high removal efficiencies.	Dramatic decrease in the biofilter performance with P.	Sorial et al., 1997
	Nutrient control N	Biofilter	Toluene	Use of alternating solution enriched with N solution, without or poor N.		The biomass accumulation could not be avoided through nutrient controls.	Delhoménie et al., 2003
	Ozone	Biofilter	Toluene	The injection of O_3 had no adverse effect on toluene removal.	The injection of O_3 could effectively keep the pressure drop stable.	Operation cost increase by O_3 addition.	Xi, Saingam, Gu, Hu, & Zhao, 2014
	Ozone	Biofilter	Chlorobenzene	The average chlorobenzene removal efficiency increased from 40% to 70%. The thickness and extra-cellular polymer substance (EPS) content of the biofilm	Improvement of the removal efficiency. The reduction of EPS content promotes mass transfer in the biofilm.	Operation cost increase	Wang, Xi, Hu, & Yao, 2009

				were remarkably reduced.			
Physical	Bed stirring and bed washing H_2O	Biofilter	Toluene	Mechanical methods gave rise to improved performance 80%.	Mechanical methods no affect the performance.	Bed washing energetically unfavorable (water fed by a centrifugal pump).	(Delhoménie et al., 2003)
	Step-feed Biofilter Configuration	Packed biofilter	Toluene	The air emission was supplied in either two or three locations along the biofilter height. The step-feed biofilter exhibited 75% lower compression energy requirement.	Promising operational strategy reducing operating cost and increased packing material lifespan.	Higher investment costs.	Estrada et al; 2013
	Stagnation (no nutrient and air) and flow switching	Trickle Bed Air Biofilter	n-Hexane	Flow switching involves direction of the gas flow co-current with the nutrient liquid flow downwards then countercurrent upwards. Stability of the results was more pronounced by using flow switching.	Flow switching together with stagnation could successfully replace backwashing for low microbial yield compounds such n-hexane.	Complex operation	Hassan & Sorial 2010
	Filling with water and draining.	Biofilter	Toluene	Filling/draining method the least efficient.	No inhibitory effect on the activity of the biofilm.	Little biomass could be removed	Mendoza et al., 2004
	Air sparing. Temperature (30-60)	Biofilter	Toluene	Higher flow rates and higher temperatures allowed the removal of more biomass.	Less inhibitory effect with heat than when adding chemicals.	Energetic Requirements	Mendoza et al., 2004

Physical methods to control biomass are effective, however, within their main disadvantages are the high energy consumption and their complex operation. Biological methods such as biological predation are considered one of the most promising methods because as they are based on trophic levels. However, the main challenge in these methods is maintaining stable the reactor, as microorganisms are mobile and sensitive to changes in the media. On the other hand, chemical methods for controlling biomass are the most used (backwashing and washing). Chemical methods allow to remove large amounts of biomass compared with physical ones, but, in most cases, the addition of a chemical results in a total or partial loss of the biological activity. Moreover, the leachate can be more toxic than the treated pollutant itself. Other chemical methods for biomass removal are the limitation of nutrients for a certain period of time or the application of a continuous flow at different concentrations. Cox and Deshusses 1998 and Mendoza et al., 2004 used chemicals such as water, sodium hydroxide, sodium hypochlorite and hydrogen peroxide. Except for water, all these reagents were shown to be very efficient for biomass removal. However this was achieved at the expense of biological activity. Smith et al., 1996 used water as washing solution and ammonium nitrate as a nutrient. The use of nitrate reduced biomass yield. Sorial et al. (1997) varied the frequency and duration of backwashing (water at 32 °C) and phosphate nutrient test as a control. Phosphate failed as a control of biomass. Generally, biomass accumulation cannot be controlled by nutrients limitation. Wang et al. (2009) used O_3 pulsations with different concentrations to control biomass degradation of chlorobenzene in a biofilter, the thickness of the biofilm and the exopolysaccharides (EPS) were remarkably reduced while the O_3 concentration was increased, and O_3 addition increased the removal efficiency of 40% to 70%. Xi et al. (2014) also used O_3 as biomass control for toluene degradation. O_3 injection had no adverse effects on the removal efficiency and could maintain stable pressure drop. García-Pérez et al. (2013) applied O_3 pulses during high inlet load of formaldehyde (65 $gm^{-3}m^{-1}$). At this inlet load, the microbial activity was inhibited by the formation of acid products (pH <4). Adding O_3 pulses helped to increase the inlet load to 74 g m^{-3} h^{-1} and maintain stable the biofilter performance; However, the effect of O_3 on EPS could not been

verified due to the low amount of biomass produced. Furthermore, O_3 pulses worked as a buffer, preventing acidification. On the other hand, Maldonado-Diaz & Arriaga (2014) reported formaldehyde biofiltration with O_3 addition, it was found that O_3 helped to recover the removal efficiency and also mentioned that O_3 addition maintained an optimum pH of 7.4-8.2.

Based on these studies, the addition of O_3 can maintain stable the biofilter performance, regulate the pH, decrease the amount of biomass and thus, decrease the pressure drop, helping to achieve higher removal efficiencies at higher inlet loads. Nevertheless, all these studies have treated recalcitrant pollutants, which produce low amounts of biomass. For all these, and as O_3 is a promising alternative for the control of biomass, it is important to better prove the O_3 effectiveness in a more readily degradable VOC, such as EA.

2. Justification of the thesis work

Volatile organic compounds (VOCs) are common air pollutants that can adversely affect human health and the environment; because of this, several technologies have been developed in order treat this issue. Biofiltration is a cost-effective and eco-friendly technology to treat these streams. However, long term operation or high inlet loads of pollutants may cause an excess in the biomass amount and a “clogging” phenomenon, which, could lead to problems like channeling, dead zones and high pressure drops, resulting in a decrease of biofilter performance. It is thus primordial to control the excess of biomass and thus, improve the biofilter performance and increase the half-life of the filter medium. On the other hand, ozone as a strong oxidant and antimicrobial agent has been used at low concentrations in biofilter systems to control the excess of biomass. These studies have reported that ozone addition has helped to achieve higher removal efficiencies with higher inlet loads of pollutant. It is important to mention that all these studies have treated recalcitrant VOCs, which, do not produce excessive biomass to effectively prove the clogging prevention. Above all, the aim of this study is to evaluate the effect of the continuous addition of O_3 at low concentration (90 ppb) in a biofiltration system, treating Ethyl Acetate, a readily degradable molecule.

2.1 Hypothesis

The continuous addition of ozone will affect the activity of the microbial biofilm, enhancing its metabolic capacity of consumption and avoiding the clogging in a biofilter treating ethyl acetate. This will improve system performance.

2.2 General Objective

To study the effects of the continuous addition of ozone in eliminate biomass clogging in a biofilter treating ethyl acetate vapors.

2.3 Specific Objectives

i. Evaluate if the continuous addition of ozone enhances the biofilter performance.
ii. Assess the continuous addition of ozone as a biomass control method.
iii. Study the biomass composition along the biofilter systems and the operation time.
iv. Identify the microbial community resemblances between a biofilter with continuous ozone addition and without.

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Chapter II. EFFECT OF THE CONTINOUS ADDITION OF OZONE ON BIOMASS CLOGGING CONTROL IN A BIOFILTER TREATING ETHYL ACETATE VAPORS.

ABSTRACT

Biofiltration systems have been recognized as a cost-effective and environmental friendly control technique for volatile organic compounds (VOCs) removal. However, the long-term operation of biofilters causes biomass accumulation and thus, the occurrence of bed clogging, leading to a major decrease in biofilters performance. Control methods have been carried out in order to solve clogging problems, including, backwashing, bed stirring, modification of flow patterns, predation, starvation and others. Ozone (O_3) has been used in biofiltration systems at low concentrations to control the excess of biomass. It is worth mentioning that all these biofiltration studies involving O_3 treated recalcitrant pollutants like chlorobenzene, formaldehyde and toluene, which do not produce enough biomass to effectively prove the clogging prevention. Thus, this study evaluated the effect of the continuous addition of O_3 as a chemical oxidant at a very low concentration (90 ppb) as a practical solution to overcome clogging in a process of biofiltration of ethyl acetate (EA), a readily degradable molecule. The maximum elimination capacities achieved were 200 g m^{-3} h^{-1} and 120 g m^{-3} h^{-1}, with and without O_3, respectively. The biomass concentration in these systems were, 23.33-180.1 mg biomass $g_{perlite}$ with O_3 addition and 43.31-288.46 mg biomass $g_{perlite}$ without O_3 addition. Based on the results, it was concluded that the continuous addition of O_3 could be an attractive solution to improve biofilter performance and extend the half time life of the filter bed.

Keywords: biofilter; clogging; ethyl acetate; ozone; biomass

1. Introduction

Volatile organic compounds (VOCs) are among the most common air pollutants emitted from chemical, petrochemical, and allied industries. VOCs are the main sources of photochemical reaction in the atmosphere leading to various environmental hazards. Two main group of technologies have been developed in

order to treat these emissions, biological and physical-chemical processes. Even if physico chemical processes are highly efficient, no technology can be considered fully satisfactory due to their different drawbacks. The economical term is important to consider, for example for physico chemical processes such as adsorption, the main issue is the cost and lifespan of the activated carbon and in the case of chemical scrubbers it is the constant chemical consumption (Estrada et al. 2012). Other drawbacks in this group are related with the high energy consumption and the use of hazardous and toxic chemical substances (Estrada et al. 2011). Also, it is worth mentioning that some of these methods are not destructive, and only transfer the pollutant from one phase to another. Biological treatments of VOCs offer potential advantages over physico chemical processes, since they need lower investment and lower operating cost. Microorganisms are used to metabolize organic compounds at ambient temperature and pressure, which represent a smaller carbon fingerprint due to the lower energy required. The most common types of biological treatment units are, biofilters, biotrikling filter, membrane bioreactor and bioscrubber. Biofilters are a cost-effective and eco-friendly technology that can treat VOCs and inorganic gases, and the by-products are innocuous. However, as it has been mentioned, each technology presents its own limitations. A major problem in biofilter systems is their instability due to rapid biomass accumulation over long term operation or when high inlet loads are applied. If no remedial action is taken, the biofilter will clog. “Clogging” phenomenon is a typical major problem which can lead to problems such as high pressure drop, channelling and dead zones, non-homogeneous microbial growth and uneven biodegradation activity along the filter bed. Moreover, excessive accumulation of biomass could lead to a negative effect on the contaminant removal efficiency (Alonso et al., 1997). Biomass growth in biofilters packed with inert carriers need to be controlled and optimized. Different physical, chemical and biological strategies have been evaluated for biomass control in biofiltration systems. Among them, splitting the feed and different speed ratios (Mendoza et al; 2003), bed stirring and nutrient control (Delhoménie et al; 2003), filing and draining with water and air sparging (Mendoza et al; 2004), step feed biofiltration (Estrada et al; 2013), starvation periods (Dorado et al; 2012), protozoa

predation (Cox & Deshusses 1999), mite predation (Woertz et al., 2002), backwashing with different chemicals and water (Smith et al., 1996; Sorial et al., 1997). Within the efforts of solving the clogging issue, O_3 treatment has also been proposed (Wang et al.2009; García-Pérez et al. 2013; Maldonado-Diaz & Arriaga, 2014; Xi et al. 2014). Even though O_3 seems to be a promising solution to control the excess of biomass in the treatment of recalcitrant VOCs, it is important to analyse its effectiveness for a readily degradable molecule, in order to produce enough biomass and effectively prove the clogging prevention. Ethyl Acetate (EA) is an irritative and explosive compound with fragrant odour that is used widespread in printing, coating facilities, paint manufacturing, pharmaceutical applications, artificial fruit essences and leather, and cleaning textiles (Budavari, 1996). Above all, the aim of this study is to prove O_3 effectiveness as a biomass control method in a biofiltration system treating a highly biodegradable pollutant, such as EA.

2. Methodology

2.1 Experimental set up of biofilters.

In brief, two biofiltration systems of 0.097 m diameter and 0.45 m high were assembled, each comprised of three identical modules of 1.1 L (total effective volume of 3.3 L) named as M1, M2, and M3, in which M1 correspond to the entrance of the saturated EA stream (**Figure 1**). One biofilter was operated without O_3 addition and served as a control and the other worked under O_3 addition. The packed material used was 3.35 mm expanded Perlite from Termolita Mexico. Reactors were inoculated with activated sludge from a waste water treatment plant of the Tangamanga Park I in San Luis Potosí, México. The mineral medium was prepared with 0.5 g L^{-1} $(NH_4)_2SO4$, 0.7 g L^{-1} KH_2PO4, 0.7 g L^{-1} K_2HPO_4, 0.3 $MgSO_4.7H_2O$ and trace elements, its pH was adjusted to 7 with NaOH solution 1N. Ethyl acetate (EA) gas phase was generated in a stripping reactor and its flux was controlled with a needle. Biofilters were fed in downward mode with an empty bed retention time (EBRT) of 60 seconds. O_3 was produced by a A2ZS-3GLAB OZONE GENERATOR system and its concentration (90 ppb) was monitored with the yodimetric method of

Rakness et al. 1996. Pressure drop was checked daily by height difference with a U-tube filled with water.

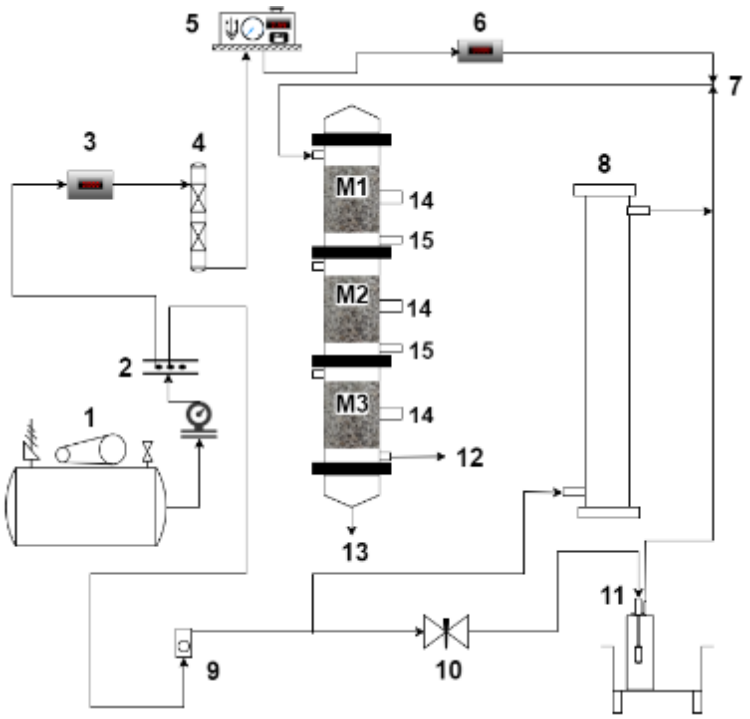

Figure 1. *Biofilter system with O_3 addition.* ***M1, M2, M3*** *(modules 1, 2 y 3).* ***1****.- Compressor;* ***2.****- Air distributor;* ***3.****- y* ***6.****- Mass flow controller;* ***4.****- Desiccator;* ***5.****- Ozone generator;* ***7****.- Union between ozone and EA gas flow ;* ***8.****- Humidifier;* ***9.****- Rotameter;* ***10.****- Needle valve;* ***11.****- EA stripping reactor;* ***12****.- Treated air;* ***13.****- Leachate;* ***14.****- Biomass sampling ports;* ***15.****- Gas phase sampling ports.*

2.2 Stages of operation.

The EA treatment experiments were carried out over a 230 day period divided into two phases (***Table 1***). The first was defined with the objective to achieve the maximum inlet load that the systems were able to work with, keeping a satisfactory removal efficiency. According to this, three stages were obtained with three different inlet loads: A=60 g m^{-3} h^{-1}, B=120 g m^{-3} h^{-1} and C=180 g m^{-3} h^{-1}. Due to that the removal efficiency dropped at stage C in the biofilter without O_3 addition, was opted to continue with the same inlet load in the next stages (D, E, F y G=180 g m^{-3} h^{-1}, phase 2) in order to continue to analyze at the same conditions the two biofilters. At the end of every stage, it was taken a representative sample from both biofilters to perform the pertinent analyzes.

Table 1. *Stages of operation.*

	Phase 1			Phase 2			
Letter	**A**	**B**	**C**	**D**	**E**	**F**	**G**
Lapsed period of operation	1-10	11-38	39-78	79-108	109-159	160-189	190-230
Difference (Days)		28	40	30	51	30	41
Inlet Load IL (g m^{-3} h^{-1})	60	120	180	180	180	180	180
IL calculated without O_3	51.2 ±3.6	120.7 ±13.5	183.7 ±25.4	184.1 ±11.3	195.7 ±30.5	185.6 ±11.8	179.8 ±27.0
IL calculated with O_3	54.2 ±9.9	127.6 ±15.5	188.9 ±32.3	190.0 ±14.8	200.7 ±24.8	182.5 ±19.4	186.9 ±8.4

2.3 Gas Chromatography

The concentration of EA was measured with a flame ionization detector (GC-FID) in a gas chromatograph equipment (Thermo Scientific Trace 1300). CO_2 concentration was measured with a thermal conductivity detector (GC-TCD) in a gas chromatograph equipment (Agilent Technologies 6850). Calibration curves of known concentrations of EA and CO_2 were made to determinate the concentration present in the samples taken at the inlet and outlet of the filters. EA and CO_2 were measured by triplicate.

2.4 Characterization of packing material

Bulk density, water retention capacity, void fraction and volatile suspended solids were estimated for the packing material (Perlite). Determinations were performed by triplicate. Supporting information ***Table S1***.

2.5 pH measurement and Total organic Carbon content (TOC)

Leachate was collected from the bottom of the biofilters each 4 days, its pH was measured with a potentiometer (Thermo Scientific Orion 4 Star), then kept at -80 °C to measure the total organic carbon (TOC) content. TOC was measured with a TOC-V_{CSN} SHIDADZU ASI-V apparatus. Samples were centrifuged at 3500 rpm for 20 min. 2 mL of the supernatant were filtered through a 0.22 µm membrane. Convenient dilutions were prepared before analysis with deionized water.

2.6 Volatile suspended solids and biomass content

The standard method (APHA, 2005) was used to determine the volatile suspended solid content of the biofilm and the leachates. Biomass content was determined by dry weight.

2.7 Abiotic reactor

In order to estimate the respective contribution of the chemical oxidation of EA and O_3, an abiotic filter was operated for 18 days within identical conditions and dimensions. Concentration of EA and CO_2 were measured by Gas Chromatography and the sub products of the chemical oxidation were identified by GC-MS.

2.8 Gas Chromatography coupled with mass spectrometry (GC-MS). Solid Phase Microextraction (SPME)

The chemical reaction products were identified with a gas chromatograph (Agilent Technologies model 7990A) coupled to a mass spectrometer (model 5975C), with the following parameters: injector 250°C, oven with a heating ramp starting at 70 °C for 1 min, then raised until 100 °C (3 °C min^{-1}) and lastly reached 250 °C (20 °C min^{-1}) for 2 min. Helium was used as carrier gas. Solid phase microextraction technique was used to analyze the samples, with a Carboxen-PDMS fiber. Basically the procedure lies in the exposure of the fiber for 5 min to the gas phase sample or in its case to the immersion in a leachate sample. The SPME fiber is directly desorbed in the GC injector.

2.9 Sorption of EA by packing material with and without biomass

Experiments were performed in serological bottles of 120 mL sealed with Mininert valves by triplicate at 25°C. The phenomenon of sorption was evaluated for perlite at ambient moisture content, water saturated perlite and perlite with biomass. The concentration of EA sorbed was estimated by taking a sample from the headspace´s bottle and then injected in GC equipment. The injections were made regularly until the response did not change (72 h). The samples of perlite with biomass were a representative mixture of the perlite of both biofilters at the end of the operation. In

all cases, 2 g of packing material were placed in the serological bottles with concentrations of EA from 1 µL to 7 µL, making sure that the drops of EA volatilize on the headspace of the bottles. To ensure that the disappearance of EA in the headspace was merely due to sorption in the biomass and not to microorganism´s activity, biomass samples placed in the bottles were previously sterilized daily for four days and CO_2 concentration were measured all along the experiment. For the wet samples, approximately 13 g of perlite were wetted in 200 mL of mineral medium for 24 h, to simulate the moisture present in the reactor. The excess of water was drained. Sorption isotherms were adjusted to the basic models, Freundlich and Langmuir.

2.10 Carbon balance

The Carbon balance was estimated globally for the 230 operation days. Degraded EA of this period was calculated with the area under the curve of elimination capacity data (EC) with the software Origin 6.0. The area obtained (g m^{-3} h^{-1} d) was multiplied by the volume of the biofilter and 24 (factor conversion days to hours), at the end we have the mass of the EA degraded (g). Then the quantity of carbon was calculated with its respective molecular weights (i.e. MW C/MW EA). Data obtained considering that the carbon content in biomass is 50%. CO_2 production was measured to evaluate the degree of EA mineralization. The reaction of complete mineralization of EA is written as follows: $C_4H_8O_2 + 5O_2 \rightarrow 4CO_2 + 4H_2O$, which corresponds to g CO_2 produced per g EA degraded in case of total mineralization.

3. Results and Discussion

3.1 Characterization of the packing material

Biofilter performance is highly dependent on the packing material, its selection is important as it is the place where biomass is adhered. Expanded Perlite is commonly used in biofiltration systems as supporting material. Perlite is a generic name for amorphous volcanic rock composed mainly of alumino-silicates that expands to a factor 4-20 when heated quickly from 760 to 980 °C. Both mineral and expanded perlite are called Perlite. The use of expanded perlite has several attractive physical

properties that can improve on the biofilter such as, low bulk density, low thermal conductivity, high heat resistance, low sound transmission, high surface area and chemical inertness (Maxim et al; 2014). Moreover, the water retention capacity of materials such as Perlite acts as a reservoir of nutrients for the microorganisms, which can been an advantage in biofiltration due to there is no need of continuously feeding a nutritive solution (Prado et al; 2002). The basic characterization of the packing material is presented in ***Table 2***. Results of the basic characterization agree with the previous reported, typical void fraction 40-50% and density of 0.1 g cm^{-3} (Kennes & Veiga, 2002).

Table 2. *Characterization of the packing material. ± Standard deviation.*

Parameter	Units	Results
Bulk density (ρ)	g L^{-1}	109.2±0.07
Water Retention Capacity (WRC)	%	64.17±3.5
Void fraction (ε)	%	51±1.6
Volatile solids (VS)	g VS g^{-1} carrier	0.0143±0.001

Void fraction values at the end of the operation are shown in ***Table 3***. Clearly, at the end of the operation, biofilter without O_3 presented a lower void fraction than the biofilter with O_3 addition. Despite this, void fraction in M2 of the biofilter with O_3 is similar to the one of M2 without O_3. This could indicate that, eventually, the biofilter with O_3 may clog, even if O_3 is added. The low void fraction at M2, in the biofilter with O_3, could be due to the microorganisms present there are producing more biomass, since the byproducts that get in that module are more easily degradable than EA. Values of void fraction without O_3 are similar to the ones reported by Xi (2014) with perlite, where a biofilter without O_3 addition presented 19% and the biofilter with the higher concentration of O_3 (300-320 mg m^{-3}) had 31% on day 66. Initial void fraction was 42%.

Table 3. *Void fraction values operation day 230.*
Module 1 (M1), Module 2 (M2) and Module 3 (M3)

Void fraction (ε)	(%)
With Ozone M1	34
With Ozone M2	13
With Ozone M3	32
Without Ozone M1	4
Without Ozone M2	11
Without Ozone M3	22

3.2 Performance of Ethyl Acetate Biofiltration with and without O_3 addition.

Biofilter performance by stages is presented in ***Figure 2***. Biofilter without O_3 addition decreased its removal efficiency (RE) to ~70% at stage C, which corresponds to the highest inlet load applied (180 g m^{-3} h^{-1}). This inlet load is similar to previous studies reported of EA biofiltration with 195 g m^{-3} h^{-1} where a decrease in RE until 60% was reported (Álvarez Hornos et al; 2007). Biofilter performance of this biofilter was quite constant in the next stages D, E and F; at stage G RE dropped until 40%. Biofilter with O_3 presented higher RE and EC since stage C. The maximum elimination capacity of the system with O_3 was 200 g m^{-3} h^{-1} and without was 120 g m^{-3} h^{-1}. O_3 addition improved the system performance along its operation. Wang et al., (2009) indicated that an O_3 addition below 120 mg m^{-3} could notably enhance the biofilter performance; Xi et al., (2006) specified that concentrations lower than 220 mg m^{-3} did not adversely affect toluene removal. Otherwise, García-Pérez et al., (2013) and Maldonado-Diaz & Arriaga (2014) added O_3 pulses of 90 ppb in a biofilter treating formaldehyde; they found that O_3 addition allowed a stable EC with higher inlet loads. A decrease in the O_3 concentration added could mean a lower cost, but this will depend on the pollutant treated and the system conditions. Additionally it is important to analyze the merely chemical O_3 oxidation, which is discussed later.

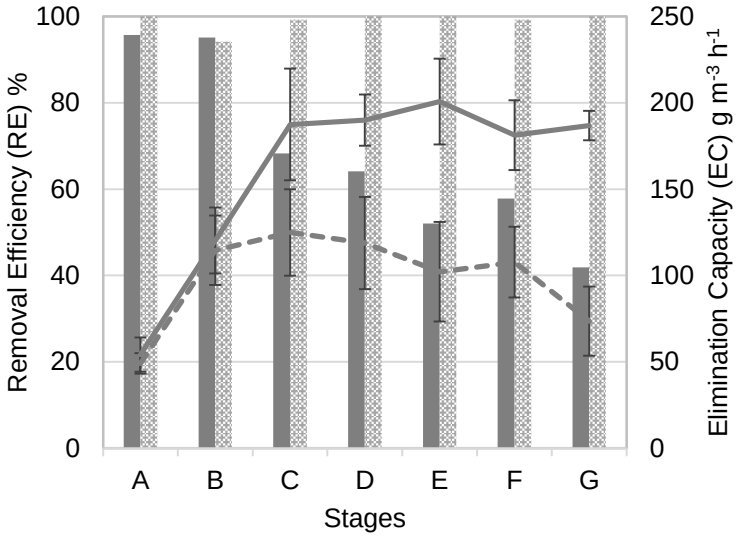

Figure 2. *Biofilter performance by stages with/without ozone addition. (■) RE without O_3; (▩) RE with O_3; (▬) EC with O_3; (▬ ▬) EC without O_3.*

3.3 Pressure drop profile and biomass content

The increase in pressure drop is a result of the biomass development in the biofilters, which can be explained by the decrease in the void fraction space. A biofilter treating EA with intermittent inlet loads of pollutant reported a variation of 20-100 mmH_2O (Álvarez-Hornos et al., 2007) in 9 month operation (274 days). As it can be observed in ***Figure 3***, the pressure drop in the reactor with O_3 was quite stable, with values around 5-20 mm H_2O. For the biofilter without O_3 addition values varied between 12-63 mm H_2O. Comparing both biofilters, a continuous addition of O_3 (90 ppb) helped to control the pressure drop; biofilter with O_3 has a pressure drop 6 times lower than the biofilter without. This agrees with another study of a biofilter treating toluene, where the continuous O_3 injection (180-220 mg m^{-3}) could effectively keep the pressure drop stable with no adverse effect on the toluene removal concentration (Xi et al., 2014). Also in an hybrid system, an UV treatment and a biofilter treating a mixture of toluene and o-xylene, showed that the pressure drop was lower under O_3 addition than the one without O_3 (Moussavi & Mohseni, 2007). Biomass concentration pattern (***Figure 3***), is similar to the pressure drop profile. Difference in biomass contents between biofilters with and without O_3 started to be significant at day 80 with an inlet load of 180 g m^{-3} h^{-1}. In general, as previously mentioned clogging appears when there is an excess of biomass, which can lead to a negative effect on the contaminant removal efficiency (Alonso et al., 1997). However, some studies have showed that conditions most favorable for toluene biodegradation are equally those that contribute strongly to excess of biomass formation and biofilter clogging (Delhoménie et al., 2003). Regardless, the optimal utilization depends on biomass distribution within the filter media and not just on the amount of biomass growing (Morgan-Sagastume, 2001). The void fraction results discussed above at the end of the operation (***Table 3***), suggest that the biofilter without O_3 has a more uneven distribution of biomass between modules compared with the other.

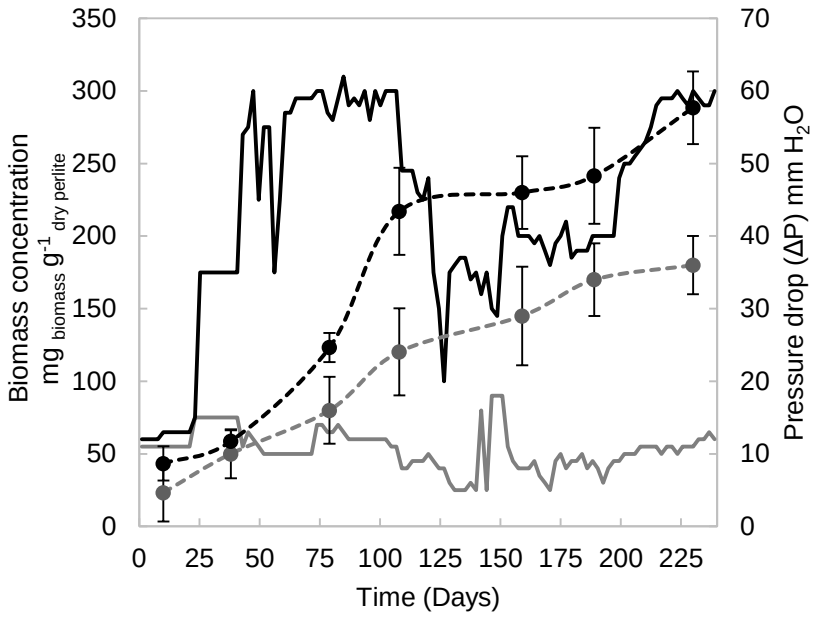

Figure 3. *Progress of biomass clogging. (——) ΔP with O_3; (——) ΔP without O_3; (--●--) biomass with O_3; (--●--) biomass without O_3;*

It is important to mention that the amount of biomass produced may depend on several factors, such as the type of pollutant, inlet load applied, the bactericides aggregated (as O_3), the bioreactor configuration (volume, type, etc.), the operational parameters (pH, mineral medium addition, EBRT, etc.) and the inoculum. Xu et al; (2016) operated 6 biofilters in parallel packed with perlite treating toluene with different O_3 concentrations (40-300 mg m^{-3}) for 97 days and an effective volume of each reactor of 2.6 L, the range of the biomass concentration reported with O_3 was 33-15 g L^{-1}. On the other hand, García-Pérez et al., (2013) measured a biomass concentration in the range of ~7.5-24 mg biomass $g^{-1}_{perlite}$ in a biofilter treating formaldehyde with O_3 (90 ppb) pulses for 220 days in a volume of 1.1 L. Maldonado-Diaz & Arriaga (2014) also studied the same pollutant with O_3 additions of O_3 (90 ppb) in a biofilter of 3.3 L for 310 days, reporting a biomass concentration between 24-60 mg biomass g $_{perlite}$. In the biofiltration systems of this paper, the ranges obtained were 23.33-180.1 mg biomass g $_{perlite}$ with O_3 addition and 43.31-288.46 mg biomass g $_{perlite}$ without O_3 addition. According to this, the biomass amounts were quite higher that the reported with formaldehyde and, if the biomass concentration of this study is multiplied by the bulk density of the perlite, the biomass

range would be 2.55-19.67 g biomass L^{-1} with O_3 addition and 4.73-31.50 g biomass L^{-1} without O_3 addition, higher than the concentrations reported by Xu. Thus, in the present study with EA as VOC, more biomass has been produced than in the other studies (allowing to effectively analyze the clogging prevention). At the end of operation the biomass amount with O_3 addition was 180.1 mg biomass g $_{perlite}$, 1.6 times lower than the biomass of the biofilter without O_3 (288.46 mg biomass g $_{perlite}$).

3.4 pH profile and TOC content

The pH in leachates in the biofilter with O_3 varied in a rage of 7-8 during 120 days, then started to drop until a pH of 2 in the last 110 days. Despite acidification removal efficiency of 100% did not decrease. It is well reported that acidification in biofiltration systems may lead to a decrease in the removal efficiency and in order to control this issue, pH is controlled, which represents a cost in the reagents consumption. The relation between pH and TOC was, the lower the pH the higher TOC content. Wang et al., (2009) suggested that O_3 could destroy part of the biofilm, and then this could be washed out with the nutrient solution, thus, increasing the TOC content in the leachates. García-Pérez et al; (2013) and Maldonado-Díaz & Arriaga (2014) found that O_3 addition is working as a pH regulator, this may be happening in the first 120 operation days. According to literature a proposed pathway could be: ethyl acetate →ethanol + acetate (Fornet & Markovetz, 1971; Eubanks et al; 1974). Acetate/acetic acid concentrations are changing the buffer capacity in these systems. Koutinas et al; (2005) concluded that ethanol concentrations at 2 g L^{-1} may be inhibitory and speculated that ethanol biodegradation is the slowest ad rate-limiting step in a bioscrubber treating EA and a sharp decrease in EA removal efficiency (below 80%). In this study no concentration of ethanol was measured, but it may be possible that ethanol concentration was high enough to reduce the RE from 100% to 70% in the biofilter without O_3 addition. At the same time biofilter with O_3 addition is helping to degrade ethanol, as acetate/acetic acid is resulting from ethanol oxidation and acetic acid is more readily biodegradable, leading to keep a 100% RE within this biofilter even if the pH is low. The presence of ethanol and acetic acid were corroborated by GC-MS (discussed below).

3.5 Carbon recovery

Table 4. *Estimation of carbon recovery.*

With Ozone	Eliminated EA	CO2	Biomass	TOC leached	Adsorbed EA	Biomass leached	Other
g C	1719.9	1404.2	138.5	18.3	1.3	3.3	
%	100.0	81.6	8.1	1.1	0.1	0.2	9.0
Without Ozone	Eliminated EA	CO2	Biomass	TOC leached	Adsorbed EA	Biomass leached	Other
g C	1004.8	700.4	216.7	11.4	1.3	12.2	
%	100.0	69.7	21.6	1.1	0.1	1.2	6.3

Mineralization obtained in the biofilter with O_3 is ~10% higher than in the biofilter without O_3. There is little information about EA mineralization in biofiltration systems. The biomass contribution in the biofilter without O_3 is 1.56 times the biomass of the biofilter with O_3. Soluble carbon, represented by TOC leached did not presented a difference but, biomass leached was significantly higher in the biofilter without O_3. Sorption of EA is described below. This results tally with a carbon balance reported by Xi et al; (2014), where it was shown that mineralization ratios were remarkably larger with O_3 injection than without. The study also indicated that mineralization ratios increased with higher O_3 concentrations and the ratios of carbon in leachate and biomass were lower with and when O_3 concentrations were increased. On the other hand, García-Pérez et al; (2013) did not find significant recovery of CO_2 and noted a slight tendency of biomass content to decrease when O_3 pulses were added, but biomass effect was no clear due to the low amount of biomass present, in regard to the biomass leached they found that more biomass was detached in the presence of O_3. Another study, when first no O_3 was added, and then continuous O_3 was added, reported that mineralization was reduced within O_3 addition, also the percentage of soluble carbon in leachates, biomass in leachates and in the carrier was greater during the period without O_3 (Maldonado-Diaz & Arriaga, 2014). Overall, results in the carbon balance of EA biofiltration in this study agree with the fact that O_3 addition is controlling the biomass production. The higher CO_2 production with O_3 could be due to the fact that O_3 may react with some organic matter, including extracellular polymeric substances (EPS), dead cells and cell debris and convert them to more readily biodegrable matter and finally CO_2 (Xi et al., 2006).

3.5 Abiotic control

Results of the abiotic control showed that the maximum removal efficiency was 23.09 ± 4.15% with an inlet load of 60 g m^{-3} h^{-1}, this higher value compared with 6.33 ± 1.01% (120 g m^{-3} h^{-1}) and 6.53 ± 2.31 (180 g m^{-3} h^{-1}) could be because the system reached the saturation point of the startup of the reactor until the equilibrium is reached. With these results it can be ensured that the main removal of the pollutant in the biofilters is due to the activity of microorganisms. S*upporting information Table S2 and Figure S1.*

3.6 GC-MS analysis

GC-MS analysis was performed in different samples: gas samples of the abiotic filter, liquid samples of some operation days of biofilter leachates working with and without O_3, and samples of perlite (carrier with biomass) of the last operation day of the biofilters with and without O_3. In leachates of both biofilters were not possible to identify the sub products, only water and EA were identified, this could be attributed to the short half-life of ethanol (6.5 -24 h) and acetic acid (24 min) on the water surface (Howard, 1991; VanDerMaas, 1972). On the other hand, sub products in the abiotic reactor and perlite samples of both biofilter were identified; acetic acid, ethanol and CO_2. The subprodutcs identified in the perlite samples with biomass samples corroborated the metabolic pathway of EA degradation of the biofilters: ethyl acetate → ethanol + acetate (Fornet & Markovetz, 1971; Eubanks et al; 1974;). Koutinas et al., 2005 compared the performance of bioscrubbers and biotricking filters for the degradation of EA; they also found that ethanol was an intermediate in the EA degradation. Supporting information Figures S2.

3.7 Sorption isotherms

Literature reports indicated that perlite has a low adsorption capacity of VOCs in gas phase (Kennes et al;1996; Prado et al., 2002). Notwithstanding, sorption isotherms in this study were accomplished. The correlation coefficients between Langmuir and Freundlich models were similar, except for water saturated perlite, which was lower

in Langmuir model. According to these results, the most likely model was Langmuir. Overall, sorption contribution was not significant. Supporting information Table S3.

4. Conclusion

The continuous addition of O_3 had a clear effect over the biomass concentration, as well as in the pressure drop. At the same time O_3 addition helped to keep stable the 100% removal efficiency of the system. Although it is needed to treat higher inlet loads of EA in order to find the maximum capacity of the O_3 biofiltration system. Still, the continuous addition of O_3 could be an attractive solution to improve biofilter performance and extend the half time life of the filter bed, but the results of void fraction indicate that eventually, depending of the system, the biofilter will clog. Acetate, a conjugate base of acetic acid could be responsible of the buffer capacity in the first 120 days of the operation in the biofilter with O_3. Although the system reached a low pH (2), the RE remain 100%; acetic acid concentration could be responsible for the low pH and as being a more readily degradable molecule than ethanol, the rate-limiting step was overcome. On the other hand, the drop in the RE in the biofilter without O_3 addition could not be due to the ethanol was as high to limit the degradation, but that the microorganisms present achieved the maximum amount of substrate that were able to metabolize.

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6. Supporting information

Table S1. *Equations used for characterizing the packing material.*

Parameter and Equation	Specifications
Bulk density (ρ) (g/mL) $\rho = \frac{W}{V}$	**W**= weight of perlite (g) **V**= volume of the test tube (mL)
Water Retention Capacity (WRC) (%) $WRC = \frac{W_{wp} - W_{dp}}{W_t} x\ 100$	**Wwp**= wet weight perlite (g) **Wdp**= dry weight perlite (g) **Wdp - Wwp**= weight retained water (g) **Wt**=**Wwp**= total weight (g)
Void Fraction (ε) (%) $\varepsilon = \frac{V_{H2O}}{V} x\ 100$	V_{H2O}= water volume added (mL) **V**= volume of the test tube (mL)
Volatile suspended solids (SSV) ($g_{ssv}\ g_{Perlite}^{-1}$) $SSV = \frac{W_{s105°C} - W_{s550°C}}{W_s}$	$W_{s105°C}$= Sample weight after exposure to 105 °C $W_{s550°C}$= Sample weight after exposure to 550 °C **Ws**= Initial sample weight

Table S2. *Abiotic control; (IL) Inlet Load applied; (RE) Removal efficiency; (std) standard deviation.*

IL ($g\ m^{-3}\ h^{-1}$)	Average RE (%)	std RE	Average IL ($g\ m^{-3}\ h^{-1}$)	std IL
60	23.09	±4.15	62.31	±3.55
120	6.33	±1.01	127.78	±5.62
180	5.53	±2.31	176.98	±4.32

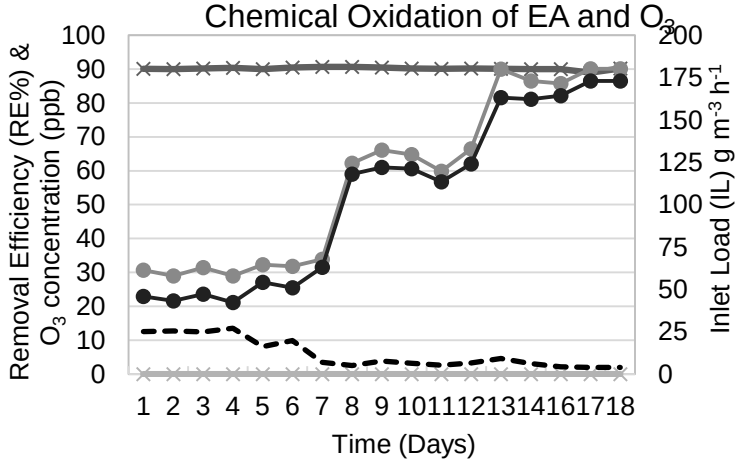
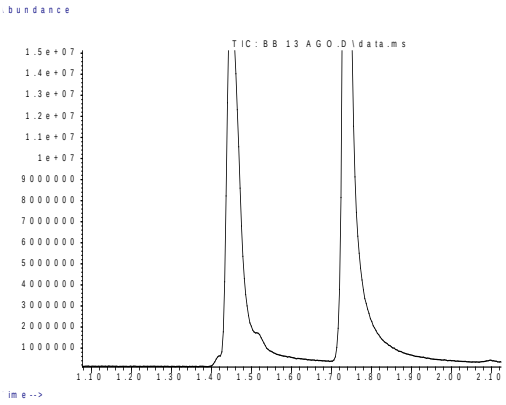


Figure S1. *Figure Abiotic control; (....) Removal efficiency; (—●—) Inlet load EA; (—●—) Outlet load EA; (—×—) O_3 inlet concentration; (—×—) O_3 outlet concentration.*

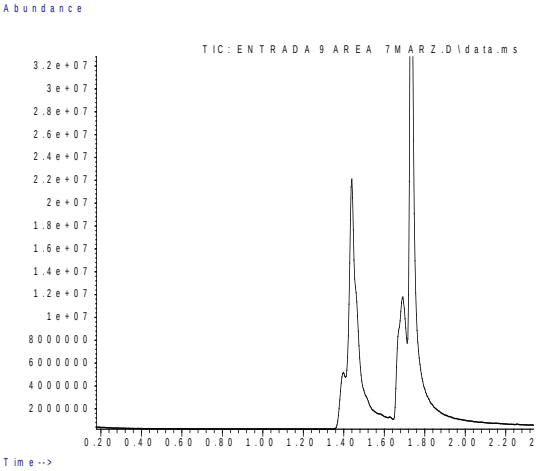
Figures S2. Chromatographs of G-C mass; A) Leachates; B) Abiotic control; C) Carrier with biomass from both biofilters at the end of the operation.

A) *Leachates*

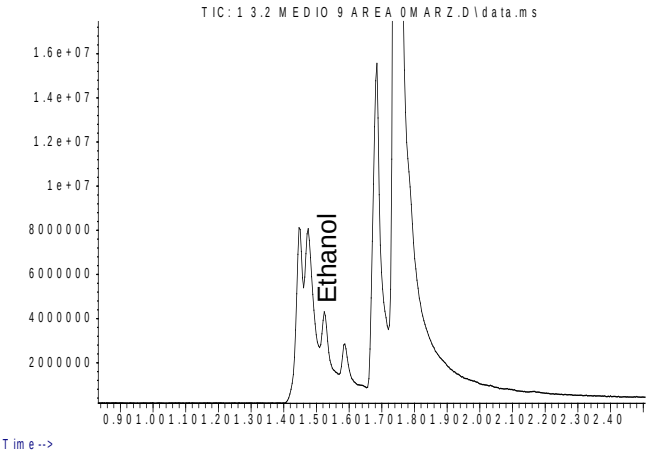


B) *Abiotic control*

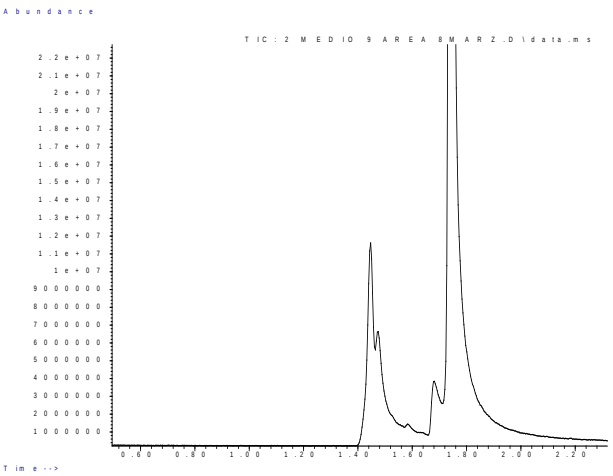
Abiotic Control. IL= 60 g m^{-3} h^{-1}. Inlet.



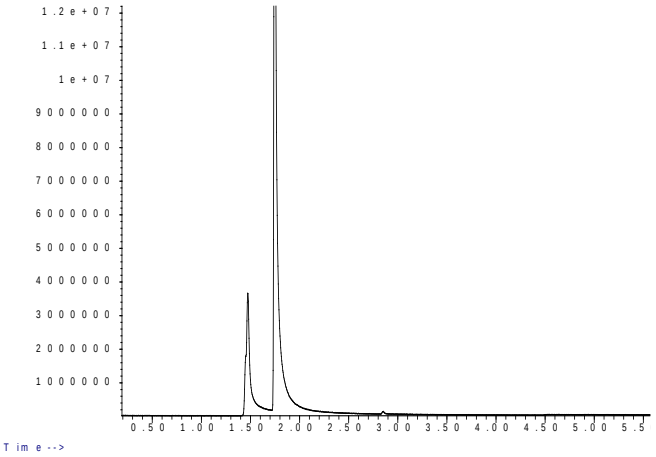
Abiotic Control. IL= 60 g m^{-3} h^{-1}. M1



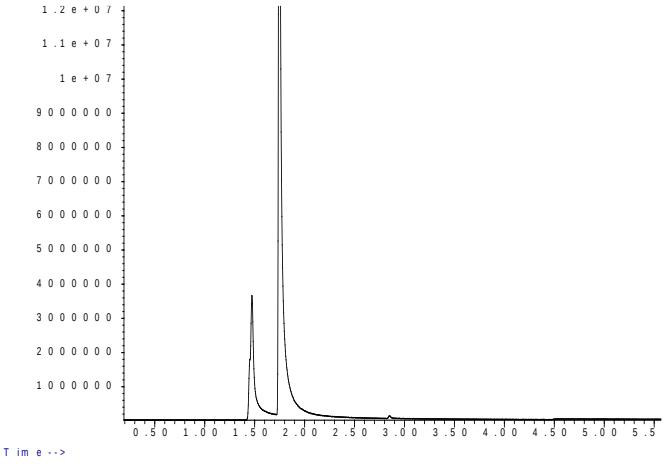
Abiotic Control. IL= 60 g m^{-3} h^{-1}. M2



Abiotic Control. IL= 120 g m^{-3} h^{-1}. Inlet



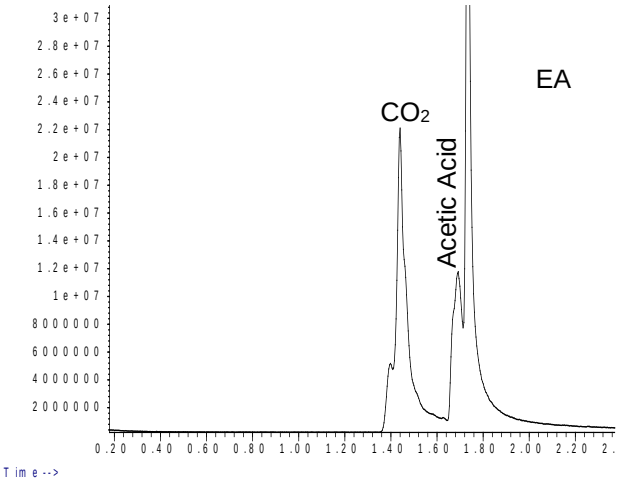
Abiotic Control. IL= 180 g m^{-3} h^{-1}. Inlet



C) *Carrier with biomass at the end of the operation*

EA

With O_3; M3



Without O_3; M3

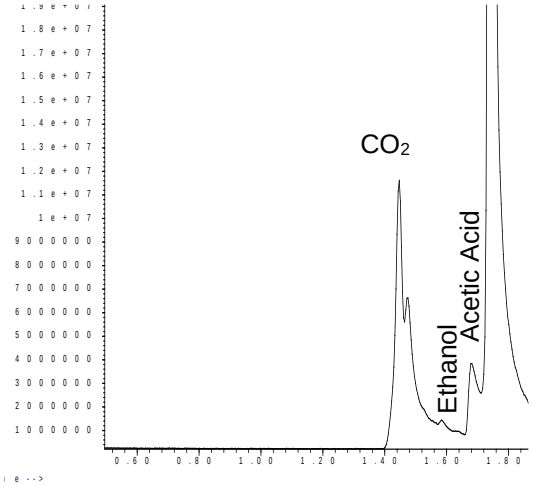


Table S3. Sorption Analyzes.

Equations of the models of sorption isotherms.

Carrier	Model	
	Langmuir	Freundlich
Perlite ambient moisture content	y = 116.18x - 0.9813 **R^2 = 0.9357**	y = 1.0463x - 2.0161 **R^2 = 0.9381**
Water saturated Perlite	y = 8.789x - 6.0016 **R^2 = 0.8306**	y = 6.8139x - 0.5721 **R^2 = 0.9627**
Perlite with biomass	y = 9.0137x + 0.154 **R^2 = 0.9811**	y = 0.9727x - 0.9941 **R^2 = 0.9706**

Langmuir model is written as follows:

$$\frac{1}{q} = \frac{1}{bq_m ec} + \frac{1}{q_m}$$

q_m=maximum adsorption capacity, q=amount of solute adsorbed, b=Langmuir constant, ec=equilibrium concentration.

With the intention to find the adsorption contribution, was calculated all *qm* of all carriers with this model. Perlite with biomass sample had the highest *qm*, *qm*=6.49 mg EAg^{-1} perlite. This, as the higher adsorption capacity was used to calculate the adoption contribution of EA in the reactors; the value was multiplied by reactor volume (3.3 L) and bulk density, then transformed to g of C.

Chapter III. BIOFILTER LONGITUDINAL BEHAIVIOR PERFORMANCE AND BIOMASS COMPOSITION IN SYSTEMS WORKING UNDER OZONE ADDITION.

ABSTRACT

The present paper is focused 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 worked under continuous addition of O_3 (90 ppb) and the other without. Throughout the operation time, the exopolysaccharides (EPS) from the biofilm were extracted for their characterization, qualitatively using Fourier transform infrared spectroscopy with attenuated total reflectance (FTIR-ATR), and quantitatively by its main constituents: carbohydrates, proteins and glucuronic acid. EPS characterization has been attempted mainly with biofilm aggregates related to water treatment, not 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 is added. This study revealed that O_3 addition is affecting only the amount of EPS not its composition and that the greater effect is on carbohydrate, since they are the main components in EPS. Qualitative analisis of EPS by FTIR-ATR showed the slightly movement of the wavenumber identified in the spectra and only shows a difference in abundance intensity. All about suggest that the EPS are only being reduced by O_3 addition and that such a low concentration of O_3 is not affecting the composition of the EPS structure.

Keywords: exopolysaccharides; ozone; biomass composition; ethyl acetate, biofiltration

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) microorganisms (Wingender et al;1999). Furthermore biofilms can exist in technical systems such as heat exchangers, plumbing systems and filters. Particularly, biofilms in a biofilter 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 and 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. Recently, O_3 has been added to biofilters at low concentrations in order to study its effects on biomass clogging and improving the system efficiency. 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) indicated that low concentrations of O_3 (90ppb) only affect 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 in a biofilter treating formaldehyde. At the same time, the component characterization of the EPS was tried by proteins, carbohydrates and glucuronic acids in the same study. They found a major effect for the proteins. Wang et al; (2009) reported that O_3 (40-120 mg m^{-3}) could lower the EPS content in a biofilter treating gaseous chlorobenzene. Several studies have focused on the characteristics of EPS and the influence of thermochemical and oxidation mechanisms on degradation and flocculation of EPS but not in biofiltration systems with O_3 addition. Therefore, as few studies have been carried out to provide

information to better understand the effects of O_3 addition on EPS, its production and composition. It is necessary to disclose more tangible evidence of the effects that O_3 has on the EPS matrix, since the biomass production is a limitation in the previous biofiltration studies.

2. Experimental section

2.1 Biofilter System

Ethyl acetate (EA) biodegradation was carried out in two identical laboratory biofilter of 3.3 L divided into three identical modules (1.1 L). Between each module was 2 sampling points, one for biomass in the middle of the module filter bed and another after the end of the module filter bed for gas sampling. Each biofilter was made of glass, with a total length of 0.45 m and an internal diameter of 0.097 m. The packing material used was perlite with an average diameter of 3.3 mm inoculated with activated sludge obtained from a wastewater treatment plant. For the biofilter with O_3 addition, a controlled airstream with a mass flow controller (GFC17; Aalborg, Orangeburg NY) passed through an EA solution (99%) contained in a stripping reactor and was mixed with an airstream from humidifier and with O_3 airstream. The same with the biofilter without O_3 addition but, no O_3 was added. Generation of O_3 was produced with a technology called “Corona Discharge Technology”, which consists in the use of a high frequency that causes the breaking of oxygen molecule due to the electrical field with a A2ZS-3GLAB OZONE GENERATOR system. O_3 concentration was estimated with the yodimetric method of Rakness et al. 1996.The operational stages were divided as follows: ***Table 1***.

Table 1. *Operational Stages.*

Letter	A	B	C	D	E	F	G
Inlet Load IL (g m^{-3} h^{-1})	60	120	180	180	180	180	180

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. The operation

temperatures were 230, 100, 230 °C for the injector, column and detector, respectively. CO_2 concentration was measured with a gas chromatograph equipped with a thermal conductivity detector Agilent Technologies 6850, the temperatures of the injector, column, and detector were 200, 50 and 250 °C, respectively. Calibration curve of each gas was prepared with different concentrations. Inlet, outlet and middle concentrations of EA and CO_2 were measured, after sampling each module sampling port.

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 with the next equation (mg biomass per g of perlite):

$$B = \frac{W_{105°C} - W_{550°C}}{W_{105°C}} * 1000$$

2.4 EPS extraction and its characterization

EPS content was extracted from 1 g of sample consisting in perlite and biomass (H. Liu & Fang, 2002). EPS were characterized in terms of proteins (Lowry et al. 1951), carbohydrates (DuBois et al. 1956) and glucuronic acid (Blumenkrantz & 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 were made powder 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 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 is shown in ***Figure 1*** a) without O_3 and b) with O_3.

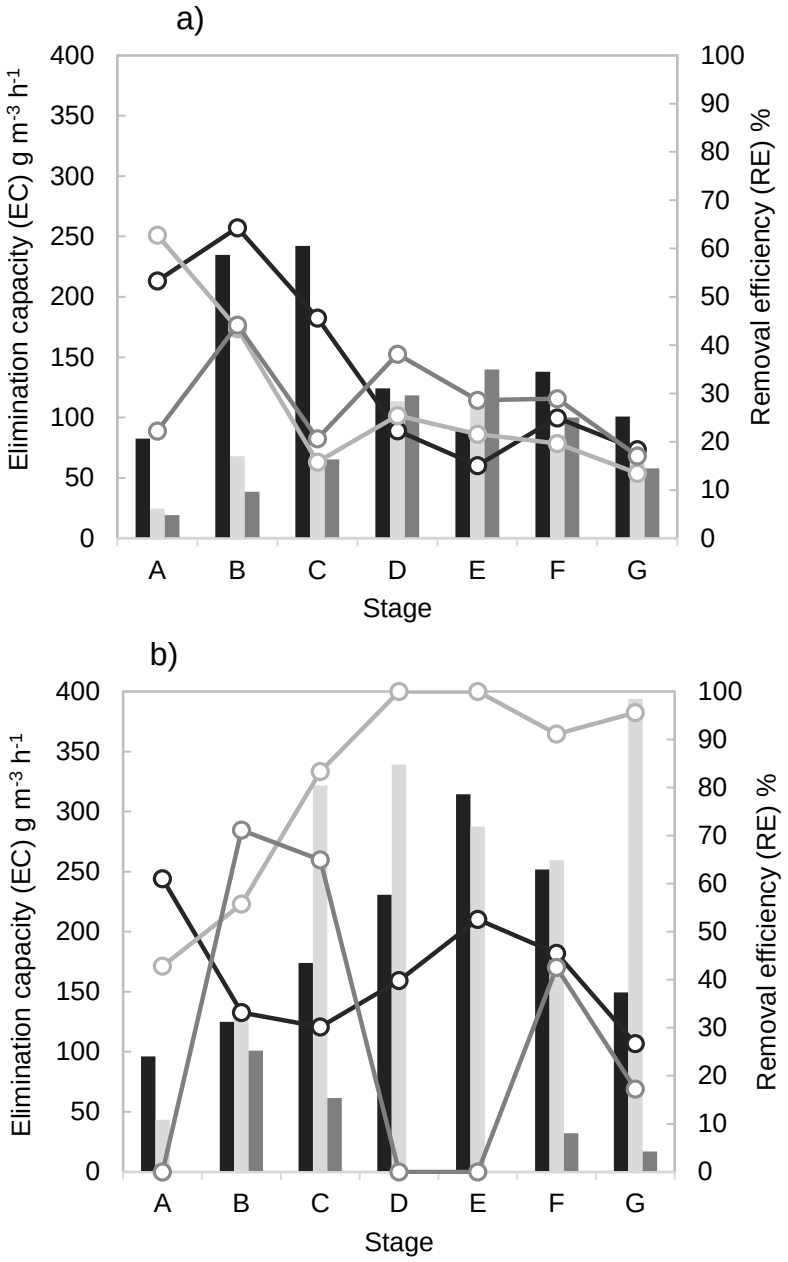


Figure 1. ***a)*** *without O_3;* ***b)*** *with O_3; Elimination capacity (EC) and Removal efficiency (RE); EC M1; EC M2; EC M3; RE M1; RE M2; RE M3. Days; A=10, B=38, C=78, D=108, E=159, F=189, and G=230.*

M2 of the biofilter with O_3 addition presented the higher RE and EC along the operation time, whereas removal was more even in all modules in the biofilter without O_3 addition. M3 in the biofilter with O_3 addition was useless, since what enters in M2 the pollutant is removed almost 100% from stage D. At the final stages F and G this biofilter present a small contribution in the RE and EC. O_3 addition allows a better RE and it can treat EA over a stretch of shorter filter, which could represent an advantage over the biofilter without. Only Maldonado-Diaz & Arriaga (2014) and Xi et al., (2014) have studied biofiltration systems with modules and O_3 additions, three and two, respectively, however no information of EC or RE by module have been reported. Overall, biofilter performance with O_3 addition achieved higher EC and RE by modules comparing by modules with the biofilter without, except for M3 in the stages D and E of the biofilter with O_3.

3.2 CO_2 production profile

The plots of CO_2 production throughout the modules are showed in the ***Figure 2***.

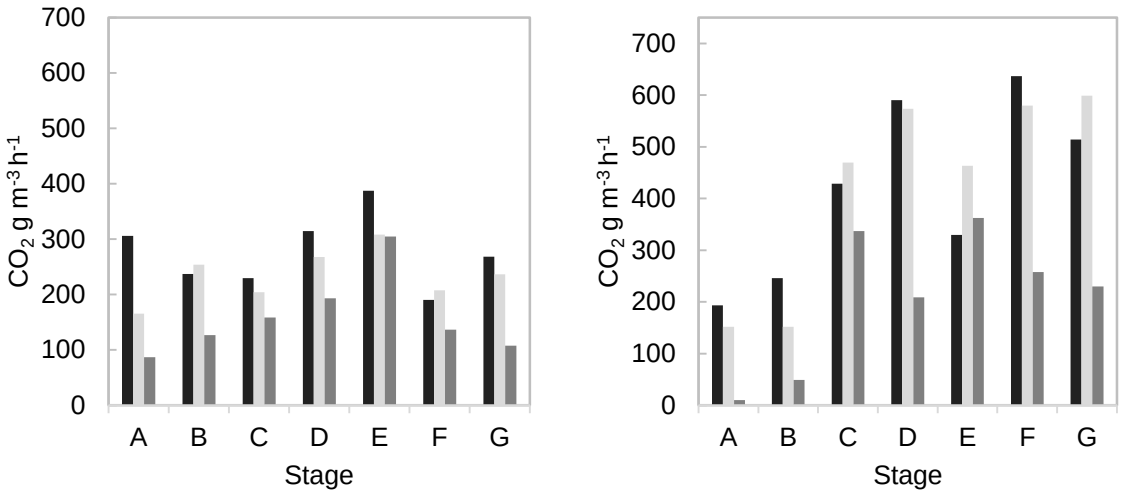


Figure 2. **a)** Without O_3; **b)** with O_3; *CO2 production g m^3 h^{-1};* *CO_2* M1; CO_2 M2; CO_2 M3. *Days; A=10, B=38, C=78, D=108, E=159, F=189, and G=230.*

Álvarez-Hornos et al., (2007) reported values of CO_2 production around 550 g m^3 h^{-1} in a biofilter with EA. Also in another study, Álvarez Hornos, (2007b) reported values as high as 150 g m^3 h^{-1} from a mixture of EA and toluene in the first quarter, half and three quarter of the biofilter. The highest CO_2 production in the biofilter

without O_3 addition in this study 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 and B, then the CO_2 increased as high as 650 g m^3 h^{-1}in the next stages. CO_2 in M1 and M2 were similar and presented the highest values at stages C and forward. The higher production of CO_2 in the biofilter with O_3 could be due to O_3 may be reacting with some organic matter, including extracellular polymeric substances (EPS), dead cells and cell debris and convert them to more readily biodegrable 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 values calculated were: without O_3, M1= 0.51, M2= 0.35 and M3= 0.21 (g CO_2/ g EA fed); with O_3, M1= 0.84, M2= 1.0 and M3= 0.35 (g CO_2/ g 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 as an indicator of the intensity of the microbial activity in the biofilters can be concluded that the biofilter with O3 presented a better removal efficiency, since more EA was mineralized.

3.3 Biomass content

Table 2 summarizes the biomass content by modules (M1, M2 and M3) along the time. M1 presented the lower biomass amount compared with the other modules in the biofilter with O_3, this could be due to the fact that it was the module more directly exposed to O_3, even though O_3 concentration was only measured in the entrance and the outlet of the biofilter (not between the modules), the most probable is that O_3 concentration was decreasing along the biofilter, and as O_3 is a strong oxidant and disinfectant the higher concentration in the first module was the responsible of inactivating some of microorganisms present, so there was less amount of biomass. Some authors 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 (Chang, 1971; Khadre et al; 2001). M2 in the same

biofilter (with O_3) presented the higher values of amount of biomass. Increasing with the time and then dropped in stage F and G. This could be due to the fact that this module receives more degradable substrate than EA, which allows the microbial growth. This happened with M1 in the biofilter without O_3. On the other hand, biomass content in M3 increased with the time then dropped at stage D. In stages E, F and G continue increasing in the biofilter with O_3. In general, the biofilter without O_3 addition clearly increased its biomass amount 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 & 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 range of biomass concentration they presented was ~7.5-24 mg biomass $g^{-1}_{perlite}$ from one biofilter with O_3 pulses, the range in this study of both biofilters is 19.9-302.3 mg biomass $g^{-1}_{perlite}$, which are far higher. The results by module in this study allowed to analyze better the effects of O_3 over the biomass production. Comparing both biofilters, the grater difference is between modules 1. Also, as can be seen, with the time the biomass amount is increasing at some modules, which could still lead to a clogging problem.

Table 2. *Biomass content by module/stage (mg biomass / g dry perlite) ± Standard deviation. Days; A=10, B=38, C=78, D=108, E=159, F=189, and G=230.*

Module **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
Module **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

3.4 EPS content and its characterization

Figure 3 shows the EPS content and its characterization by module. According to the results, EPS production (gray area) decreased in M1 since stage E (day159), the same happened in M2 but in M3 EPS decreased until stage D (day 79) in the biofilter with O_3. 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 & Arriaga (2014) 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).

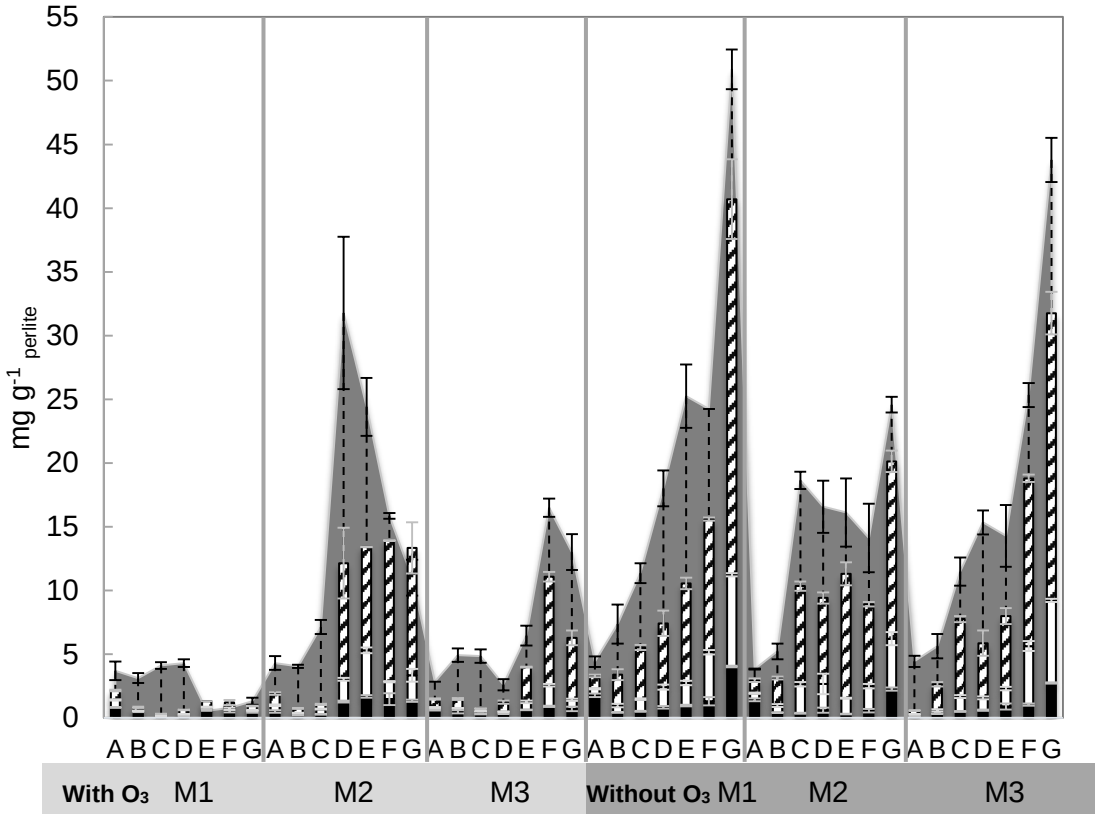


Figure 3. *EPS content and its characterization, by module/stage. (■) EPS content; (■) Proteins; (□) Glucuronic acid; (▨) Carbohydrates; Days; A=10, B=38, C=78, D=108, E=159, F=189, and G=230.*

With respect to the characterization of EPS, it can be seen in the stacked column there was a greater effect on carbohydrate, which is the main component of EPS matrix (Wingender et al; 2001). 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 due to as 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 is fed, which allows more microbial growth and thus more biomass and EPS content.

To our knowledge, no information has been reported for relations between EPS amount and biomass production in biofilters with O_3 addition. Analyzing this relation in this study (***Table 3****), it* can be seen that the biofilter without O_3 addition presented a 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 that more EPS are being secreted by the microorganisms depending in the operational conditions to protect themselves.

Table 3. *Ratios by module/stage of EPS and biomass amount produced (mg EPS / mg biomass). Days; A=10, B=38, C=78, D=108, E=159, F=189, and G=230.*

Module **with O_3**	A	B	C	D	E	F	G
M1	0.18	0.09	0.10	0.08	0.005	0.006	0.01
M2	0.17	0.06	0.05	0.14	0.08	0.05	0.05
M3	0.11	0.10	0.05	0.03	0.05	0.10	0.06
Module **without O_3**	A	B	C	D	E	F	G
M1	0.08	0.11	0.09	0.07	0.10	0.07	0.15
M2	0.10	0.10	0.15	0.07	0.08	0.09	0.11
M3	0.13	0.10	0.10	0.11	0.07	0.12	0.14

3.5 EPS analysis by FTIR

FTIR spectra of EPS extracted are showed in ***Figure 4 a) b)***. In this study the whole spectra is presented. The peaks in the range of 3800-3100 cm^{-1} correspond to H-O stretching (Alvarez & 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 there was found only a peak at 1030 cm^{-1} on day 10 and 1010 cm^{-1} on day 108, 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. In 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. 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 signals were found a signal at 1400 cm^{-1}, which did not changed 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 changed, which correspond to Amides II and 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. The bands assignments reported were 1645 cm^{-1} (amine I), 1450 cm^{-1} (CH_3), 1400 (C-O), 1260 and 1080 cm-1 (P=O) (Eboigbodin & 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 (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 mentioned, 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 is unchanged.

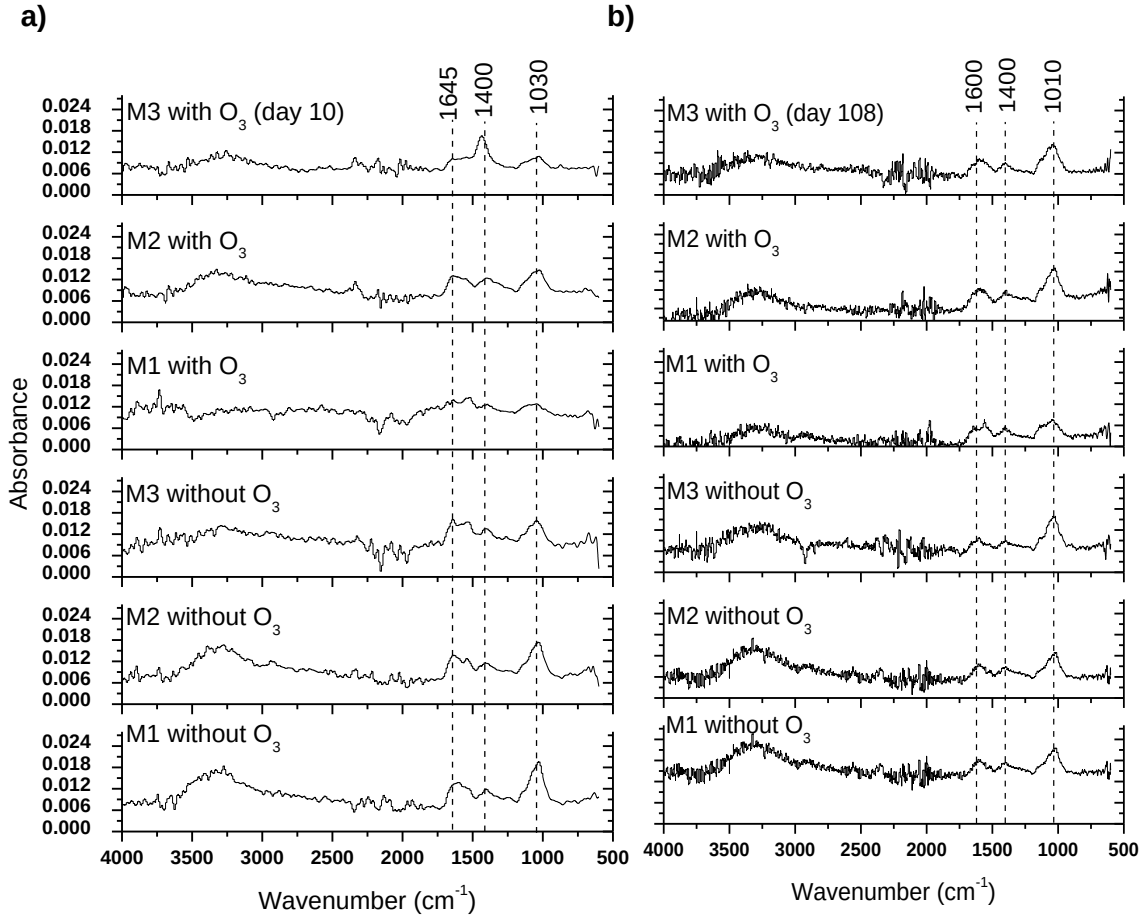


Figure 4. - a) *EPS spectra of operation day 10, inlet load 60 g/m^{3}h*. **b)** *EPS spectra of operation day 108, inlet load 180 g/m^{3}h.*

4. Conclusion

The present paper provides further information into EPS identification when O_3 is added in a biofilter. The results of FTIR analysis suggested that O_3 addition on EPS did not present a significantly difference in the functional groups identified. The quantification of each component of EPS analyzed indicated that O_3 addition makes greater effect on carbohydrate amount. In conclusion, with this study, O_3 is affecting only the amount of EPS, and not its composition. Regarding the biofilters performances, the biofilter with O_3 in this study presented a more stable tendency, global and by module but it would be interesting to find the limiting inlet load that the system with O_3 could withstand without affecting the system stability. Overall, biofilter with O_3 addition presented higher EC by modules and globally.

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Chapter IV. EFFECTS OF OZONE ON THE MICROBIAL COMMUNITY COMPOSITION IN BIOFILTRATION SYSTEMS TREATING ETHYL ACETATE VAPOURS.

ABSTRACT

Several investigations have inferred that ozone (O_3) addition could be an effective strategy to eliminate clogging in biofilters and thus improve air biofiltration. However, to our knowledge few studies have focused on the analysis of the microbial composition in biofilters working under O_3 addition. The importance of obtaining a highly improved and more complete overview of the effects caused by continuous addition of O_3 in the biofilters will allow a much better understanding of the factors influencing the removal efficiencies of certain pollutants from waste gas and further enhance their performance. Two biofilters treating Ethyl Acetate (EA) were operated for 230 days, one biofilter worked under a low continuous O_3 addition [90 ppb] and a control without. In general, the biofilter with O_3 addition achieved higher removal efficiencies (100%) than the biofilter without O_3 addition (~70%), for the three inlet loads applied (IL= 60, 120 and 180 g m^3 h^{-1}). The pH and TOC (total organic carbon) in the leachates were measured throughout the biofilter operation. After 120 days of operation the pH in the biofilter with O_3 dropped until 2 and in the control until 4. The microbial community composition of the biofilters was investigated by amplicon sequencing of the 16S rRNA gene throughout the 230 days. OTUs richness, algorithm NMDS and heat maps of fungi and bacteria were analysed. Results indicated similar behaviour in the microbial community between the two biofilters. Main genus of bacteria identified were *Beijerinckia, Gluconacetobacter* and *Acidocella*. Amplicon sequencing of the fungal ITC 1 region revealed the presence of *Rhinocladiella similis*, *Trichosporon veenhuissi*, *Exophilia oligosperma* and others. Thus, the study shows the diversity and development of bacterial and fungal communities and how they are affected by continuous addition of O_3.

Keywords: microbial community; biofilter; ozone treatment; ethyl acetate.

1. Introduction

O_3 is a powerful antimicrobial agent that has been suitable for application in food in the gaseous and aqueous states, and processing environment (Khadre et al; 2001). Quite recently O_3 treatment has been applied in biofiltration systems of volatile organic compounds (VOCs) as a mean to reduce biomass overgrowth and thus improve the performance of the system. Recalcitrant compounds and high inlet loads of VOCs were treated in biofilters working under O_3 addition, such as, formaldehyde, chlorobenzene and toluene (García-Pérez et al. 2013; Maldonado-Diaz & Arriaga, 2014; Wang et al. 2009; Xi et al. 2014). These investigations have inferred that O_3 addition could be an effective strategy to improve air biofiltration. However so far, these studies have not assessed the effects of O_3 on the microbial community composition. Some Gram-positive bacteria with thick cell wall became the dominant species in a biofilter treating chlorobenzene at different O_3 concentrations (40, 80 and 120 g m^{-3}), as it was found that the exposure of O_3 can affect the microbial community composition, resulting in higher metabolic activities (Wang et al. 2009). On the other hand, the viable cell concentration was estimated in a biofilter comprised of three modules treating formaldehyde with pulses of O_3 (90ppb) (Maldonado-Diaz & Arriaga, 2014), the cell viability showed that the module subjected to the highest concentration of the pollutant and O_3 presented the lowest cell viability compared with the other two modules. Nevertheless, no clear relationship was found between the O_3 addition and inlet load with the cell viability. Saigam et al; (2016) found that O_3 addition significantly enhance the ratio of viable cells and allowed the dominance of Gram-positive bacteria in a biofilter treating toluene. A biofilter can be considered as an ecosystem with a complex microbial community structure. Typical numbers of cultivable bacteria in biofilter samples can range from 10^8 up to 10^{10} per gram (Álvarez-Hornos et al., 2007; Maldonado-Diaz & Arriaga, 2014; Medina et al., 1995), which is similar to microbial densities obtained for a natural ecosystem such as soil (Robert and Chenu, 1992). The diversity and relative abundance of microbial species present in a biofilter is influenced by the inoculum source, the composition of the waste gas, environmental conditions, oxygen, pollutant concentration and the overall operating strategies employed.

Several biofiltration research works have focused on bacteria and mixed cultures, in biofilters treating organic pollutants, heterotrophic eubacteria, actinomycetes, fungi, yeast, algae and protozoa have all been detected (Singh & Ward, 2005). Although a wide range of microbes are present in biofilters, some of them may be inactive or do not grow on the pollutant present in the waste gas (Christian Kennes & Veiga, 2002). Study of the microbial community composition has been accomplished with different techniques, such as, analysis of biomarkers, fluorescence in situ hybridization and cloning and sequencing, to name a few. Two biofilters exposed to ethyl ketone highlighted the differences in bacterial community structure as a function of the spatial location, molecular techniques used revealed that microbial community structures differed significantly according to the operational strategy mode along the biofilters (Li & Moe, 2004). Other studies have analyzed the vertical pattern of the microbial community. Friedrich et al; (2003) did not found a vertical gradient of the microbial community in a full-scale industrial biofilter used for waste gas abatement in an animal-rendering plant, but they found that the lower 50 cm of the biofilter presented the most active part in the degradation. Otherwise, Krammar et al; (2005) did find a vertical community composition gradient, in terms of density and diversity in two biofilters treating complex mixtures of VOCs. Also Cabrol et al; (2012) studied the stratification of the microbial structures along the filter bed and statistically correlated it to the longitudinal distribution of environmental conditions, such as concentration and elimination capacity.

Molecular methods have been employed to investigate the microbial community of biofilms obtained from different bioreactors and 16S ribosomal RNA genes (rRNA genes) amplicon analysis has become the preferred approach for the cultivation-independent investigation of microbial diversity (Friedrich et al., 2003; Gabor et all; 2003). The current use of culture-independent studies will enable a much better determination of the composition of the microbial communities and provide a better understanding of the complex interactions between microbial community structure and biodegradation functions.

In general, all microbial community investigations in biofilters so far have shown the necessity and importance to study the microbial ecology of biofilters treating waste

gas in order to understand, improve and monitor this biological process. This may open new possibilities to control and create new biofilters with more favorable treatment performance. The aim of this report is to study the effect caused by continuous addition of O_3 on the microbial community composition in order to better understand the effectiveness of O_3 addition and identify the main resemblances of two biofilters, one working under the O_3 addition and the other without. The microbial community composition of the biofilters was investigated by amplicon sequencing of the 16 rRNA gene throughout the operational performance.

2. Materials and Methods

2.1 *Biofilter setup*

The gas biofiltration experiment was performed in two identical biofilters, comprised of three modules, each module with a working effective volume of 1.1 L. The modules were packed with Perlite-pellets sieved at 3.35 mm, previously inoculated with activated sludge obtained from a wastewater treatment plant in San Luis Potosi México. An airstream passed through a 99% ethyl acetate solution in a gasifier and it was introduced to the biofilter together with and airstream from a humidifier in downward mode (***Figure 1***). One biofilter was operated without O_3 addition and another worked under O_3 addition. In order to deliver the O_3, a supplementary air stream containing O_3 was mixed with the regular ethyl acetate air stream, before entering the biofilter, without modifying the empty bed retention time of 1 min. O_3 (90 ppb) was generated by an A2ZS-3GLAB OZONE GENERATOR and EA concentration in gas phase was measured with a gas chromatograph (Agilent Technologies-6890) equipped with a flame ionization detector. Liquid samples from the leachates were characterized in terms of pH (Thermo Scientific Orion 4 Star) and total organic carbon content (TOC-V_{CSN} SHIMADZU model ASI-V).

The operation strategy was divided into 3 stages (SI, SII and SIII) along 230 days, according to three inlet load tested, SI, IL=60 gm^3h^{-1}, day 0 to 10; SII, IL=120 gm^3h^{-1}, day 11 to 38 and SIII, IL=180 gm^3h^{-1}, day 39 to 230.

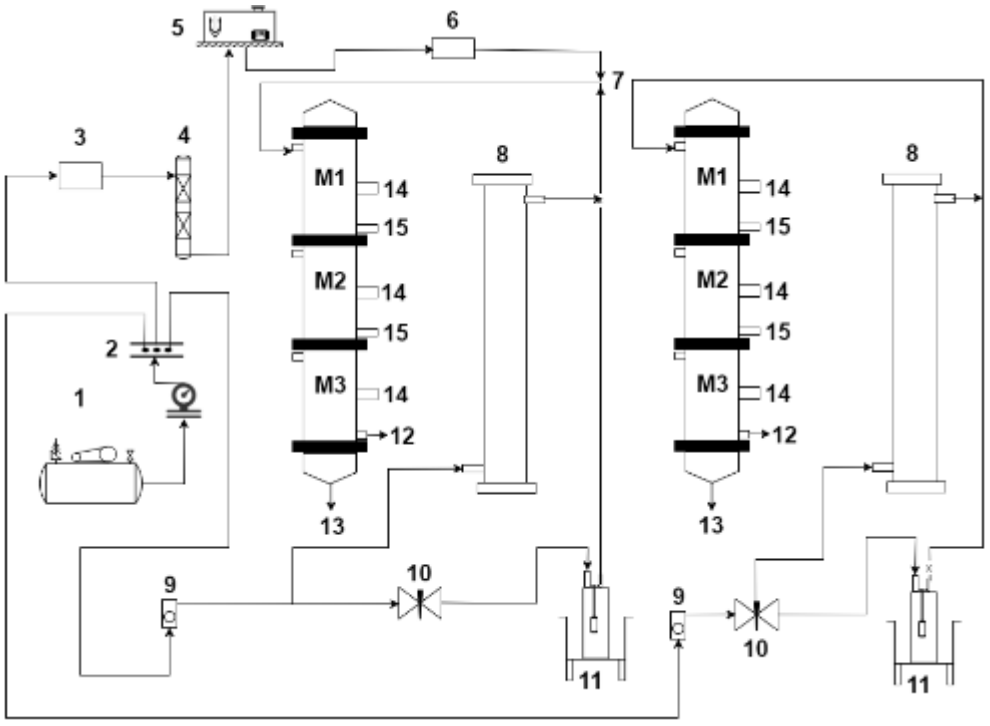

Figure 1. - Schematic reactors system; 1. - Compressor; 2. - Air distributor; 3. - Mass flow controller; 4. - Desiccator; 5. - O_3 generation; 6. - Mass flow controller; 7. - Mixing point (EA and O_3); 8. - Humidifier; 9. - Rotameter; 10. - Needle valve; 11. - Gasifier; 12. - Treated air; 13. - Leachate; 14. - Biomass sampling point; 15. - Sampling points between gas phase modules.

2.2 *Samples*

Biomass samples were collected from both biofilters along 230 days of operation. The samples were stored immediately at -80°C for their further analysis. Samples were transported in Eppendorf tubes of 1.5 mL with 700-800 µL of a buffer solution of DNA/RNA Shield™ of ZYMO Research, which helped to preserves the genetic integrity, to the Center for Microbial Communities of Aalborg University.

2.3 *Preparation of the samples for DNA extraction*

Thawed samples were homogenized by addition of 1.5 mL of 1x of PBS buffer (HyClone Thermo Scientific 10x) and vortexed for at least 10 s to mix. Biomass was removed from the carrier by transferring the samples to 50 mL glass pyrex tubes and placing them in an ultrasonic bath (Branson 2510) for 15 min. Representative sample of the obtained suspension (300 µL) was transferred to new Eppendorf tubes for DNA extraction.

2.4 *DNA extraction*

Total genomic DNA was extracted using FastDNA SPIN kit for Soil (MP Biomedicals, USA), according to manufacturer's recommendation with an amended bead-beating step of 4x40 s at 6 m/s. DNA quality was evaluated using TapeStation 2200 and Genomic DNA ScreenTapes (Agilent, USA), quantity was determined using Quant-IT dsDNA assay (Thermo Fischer Scientific, USA) on an Infinite M1000 Pro (TECAN, Switzerland) plate reader.

2.5 *16 rRNA gene and ITS1 amplicon sequencing*

The Procedure for 16SrRNA gene (variable region V1-V3) and ITS1 region amplicon sequencing was performed as described elsewhere (Bokulich & Mills, 2013; Caporaso et al., 2012). Amplicon library PCR was performed in 10 ng template DNA per 25 µL PCR reaction (2mU Platinum Taq DNA polymerase HF and 1X Platinum High Fidelity buffer; Thermo Fischer Scientific, USA), 400 nM of each dNTP, 1.5 mM $MgSO_4$, and 400 nM of barcode library adapters for the 16S rRNA gene. The ITS1 libraries were generated using the sample protocol, with an added secondary PCR step to attach the adapters in accordance with Nextera barcoding (Illumina, USA). 16S rRNA gene V13 primers: 27F AGAGTTTGATCCTGGCTCAG and 534 R ATTACCGCGGCTGCTGG. ITS1 primers: BITS F ACCTGCGGARGGATCA and B58S3 R GAGATCCRTTGYTRAAAGTT. Thermocycler settings: Initial denaturation for 2 min at 95 °C; 30 cycles of 95 °C (for 20 s), annealing (at 56 °C for 16SV13 and 50 °C for ITS1) for 30 s, elongation at 72 °C for 60 s, and a final elongation step at 72 °C for 5 min. Obtained libraries were purified using Ampure XP bead protocol (Beckmann Coulter, USA) with sample/bed solution ratio 5/4, an elution to 23 µL nuclease free water. Library quality was evaluated using TapeStation 2200 (Agilent, USA). Library concentration was measured using Quant-IT HS dsDNA assay (Thermo Fischer Scientific, USA) on an Infinite M1000 Pro (TECAN, Switzerland) plate reader. Samples were pooled in equimolar concentrations and sequenced using Illumina MiSeq technology with 20% PhiX control spike-in and a final library pool concentration of 20pM.

2.6 *Bioinformatic processing*

Sequenced libraries were quality checked and trimmed using trimmomatic (v0.32) (Bolger et al. 2014) and merged using FLASH, (v1.2.7) (Magoč et al. 2011). Reads were formatted for use with the UPARSE workflow (Edgar, 2013), then de-replicated and clustered into Operational Taxonomic Units (OTUs) at 97% sequence similarity. Taxonomy was assigned using RDP classifier as implemented in QIIME (Caporaso et al., 2010) using MiDAS taxonomy version 1.20 (McIlroy et al., 2015), based on SILVA taxonomy (Quast et al., 2013) for 16S rRNA gene libraries, and BLAST as implemented in QIIME using UNITE (version) taxonomy (ref) for ITS1 libraries.

2.8 *Identification and taxonomic classification of gene fragments*

The phlylogenetic relationships of the microbial consortia were determined by comparing their 16S rRNA gene sequences using bioinformatics pipelines available in the Nielsen lab (Aalborg University). The identified sequences were used to compare the microbial communities in the two biofilters and how they evolve over time and were affected by the O_3 addition.

3. Results

3.1 *Biofilters operation*

In general, biofilter with O_3 presented higher removal efficiency (RE) and elimination capacity (EC) than the control system. Biofilter without O_3 addition achieved its maximum EC during SIII, operated with an inlet load of 180 g m^{-3} h^{-1} (day 40), varying its RE from 100% to 50-70% until the end of the experiment. Last 5 days RE dropped until 27% with an EC of 20 g m^{-3} h^{-1}. (***Figure 2***).

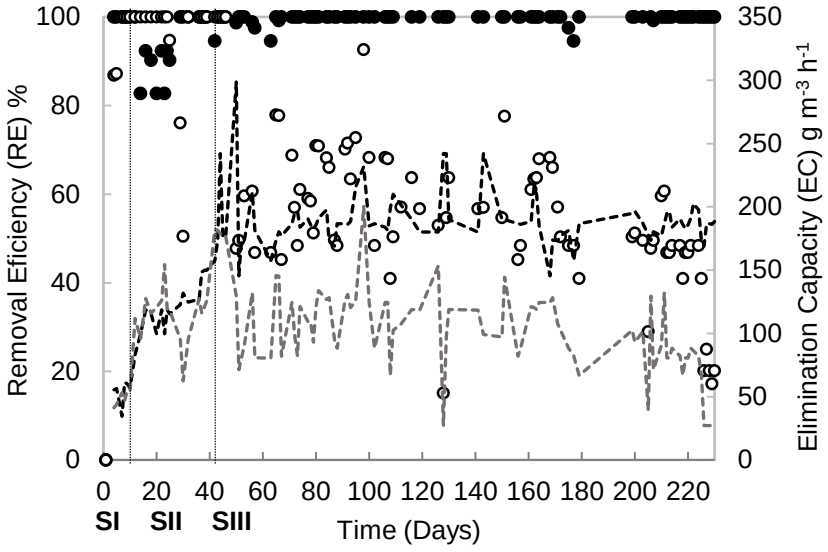

Figure 2. Biofilter Performance: Overall removal efficiency (RE) and Elimination Capacity (EC); (●) RE with O_3; (○) RE without O_3; (▪▪▪) EC with O_3; (▪▪▪) EC without O_3.

The pH and TOC in the leachates were measured throughout the biofilter operation (***Figure 3***). During 120 days biofilter with O_3 presented a quite stable pH with average value of 7, the other 110 days the pH was as low as 2. Meanwhile biofilter without O_3 had a pH of 5 until day 120 and onwards a mean of 4.23. TOC content increased when pH dropped. TOC content in the biofilter with O_3 addition was higher than the control.

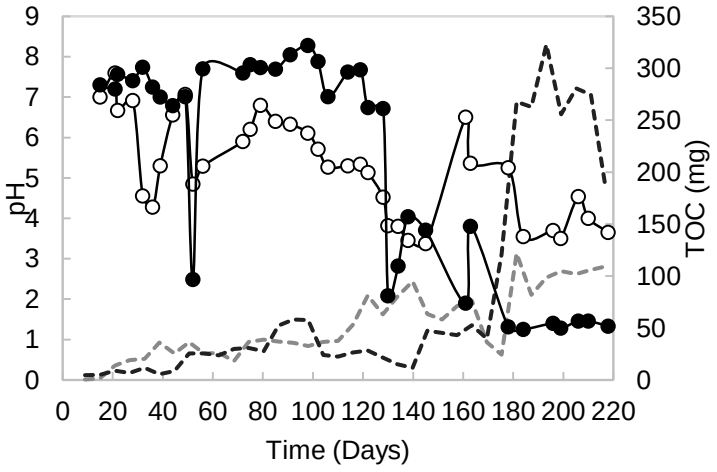

Figure 3. Total organic carbon (TOC) and pH evolution along biofilter performance. (▪▪▪) TOC without O_3; (▪▪▪) TOC with O_3; (—○—) pH without O_3; (—■—) pH with O3 addition.

Analyzing the performance of the biofilters by module (***Figure 1*** a) without O_3 and b) with O_3), it can be seen that the EC in M3 of the biofilter with O_3 was most of the time null, this is be due to almost the 100% what enters in M2 is being removed. In general EC in M1 and M2 of the biofilter with O_3 addition was higher than the biofilter without. O_3 addition allowed a better EC and it could treat EA over a stretch of shorter filter, which could represent an advantage over the biofilter without. Only Maldonado-Diaz & Arriaga (2014) and Xi et al., (2014) have studied biofiltration systems with modules and O_3 additions, three and two, respectively, however no information of EC or RE by module have been reported.

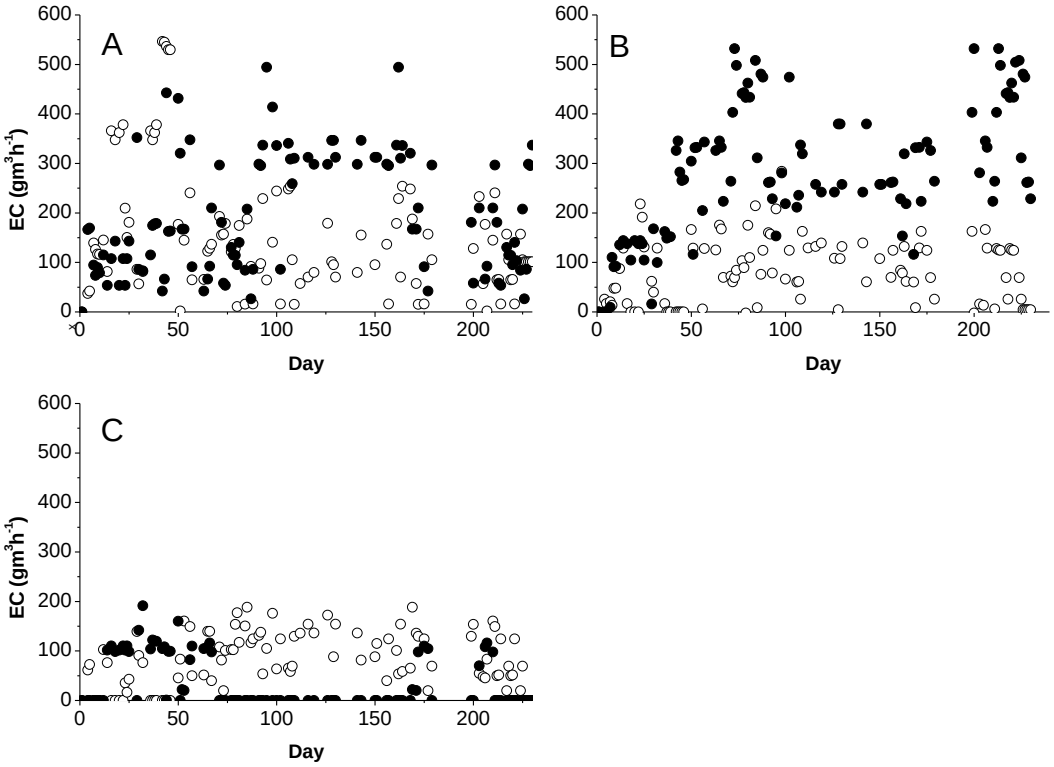

Figure 4. - Elimination Capacity (EC) in module A (M1), B (M2) and C (M3) along the time. (●) EC with O_3; (○) EC without O_3.

3.2 *Bacterial and fungal richness*

The species richness of the fungi and bacteria were analyzed in the two biofilters by OTUs (operational taxonomic unit), which is used to classify groups of closely related

individuals “clusters” grouped by DNA sequence similarity. According to the box and whisker plot, ***Figure 5 A)***, biofilter without O_3 had a median of 340 OTUs with a range of 440 OTUs, while the median of the biofilter with O_3 was 440 OTUs with a range of 425 OTUs. Biofilter without O_3 addition appears to have a higher richness that the one with O_3 addition. The medium value of the biofilter without O_3 addition is closer to the lowest richness, on the other hand the medium in the biofilter with O_3 is closer to the highest richness. Biofilter without O_3 addition has a higher dispersion between the 50% and the 75% of the data than the 25% and 50%, whereas in the biofilter with O_3 addition there is a higher dispersion in the data between the 25% and 50%. Regarding fungi, its box shows a quite lower richness distribution in the biofilter without O_3 than with O_3. As it can be seen in ***Figure 5 B)***, richness of both biofilters decreased with time, from 620 OTUs until almost 160 in biofilter without O_3. The richness in the biofilter with O_3 addition decreased from 600 to 200 until day 100 of operation and then increased in day 150.

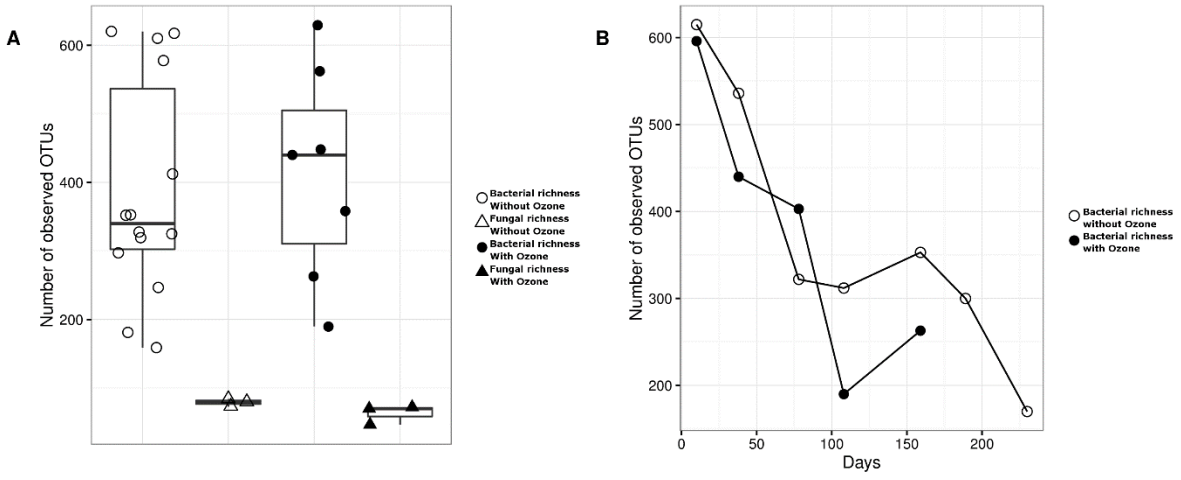

Figure 5. Bacterial and fungal richness of the biofiltration systems. A) Box and whisker plot of number of richness. B) Richness behavior throughout the operational time.

3.3 *Microbial community behavior*

Clearly, the evolution of the bacteria along the operational time was disturbed by the treatment and continued changing along the biofilters performance. ***Figure 6*** of NMS (Non-metric multidimensional scaling) shows us the more similar points together

according to its MDS (multidimensional scaling, mathematical technique which generated a spatial configuration map where the distance between data point reflects the relationship between individual variables). The different clusters indicated that there was no significantly difference in microbial composition between the modules, but there was a difference in the time of the sample was taken. Inoculum sample was significantly different from the others samples, started to change since the beginning. On day 10 both biofilters were quite similar (SI, IL= 60 g m^{-3} h^{-1}), but different from the inoculum. Then in operation day 38, biofilters started to stratify with the double of the inlet load (SII, IL=120 g m^{-3} h^{-1}). Operation days 78,108, 159 and 189 in the biofilter without O_3 addition behaved very stable (same inlet load, SIII, IL= 180 g m^{-3} h^{-1}) and in the final point (day 230) the microbial community changed. On the other hand, the biofilter with O_3 addition in the next sample points continued changing without showing the stable performance at 180 g m^{-3} h^{-1} of inlet load.

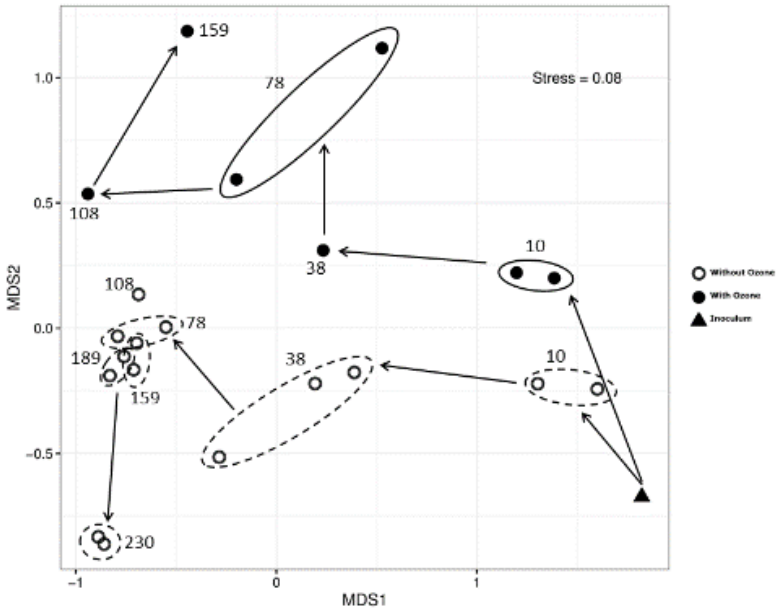

Figure 6. Non-metric multidimensional scaling (NMS). Microbial community behavior.

3.4 *Heat-map genera bacteria and fungi*

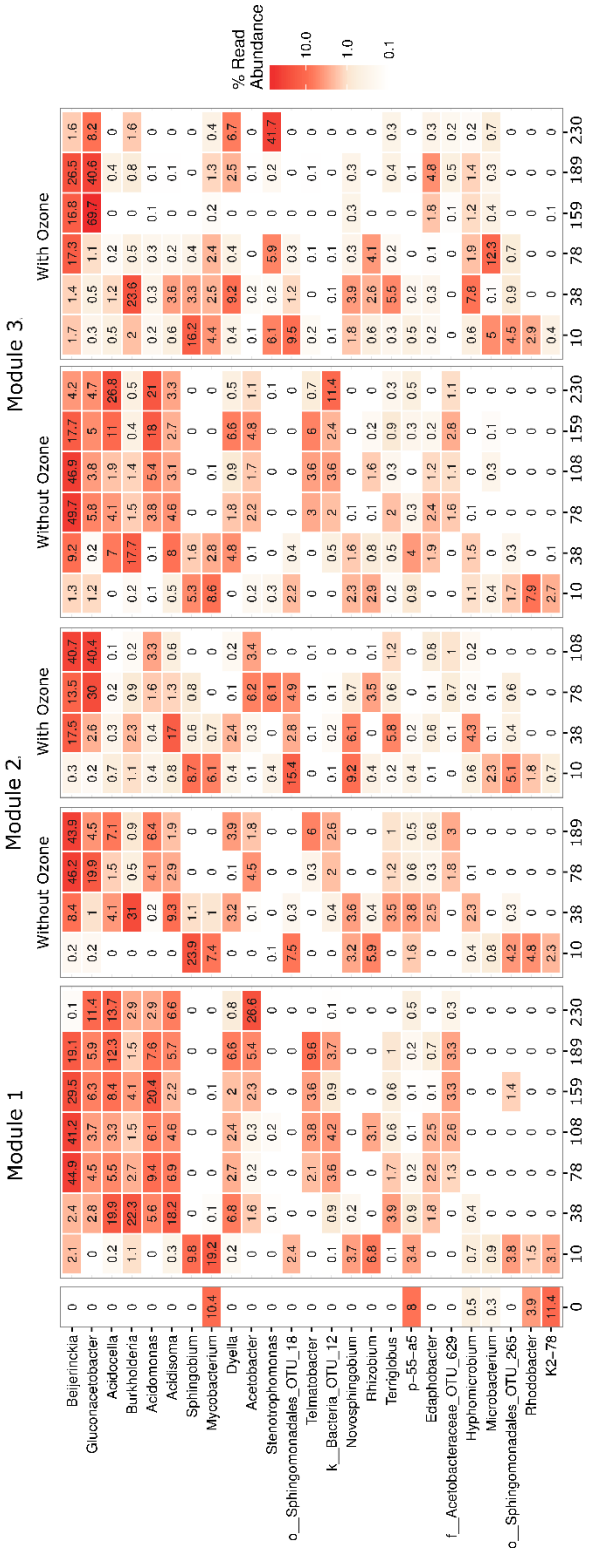

Figure 7. - Heat-map bacteria

As we can observe in the heat-map (***Figure 7***) the inoculum was remarkably different from the other samples, the main bacteria identified were *Mycobacterium, p-55-a5, Rhodobacter* and *K2-78*, these bacteria disappear along the operational performance of both biofilters. *Beijerinckia* was the main bacterium identified in both biofilters. This bacteria in the biofilter without O_3 started to increase in the three modules, days 10 and 38, with inlet loads of 60 g m^{-3} h^{-1} and 120 g m^{-3} h^{-1} respectively. Days 78,108,189 in the three modules presented a quite stable abundance, with a mean of 45.5%, from day 39 and onwards the inlet load was 180 gm^3h^{-1}. Interestingly from day 159 the abundance started to decrease (M1 and M3) until values nearer to 0. On the other hand, the abundance of *Beijerinckia* in the biofilter with O_3 was increasing with time at M2 (highest value of 40.7, day 108) and in M3 (highest value of 26.5, day 189), but in M3 drop at the end of the operation at day 230.

Gluconacetobacter was the other main genera found in both biofilters. Overall, *Gluconacetobacter* abundance was higher in the biofilter with O_3 than without. The abundance in the biofilter with O_3 increased with the time in M2 and M3, presenting the highest abundance of 69.7 in M3 at day 159 and then dropped the onwards days. Otherwise biofilter without O_3 increased with time in M1, and, in M2 increased and then dropped at day 189, and last in M3 was quite stable since day 78.

The genera *Acidocella* was mainly present in biofilter without O_3, increasing with the time in M2 and M3. In M1 dropped at day 78 and then increased on day 189 and 230. *Acidomonas*, also presented higher abundance in the biofilter without O_3, increased with the time in M2 and M3 and in M1 was varied. *Acetobacter* presented an abundance of 26.6 at the end of the operation in M1, biofilter without O_3. *Burkholderia, Acidosoma, Spingobium*, and others tended to disappear along time.

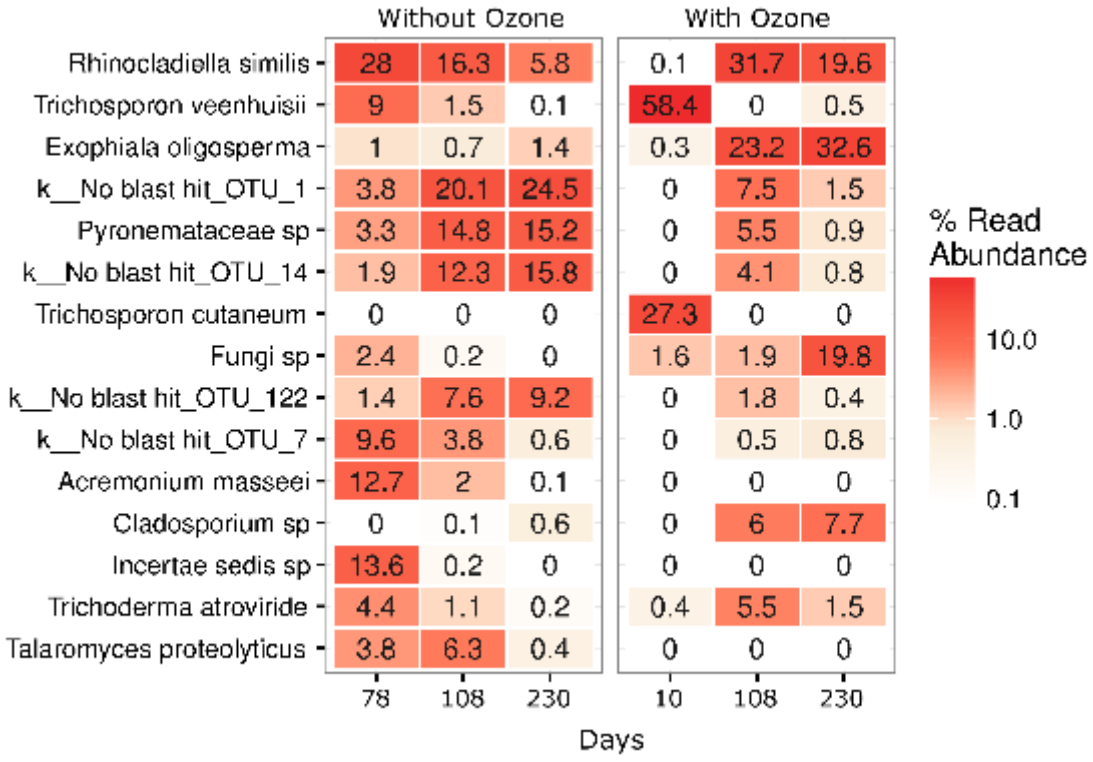

Figure 8. - Heat-map fungi

In summary, the abundance of each genera of fungi were higher in the biofilter without O_3 treatment (***Figure 8***). It is important to mention that these heat-map comes only from samples of module 2 from both biofilters. The genera *Exophiala oligosperma* has a higher abundance in the biofilter with O_3 and is increasing with time. In comparison, biofilter without O_3 has a low abundance. *Rhinocladiella simmilis* decreased with time in biofilter without O_3 and in biofilter with O_3 increased in day 108, but on day 230 decreased. Species as *Acremonium massrri, Incertae sedies, Trichosporon veemhuisii, Trichosporon cutaneum* the others disappeared with time.

4. Discussion

Due to the low pH of the system from day 120 forward, the most likely microorganisms to be found were the ones capable to survive in strongly acidic environments. The NMS figure showed clearly, that there is no significantly difference in the microbial composition between modules, but there was a difference

according to the time of sampling. According to literature, the bacteria identified in our system, named *Acetobacter, Acidomonas* and *Gluconacetobacter* belong to the family *Acetobacteraceae*. All members of this family are obligately aerobic and their metabolism is strictly respiratory with oxygen as the terminal electron acceptor. One characteristic of this acid bacterium is the aerobic oxidation of ethanol to acetic acid, with accumulation of the latter medium. When the oxidation of Ethanol is completed, strains of *Acetobacter, Gluconacetobacter* and *Acidomonas* oxidize acetic acid further to CO_2 and H_2O (Kersters et al; 2006).These bacteria identified in our biofilter corroborate the acidification and agrees with the degradation pathway of Ethyl Acetate, which is ethanol and acetic acid (Eubanks et al., 1974).

The genus *Beijerinckia* identified in both biofilters, is characterized as nosymbiotic, aerobic, chemoheterorophic bacteria with the ability to fix atmospheric nitrogen. Moreover, it had showed great acid tolerance, being able to grow and fix nitrogen at pH 3.0-4.0 (Becking 1968). They usually produce highly raised colonies of very tenacious and elastic slime, and on liquid media they turn the whole medium viscous (Becking, 1968). Another genus identified is Acidocella, which is phylogenetic neighbor of the *Acetobacteraceae*, *Acidocella* has been found mainly in acidic reducing environments, with relatively high concentrations of metals, such as coal fields and sites affected by acid mine drainage (pH 2.7-3.7) (Johnson et al. 2001).

Interestingly biofilter with O_3 reached a lower pH than the control (Fig. 3). The more abundance of acidophiles could mean lower pH, overall *Gluconacetobacter* abundance was higher in the biofilter with O_3 than without, increasing in M2 and M3, presenting the highest abundance of 69.7 in M3 at day 159, but then dropped the onwards days. One plus of the acidification in the system with O_3 is that the removal efficiency of the pollutant did not decrease, and remained at 100%, whereas the collapse in the efficiency of biofilters has commonly be present at low pH (Wu et al; 2006). Something developed in order to overcome this problem has been the inoculation of biofilters with fungi, since bacteria are less resistant to low pH or drying environments than fungi. Moreover, the presence of fungal could be enhancing the performance of our system, since it has been hypothesized that the large surface area of hyphae would enhance the absorption and transport of hydrophobic

compounds from the contaminated gas phase to the cell surface (Christian Kennes & Veiga, 2001). As previously mentioned the main fungi identified were Rhinocladiella similis, Trichosporon veenhuissi, Exophilia oligosperma and others. Several fungi isolates have been used in biofiltration systems treating polluted air.

Exophiala oligosperma isolates have been used in biofiltration systems treating toluene polluted air (Estévez, Veiga, & Kennes, 2005), although fungi are more active than bacteria at low pH and reduce water content, this study showed a lower performance under such conditions. *Rhinocladiella simmilis* isolate has also been used in a biofiltration system treating different VOCs, it has been found that the performance of the reactors was dependent on hydrocarbon polarity (ethanol and phenol were more easily biodegraded than toluene and n-hexane). *R. similis* adapted by modifying its surface hydrophobicity and expression of hidrophobin-like molecules when grown with compounds of opposite polarities, such ethanol or n-hexane (Vigueras et al. 2009).

The less abundance of bacteria in the biofilter with O_3 could be related with that O_3 is reducing the bacterial population, since, biofilter with O_3 presented a lower pH, O_3 is more stable at low than high pH values and the inactivation of microorganism is mostly through reaction with molecular O_3 when the pH is low, O_3 stability decreased as pH increased (Khadre et al., 2001). Moreover, O_3 has been evaluated for eliminating bacterial populations during fungal cultivation on corn-processing wastewater (Sankaran et al. 2008), an O_3 dosage of about 57 mg/L was found to be the most effective in improving both fungal biomass production and soluble chemical oxygen demand (SCOD) removal (up 90%), higher and lower dosages resulted in poorer fungal growth and lower SCOD removal. Another important factor to consider in the reduction of the bacteria population is that each microorganism has an inherent sensitivity, bacteria are more sensitive than yeasts and fungi, Gram-positive bacteria are more sensitive than Gram-negative organism and spores are more resistant than vegetative cells (Pascual et al; 2007). Filamentous fungal cells have a greater resistance to disinfectants (ozone) than non-filamentous bacterial cells (Reynolds, 2002). So, in this system O_3 addition is decreasing the pH, which is helping to inactivate some microorganisms, and thus selecting the more capable to

degrade EA or their sub products more effortlessly, improving the elimination capacity of the system. It is difficult to say specifically which microorganism in the biofilter with O_3 are catalyzing the removal of ethyl acetate, since only the relative abundance was obtained and not the exact amount of each genus. At the same time it is important to consider the metabolic capacities of the microorganism involved and the environmental conditions, which change with the time.

5. Conclusions

Effects of O_3 on the microbial community composition in biofilter treating ethyl acetate vapours was attempted. The main bacteria identified in these systems are acidophiles, which are potential candidates for remediation of contaminated acidic environments. On the other hand Acetobacteraceae play a key role in the industrial manufacture of vinegar.

Even though Microbial composition is extremely depending upon the experimental conditions applied, as well as the methodological approach; each individual study of microbial community reveals important and interesting information. This study highlights the importance of the operational factors that can affect the microbial community's composition, in this case the O_3 addition in a biofilter system and the pH, which are closely related with the substrates concentrations, increasing or decreasing and acidifying the media, and finally in response to these variations changing the microbial community.

The accumulation of the information generated allowed not only monitoring, but provided data from which new hypotheses about response of community change to disturbance events can be developed. Furthermore microbial community analyses can lead to different advantages such as the identification of the optimal community for stable and efficient operation and the selection of the best organisms for inoculating new systems. A better understanding of their function is necessary for successful application.

6. References

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Chapter V. PERSPECTIVES AND CONCLUSIONS

The main conclusions were the following. The continuous addition of O_3 had a clear effect over the biomass concentration, as well as in the pressure drop; at the same time O_3 addition helped to keep stable the 100% removal efficiency of the system. Although it is necessary to treat higher inlet loads of EA in order to find the maximum capacity of this system. Continuous addition of O_3 still could be an attractive solution to improve biofilter performance and extend the half time life of the filter bed, but the results of void fraction indicated that eventually, depending of the system, the biofilter will clog. EA biofilters have produced more biomass to effectively analyze the clogging prevention in comparison to the biofiltration of recalcitrant pollutants.

The results of FTIR analysis suggested that O_3 addition on EPS did not present a significantly difference in the functional groups identified. The quantification of each component of EPS analyzed indicated that O_3 addition made greater effect on carbohydrate amount. O_3 is affecting only in the amount of EPS not the composition. Regarding the microbial analysis, the main bacteria identified in these systems were acidophiles, which are potential candidates for remediation of contaminated acidic environments. This study highlighted the importance of the operational factors that can affect the microbial community's composition, in this case the O_3 addition in a biofilter system and the pH, which are closely related with the substrates concentrations, increasing or decreasing and acidifying the media, and finally in response to these variations changing the microbial community. Overall, O_3 addition in a biofiltration system offers a promising solution to improve air biofiltration.

Perspectives:

- Since, the biofilter with O_3 addition achieved a RE of 100% along the operation stages, would be interesting to apply a higher IL of EA in order to find the limit of the system.
- Operate the biofilter under O_3 addition with the same conditions, to look for how much time the biofilter will last until reaching the bed clogging.
- Looking for lower O_3 concentration without affecting the removal efficiency in order to economize the process, due to O_3 addition represent an extra cost in a biofiltration system. Additionally, as O_3 effectiveness against microorganisms depends not only on the amount applied, but also on the residual O_3 in the medium. It would be interesting to analyze how much residual O_3 is entering to the next modules in this system (90 ppb) and do the same of the above premise of a limit concentration.
- Measure the cell viability to see how many bacteria are alive with/without O_3 treatment and with the clogging development.
- Measure the metabolic activity, since it was reported that O3 exposure showed higher metabolic activities (Xu et al., 2016).
- Real quantification of bacteria or fungi in order to find which one is present in more quantity. Maybe this could be a clue of why the better or worse performance.