Universidad de Valladolid

ESCUELA DE INGENIERÍAS INDUSTRIALES

DEPARTAMENTO DE INGENIERÍA QUÍMICA Y TECNOLOGÍA DEL MEDIO AMBIENTE

TESIS DOCTORAL:

Biotechnologies for Air Pollution Control: Overcoming Design and Operational Limitations

Presentada por José Manuel Estrada Pérez para optar al grado de doctor por la Universidad de Valladolid

Dirigida por:

Raúl Muñoz Torre
Raquel Lebrero Fernández

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Certifican que:

JOSÉ MANUEL ESTRADA PÉREZ ha realizado bajo su dirección el trabajo *"Biotechnologies for Air Pollution Control: Overcoming Design and Operational Limitations"*, en el Departamento de Ingeniería Química y Tecnología del Medio Ambiente de la Escuela de Ingenierías Industriales de la Universidad de Valladolid. Considerando que dicho trabajo reúne los requisitos para ser presentado como Tesis Doctoral expresan su conformidad con dicha presentación.

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Reunido el tribunal que ha juzgado la Tesis Doctoral titulada *"Biotechnologies for Air Pollution Control: Overcoming Design and Operational Limitations"* presentada por el Ingeniero Químico José Manuel Estrada Pérez y en cumplimiento con lo establecido por el Real Decreto 99/2011 de 28 de enero de 2011 acuerda conceder por_____ la calificación de _____.

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Resumen

La contaminación atmosférica ha recibido tradicionalmente menos atención que otras formas de contaminación como la de suelos o aguas. Sin embargo, hoy en día el mundo se enfrenta a problemas y desafíos medioambientales relacionados con la contaminación del aire que deberán abordarse desde el avance tecnológico, la concienciación y cambio de hábitos sociales, y el desarrollo de legislaciones más estrictas.

Entre los grandes problemas del siglo XXI se encuentran las emisiones de compuestos orgánicos volátiles (COVs), las emisiones odoríferas y los gases de efecto invernadero. Todas estas emisiones provienen de un amplio abanico de actividades humanas que incluyen la industria, el tratamiento y gestión de residuos, la ganadería o la agricultura. A pesar de las características específicas de cada emisión, todas ellas pueden ser generalmente tratadas mediante tecnologías "end-of-the pipe" que comparten las mismas bases fundamentales. Estas tecnologías para el tratamiento de la contaminación atmosférica se clasifican habitualmente en físico-químicas y biológicas, y cada grupo presenta sus ventajas e inconvenientes económicos, operacionales, medioambientales o sociales. Sin embargo, los datos referidos al comportamiento de las diferentes tecnologías bajo un mismo escenario son escasos. La recopilación y análisis comparativo de estos datos permitiría mejorar los criterios de selección, reorientándolos desde la perspectiva actual de viabilidad económica basada en el flujo y la concentración del contaminante hacia un enfoque holístico de sostenibilidad.

Las tecnologías biológicas han experimentado un importante desarrollo en las últimas décadas llegando a ser alternativas viables para el tratamiento de la contaminación atmosférica. Aun así, todavía existen limitaciones que impiden su implementación o limitan su rendimiento en ciertas aplicaciones. La identificación de esas limitaciones y la investigación para superarlas son factores clave para conseguir la generalización de estos tratamientos sostenibles de contaminación del aire.

Con estas bases, en esta tesis se realizaron estudios comparativos para analizar el estado del arte de las diferentes tecnologías disponibles para el tratamiento de la contaminación atmosférica, identificándose las principales limitaciones de las

tecnologías biológicas. Esta información permitió el desarrollo posterior de estrategias innovadoras de diseño y operación para superar esas limitaciones.

En un primer estudio se evaluaron las tecnologías de biofiltración, difusión en lodos activos, biofiltro percolador, lavador químico, adsorción en carbono activo, incineración regenerativa y una tecnología híbrida (biofiltro percolador acoplado en serie a un sistema de adsorción en carbono activo), teniendo en cuenta aspectos medioambientales, económicos y sociales. Se empleó el procedimiento IChemE Sustainability Metrics en el contexto de tratamiento de olores en una estación depuradora de aguas residuales. Los resultados mostraron que las tecnologías biológicas resultan más económicas y respetuosas con el medio ambiente que las físico-químicas. El biofiltro percolador y la difusión en lodos activos resultaron las tecnologías más prometedoras, pero aún están limitadas por las bajas eficacias de eliminación de contaminantes altamente hidrofóbicos.

Las ventajas de las tecnologías biológicas en términos de sensibilidad económica también fueron confirmadas. Se analizó la sensibilidad económica de las cinco tecnologías más aplicadas al tratamiento de olores frente a variaciones en parámetros de diseño y precios de materias primas. Además se estudió la influencia de la localización geográfica en los costes totales de las tecnologías y su robustez frente a fluctuaciones y problemas de operación típicos. El análisis mostró que las tecnologías biológicas presentan costes de operación hasta seis veces menores que las físico-químicas, siendo el material de empaque el parámetro clave que más afecta a estos costes (representando un 40-50% de los costes totales de operación). La adsorción en carbono activo y la tecnología híbrida mostraron la mayor robustez, mientras que las biotecnologías presentaron una robustez similar a la de los lavadores químicos.

Para mejorar el proceso de arranque y la estabilidad de las tecnologías biológicas se estudió la posibilidad de aclimatar el inóculo empleado bajo diferentes concentraciones de COVs. Se analizó así la influencia de la concentración de tolueno (utilizado como contaminante modelo) en la diversidad microbiana y en el rendimiento macroscópico de biodegradación. El aislamiento a alta concentración de tolueno en la fase gaseosa (10 g m^{-3}) provocó inestabilidad en el proceso y eficacias de eliminación por debajo del

33%, mientras que la operación a concentraciones gaseosas medias (300 mg m^{-3}) y bajas (11 mg m^{-3}) fue estable y con eficacias de eliminación entre el 74 y el 94%. La biodiversidad desarrollada fue inversamente proporcional a la concentración de contaminante. Estos resultados demostraron que las estrategias tradicionales de aislamiento/aclimatación de inóculos basadas en tolerancia a la toxicidad pueden dar lugar a bajos rendimientos y periodos de arranque largos cuando el contaminante a tratar se encuentra a baja concentración.

Otro problema clave para la implementación de las tecnologías biológicas es la limitación para la transferencia desde la fase gaseosa a los microorganismos de los contaminantes menos solubles en agua. En este sentido, mientras que los biofiltros bacterianos generalmente muestran una gran diversidad y redundancia microbiana, los biofiltros fúngicos presentan un mejor rendimiento a baja humedad y bajos pHs, siendo más eficientes en el tratamiento de COVs hidrofóbicos. Un biofiltro bacteriano y otro fúngico se operaron en el tratamiento de una mezcla de COVs (propanal, metil-isobutil cetona, tolueno y hexanol) bajo las mismas condiciones. En términos generales el biofiltro bacteriano mostró mejores resultados de eficacia de eliminación y mineralización de contaminantes. El orden de preferencia de biodegradación de los sustratos fue el mismo en ambos casos (propanal>hexanol>cetona>tolueno), con un efecto de inhibición parcial causado por el propanal. Ambos biofiltros mostraron una alta robustez frente a paradas de alimentación de contaminantes de 24 horas.

Los biofiltros son una de las tecnologías más aplicadas para el tratamiento de efluentes gaseosos. Sin embargo, la pérdida de estabilidad estructural del empaque y la obstrucción del lecho son sus desventajas principales, que conllevan incrementos en el consumo de energía y la reducción de la vida útil del relleno. Para resolver esta limitación se analizó una nueva configuración de alimentación por etapas en comparación con un biofiltro estándar, utilizando tolueno como contaminante modelo y dos tipos de empaque: uno orgánico y otro inorgánico. La configuración de alimentación por etapas alcanzó capacidades de eliminación similares a la estándar pero con reducciones del 75 y el 65% en la energía necesaria para la compresión de aire con los empaques orgánico e inorgánico, respectivamente. La alimentación por etapas

resultó una alternativa prometedora capaz de reducir los costes de operación de la biofiltración, reduciendo el consumo de energía y maximizando la vida útil del material de empaque.

Las limitaciones por transferencia de materia reducen la capacidad de eliminación y limitan la aplicación a escala industrial de los biofiltros percoladores para el tratamiento de contaminantes altamente hidrofóbicos como el CH_4. En este contexto, se desarrolló un estudio con el objetivo de maximizar la transferencia de materia en un biofiltro percolador optimizando la velocidad de recirculación de líquido e introduciendo una estrategia novedosa de recirculación de gas. Teóricamente, la recirculación puede aumentar la eficiencia en el uso de energía del reactor en un 50% bajo ciertas condiciones. La implementación de la recirculación favoreció la eliminación de CH_4 en las etapas iniciales de la operación y se alcanzaron capacidades de eliminación superiores a 30 g m^{-3} h^{-1} con tiempos de residencia de 4 min y velocidades de recirculación del líquido de 5 m h^{-1}, siendo las mayores eliminaciones de CH_4 reportadas hasta la fecha en un biofiltro percolador monofásico. Sin embargo, posteriormente se identificaron limitaciones de transferencia adicionales entre la fase líquida y la biopelícula que se atribuyeron a la acumulación excesiva de biomasa en el empaque.

Finalmente se llevó a cabo una prueba de concepto sobre la aplicabilidad de recubrimientos bioactivos de látex para el tratamiento de COVs gaseosos. Estos recubrimientos biocatalíticos mostraron capacidades específicas de degradación 10 veces superiores a las de biopelículas artificiales con alto contenido en agua. El método permitió superar las limitaciones por transferencia de materia de las biopelículas convencionales, probablemente debido a la ausencia de agua en el biofilm o sobre el mismo. Los resultados fueron muy positivos y podrían conllevar mejoras importantes en el rendimiento de las biotecnologías para el control de la contaminación atmosférica.

Abstract

Atmospheric pollution has traditionally received less attention than other forms of pollution such as soil or water contamination. Nevertheless, the world faces new environmental problems and challenges related to air pollution that will demand action from different approaches: technological advances, social awareness and habit modification, and enforcement of stricter legislations.

Volatile organic compounds (VOCs), malodorous emissions and non-CO_2 greenhouse gases are responsible of some of the major air pollution problems in the 21st century. These compounds are emitted from a wide range of activities including industry, waste management, livestock facilities or agriculture. Despite the specific features of each emission, all of them are susceptible to be treated by end-of-the pipe technologies sharing the same fundamental basis. These technologies are usually classified into physical/chemical or biological air pollution control technologies, each group presenting specific economic, operational, environmental or social strengths and drawbacks. However, there is a lack of comparative data assessing the performance of the different air pollution control technologies under similar scenarios. The compilation and comparative analysis of these data would entail a shift in the technology selection criteria from the traditional economic feasibility based on flow rate and pollutant concentration towards a holistic sustainability approach.

Biotechnologies have emerged in the past decades as viable alternatives for air pollution control. Nevertheless, there are still limitations which hinder their implementation or performance in certain applications. The identification of those limitations and the development of research to overcome them will be of key relevance in order to broaden the widespread implementation of these sustainable air pollution control technologies.

In the present thesis, the main limitations of biotechnologies for air pollution control were identified using state-of-the art comparative studies. This information allowed developing innovative design and operational strategies in order to overcome those limitations.

In a first study, biofiltration, activated sludge diffusion, biotrickling filtration, chemical scrubbing, activated carbon adsorption, regenerative incineration, and a hybrid technology (biotrickling filtration coupled with carbon adsorption) were comparatively evaluated in terms of environmental performance, process economics, and social impact using the IChemE Sustainability Metrics in the context of odour treatment in wastewater treatment plants. Biological technologies were proven to be more economic and environmentally friendly than their physical/chemical counterparts, biotrickling filtration and activated sludge diffusion being the most promising technologies, but still limited by their low removal efficiencies (RE) when treating highly hydrophobic pollutants.

The advantages of biological technologies were further established by analyzing the economic sensitivity of the five most commonly applied odour abatement technologies towards design parameters and commodity prices. In addition, the influence of the geographical location on the total costs of each technology and their robustness towards typical process fluctuations and operational upsets were assessed. This comparative analysis showed that biological techniques present up to six times lower operating costs and a lower economic sensitivity than their physical/chemical counterparts, with packing material being the key parameter affecting their operating costs (40-50% of the total operating costs). Activated carbon adsorption and the hybrid technology were the most robust technologies according to the results obtained, while biotechnologies presented similar robustness to chemical scrubbers.

In order to improve the start up and stability of biotechniques, the possibility of acclimating inocula under different VOC concentrations was studied. In this context, the influence of toluene concentration (used as a model pollutant) on the microbial diversity and the macroscopic biodegradation performance was studied during an enrichment process. Culture enrichment at high pollutant gas phase concentration (10 g m^{-3}) resulted in process instability with REs below 33%, while operation at medium (300 mg m^{-3}) and low (11 mg m^{-3}) toluene gas phase concentrations was stable, with REs ranging from 74 to 94%. Biodiversity was inversely proportional to pollutant concentration. These results also confirmed that traditional inoculum

isolation/acclimation based on toxicity tolerance may result in a poor abatement performance and long start-up periods during the treatment of diluted off-gas emissions.

Another key problem for the implementation of biological technologies derives from mass transfer limitations of the less water soluble pollutants from the gaseous phase to the microorganisms. In this context, while bacterial biofilters usually exhibit a high microbial diversity and functional redundancy, fungal biofilters have been claimed to perform better at low moisture contents and pH value, and to be more efficient coping with hydrophobic VOCs. A fungal and a bacterial biofilter for the treatment of a VOC mixture (propanal, methyl isobutyl ketone-MIBK, toluene and hexanol) were compared under the same operating conditions. Overall, the bacterial biofilter showed better results in terms of REs and pollutant mineralizations. The substrate biodegradation preference order was equal for both biofilters (propanal>hexanol>MIBK>toluene) with propanal partially inhibiting the consumption of the rest of the VOCs. Both biofilters showed a high robustness when subjected to 24 h VOC starvation episodes.

Biofilters are still one of the most applied technologies for air pollution control. However, the loss of structural stability and media clogging are often pointed out as their main drawbacks, leading to increased energy consumption and a limited packed bed lifespan. An innovative step-feed biofilter configuration, with the polluted emission supplied at different locations along the biofilter height, was tested and compared with a standard biofilter using toluene as a model pollutant and two different packing materials: organic and inorganic. The step feed configuration supported similar elimination capacities (ECs) to those of the standard biofilter. However, it exhibited 75% and 65% reduction in the compression energy when using compost or perlite as the packing material, respectively. Step-feed biofiltration constituted a promising configuration capable of reducing the operating costs of biofiltration via reduced energy consumption and an increased packing material lifespan.

Pollutant mass transfer limitations often reduce the abatement potential and hinder the full-scale application of biotrickling filters for the treatment of highly hydrophobic compounds such as CH_4. In this context, a study was conducted to maximize the mass transfer in a biotrickling filter by optimizing the liquid recycling rates and implementing an innovative gas recycling strategy. Under certain conditions, internal gas recycling can theoretically improve the energy use efficiency of a biotrickling filter by 50%. The implementation of internal gas recycling favored CH_4 abatement in the early stages of the operation and supported stable ECs above 30 g m^{-3} h^{-1} at an empty bed residence time of 4 min and a liquid recycling velocity of 5 m h^{-1}, which represented the highest CH_4 ECs reported to date in single phase biotrickling filter. However, additional mass transfer limitations from the liquid phase to the biofilm were identified, which were attributed to an excess of biomass accumulation in the packing material.

Finally, a proof of concept study on the applicability of bioactive latex coatings to air pollution control in order to maximize the biodegradation of VOCs was performed. Bioactive latex coatings showed toluene specific ECs 10 times higher than those supported by artificial water-based biofilms, overcoming the strong mass transfer limitations encountered in conventional biofilms probably due to the absence of water over or in the biofilm. These promising results may entail important enhancements in the performance of biotechnologies for air pollution control.

List of publications

The following publications are presented as part of the present thesis. Five of them are published in international journals indexed in ISI web of Knowledge (Papers I to V). Paper VI has been submitted for publication and Paper VII is an unpublished manuscript.

Paper I. Estrada J.M, Kraakman B, Muñoz R, Lebrero R (2011) *A comparative analysis of odour treatment technologies in wastewater treatment plants*. Environ. Sci & Technol. 45: 1100-1106.

Paper II. Estrada J.M, Kraakman N.J.R, Lebrero R, Muñoz R (2012) *A sensitivity Analysis of Process Design Parameters, Commodity Prices and Robustness on the Economics of Odour Abatement Technologies*. Biotechnology Adv. 30:1354-1363.

Paper III. Estrada J.M, Rodriguez E, Quijano G, Muñoz R (2012) *Influence of gaseous VOC concentration on the structure of microbial communities and their macroscopic biodegradation performance*. Bioprocess and Biosystems Engineering 35:1477-1488

Paper IV. Estrada J.M, Hernández S, Muñoz R, Revah S (2013) *A comparative study of fungal and bacterial biofiltration treating a VOC mixture.* Journal of Hazardous Materials. 250-251: 190-197.

Paper V. Estrada J.M, Quijano G, Lebrero R, Muñoz R (2013) *Step-feed biofiltration: a low cost alternative configuration for off-gas treatment.* Water Research. 47: 4312-4321.

Paper VI. Estrada J.M, Lebrero R, Quijano G, Pérez R, Figueroa I, García-Encina P.A, Munoz R (2014) *Methane abatement in a gas-recycling biotrickling filter: evaluating innovative operational strategies to overcome mass transfer limitations.* Chemical Engineering Journal, submitted for publication.

Paper VII. Estrada J.M, Bernal, O.I, Flickinger M.C, Muñoz R, Deshusses M (2014) *Biocatalytic Coatings for Air Pollution Control: a proof of concept study on enhancing the rate of VOC Biodegradation*. Unpublished manuscript.

Contribution to the papers included in the thesis

Paper I. In this work I was responsible for information gathering, calculations and data analysis in collaboration with Dr. Raquel Lebrero and MEng. Bart Kraakman and under the supervision of Dr. Raúl Muñoz. I was also in charge of the manuscript writing under the supervision of Dr. Raquel Lebrero and Dr. Raúl Muñoz.

Paper II. In this work I was responsible for gathering information, calculations and data analysis in collaboration with Dr. Raquel Lebrero and MEng. Bart Kraakman and under the supervision of Dr. Raúl Muñoz. I was in charge of the manuscript writing under the supervision of Dr. Raquel Lebrero and Dr. Raúl Muñoz.

Paper III. During the execution of this work I was responsible of the design, start-up and operation of the experimental set-up, results evaluation and manuscript writing with the collaboration of Dr. Guillermo Quijano and under the supervision of Dr. Raúl Muñoz. Dr. Elisa Rodríguez was responsible of the microbiological characterization, where I contributed in the data analysis and discussion.

Paper IV. In this work I was responsible for the design, start-up and operation of the experimental set-up and results evaluation in collaboration with MSc. Sergio Hernández. I prepared the manuscript under the supervision of Dr. Raúl Muñoz and Dr. Sergio Revah. This work was carried out in the Department of Process and Hydraulics, Universidad Autónoma Metropolitana, México City (México).

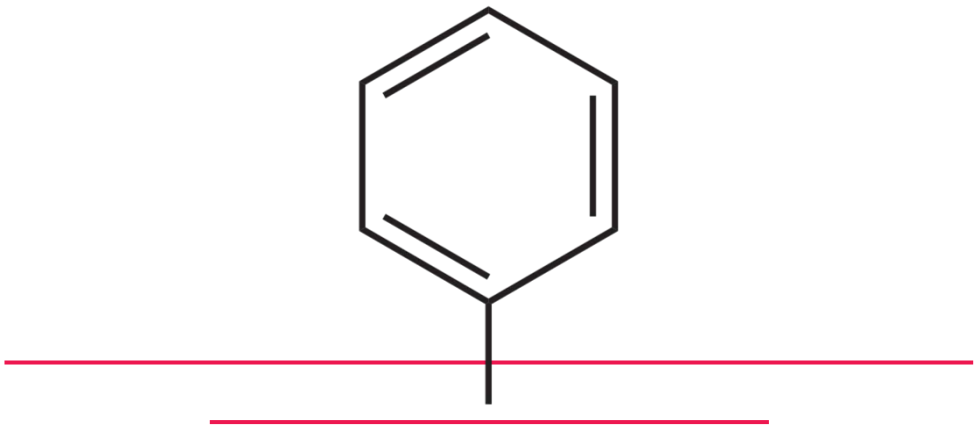
Paper V. In this work I was responsible for the design, start-up and operation of the experimental set-up and results evaluation with the collaboration of Dr. Guillermo Quijano and Dr. Raquel Lebrero and under the supervision of Dr. Raúl Muñoz. I prepared the manuscript under the supervision of Dr. Raquel Lebrero and Dr. Raúl Muñoz.

Paper VI. During this research I was in charge of the design, start-up and operation of the experimental set-up and results evaluation in collaboration with Dr. Guillermo Quijano and Dr. Raquel Lebrero and under the supervision of Dr. Raúl Muñoz. I prepared the manuscript under the supervision of Dr. Raquel Lebrero and Dr. Raúl Muñoz. Dr. Rebeca Pérez, Dr. Ivonne Figueroa and Dr. Pedro García-Encina were responsible of the microbiological analysis, where I contributed in the discussion section.

Paper VII. In this work I was responsible for the experimental methodology design, implementation and results evaluation under the supervision of Dr. Marc Deshusses and with the collaboration of MEng. Óscar Bernal and Dr. Michael Flickinger. I prepared the manuscript under the supervision of Dr. Marc Deshusses and Dr. Raúl Muñoz. This work was carried out in the Civil and Environmental Engineering Department, Duke University, Durham, NC (USA) in collaboration with the Department of Chemical and Biomolecular Engineering, North Carolina State University, Raleigh, NC (USA).

Introduction

Chapter 1



1.1 A brief introduction to anthropogenic air pollution

Atmospheric pollution is not a recent environmental problem in our society despite receiving traditionally less attention compared to other forms of pollution such as soil or water contamination. It was in the 19th century, with the consolidation of the industrial revolution, when our society started to be aware of the real extent of anthropogenic atmospheric pollution. The most important (and visible) impact at the time was caused by the smoke and particulate matter released to the atmosphere from coal burning for steam generation, furnaces and home heating. Smoke abatement was firstly addressed in Great Britain as a Health Agency responsibility [1]. In the US, air pollution was considered a municipal issue, with the first regulations and ordinances limiting emissions of black smoke and ashes, together with the first prevention concepts, dating back to the 1880s [2]. Some technologies applied to air pollution control more than 100 years ago, such as scrubbers to remove acid gases, cyclones and bag dust collectors, are still in use nowadays.

In December 1952, the city of London suffered a major air pollution episode with sulphurous and particulate matter oxidant fog covering the city area for four days, a phenomenon known as smog. At that time, 4,000 deaths were associated to this episode but subsequent research increased this figure to 12,000 deceases considering the event and the two following months. The "London Big Smoke" is often regarded as a turning point in the way air pollution was regarded all over the world [3, 4]. Cities like Los Angeles also suffered from severe photochemical smog pollution episodes, a problem which extended to many cities all over the US. The serious air pollution problems experienced by most of the big cities in Europe, Japan, Australia and New Zealand between 1950 and 1980 gradually resulted in an increased public concern and the development of important research on atmospheric pollution [1].

Along the 20th century, the replacement of coal and oil by gas in housing heating and the technological advances in electric power generation plants significantly improved the perceived air quality, with a decrease in the number of "smoke" episodes recorded in big cities. Unfortunately, other local atmospheric pollution problems arose with the massive popularization of motor vehicles [1, 5].

In the decades of 1970 and 1980, new atmospheric problems such as stratospheric ozone layer depletion and acid rain emerged. Ozone layer depletion was caused by the release of man-made chlorofluorocarbon compounds (CFCs) to the atmosphere [6]. Those compounds react and destroy the stratospheric ozone that protects the Earth from the harmful wavelengths of solar UV radiation. Legislative control efforts to limit the use of CFCs significantly reduced their global production by >80%, and their negative effects on the ozone layer are expected to be reversed during this 21st century [6, 7]. On the other hand, acid rain was mainly attributed to the emission of sulphur and nitrogen oxides from fossil fuel combustion and represented a trans-border pollution that demanded immediate and integral response from the international community [8]. Environmental policies implemented in Europe and the US have been proven to be successful tools to reduce the SO_2 and NO_x emissions, limiting the occurrence of acid rain episodes besides reducing the emissions of particulate matter [9, 10]. However, acid rain is still a major problem in countries such as China [11].

Nowadays, in the second decade of the 21st century, air pollution control faces new problems and challenges. In this context, the specialized Cancer Agency of the World Health Organization (WHO) classified in November 2013 outdoors air pollution as a human carcinogenic for lung and bladder cancer, being responsible for the death of 223.000 lung cancer patients in 2010 [12]. A single approach to control atmospheric pollution within the fast-developing society we live in, driven by globalization and exponential economic growth, will probably not be enough to tackle worldwide environmental issues. Hence, a combination of technology, social habit modifications and enforcement of stricter environmental legislations will be necessary to deal with air quality problems from a holistic sustainable approach.

1.2 Air pollution in the 21st century

1.2.1 Volatile Organic Compounds (VOCs)

VOCs include a wide range of organic chemicals which, due to their high volatility, are generally emitted as gaseous pollutants. Non-methane hydrocarbons (NMHC), oxygenated NMHC and sometimes BTEX are referred to as VOCs (including organic

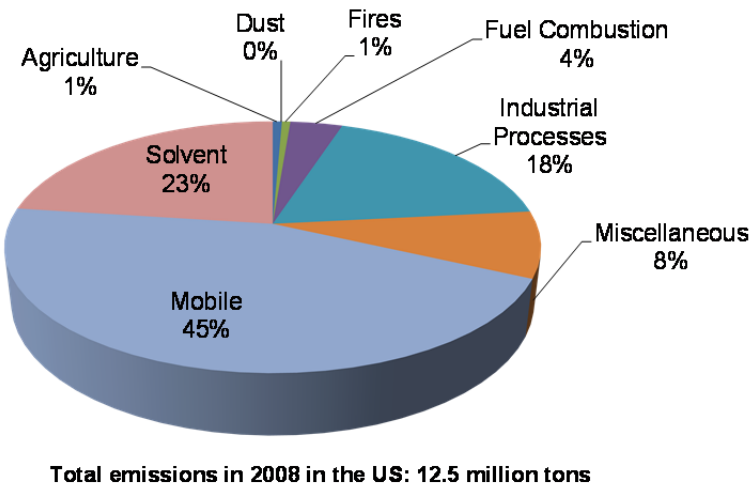


Figure 1.1 Distribution of anthropogenic VOC emissions by source in the United States in 2008. Source: US Environment Protection Agency (EPA).

acids, aldehydes, alcohols or aromatics among others) [13]. These compounds can be classified according to their boiling point (Tb) range as very volatile VOCs (Tb <0 to 50-100ºC), medium volatility VOCs (Tb = 50-100 to 240-260ºC) and semi volatile VOCs (Tb = 240-260 to 380-400) [14]. Despite some VOCs such as terpenes are naturally released to the atmosphere by plants, nature being the largest contributor to VOC emissions worldwide, the work conducted in the present thesis focused on anthropogenic sources [15]. Anthropogenic emissions occur mainly in industrial densely populated areas, where natural emissions are low, thus causing a significant impact on the local urban atmosphere [13].

Sources of VOCs can be stationary and mobile, the latter being the most important source of anthropogenic VOC emissions and mainly caused by on-road emissions from vehicles [16, 17]. Among stationary sources, solvent storage or transport and industrial processes are the main contributors to VOC releases (Figure 1.1) [17, 18]. Data collected in the US and Europe show a similar contribution of the different VOC sources, despite the difficulties found when elaborating reliable and unbiased emission inventories [18]. In 2011, 12.5 and 7.1 million ton of VOC were emitted to the atmosphere in the US and the EU-27, respectively [17, 19].

VOCs cause numerous direct harmful effects on human health: irritation, headaches, damage to liver, kidney and central nervous system, etc. Some specific VOCs can cause

cancer on animals and humans. These effects are mediated by the high concentrations of these pollutants sometimes present in indoor air environments, which are usually 2 to 5 times higher than those found outdoors [14]. The main problem associated to VOC emissions outdoors is their contribution to the formation of tropospheric ozone in the presence of nitrogen oxides and UV solar radiation, which is a main precursor of the phenomenon of photochemical smog [13]. Ozone is a strong photochemical oxidant which, at high concentrations, causes severe health problems and damage to materials and vegetation (including crops) [20]. Recent studies have proven the direct relation between its tropospheric levels and the emission of VOCs and NO_x [21].

Toluene, a natural component of crude oil mainly obtained at industrial scale from oil reforming, was employed as a reference VOC throughout this thesis. Its principal uses are benzene production (50%), gasoline blending (34%), process solvent (5%) and other chemical applications [22, 23]. Most of the emissions occurring during toluene production arise from loading operations, storage and equipment leaks. The amount of toluene emitted in production sites to the atmosphere can reach up to 7 t day^{-1} according to monitoring surveys carried out in Europe. Uncontrolled fugitive emissions from leaks account for up to 90% of those emissions. On the other hand, the main toluene emissions associated to its use derive from benzene and other derivatives production, paint and ink manufacturing. Significant toluene concentrations have also been found in emissions from vehicle manufacturing (mainly associated to coating processes) and furniture manufacturing (Figure 1.2) [22, 24]. In 2000, estimates reported total continental Europe emissions of 1090 t day^{-1} including production and downstream use of toluene [23, 25].

Countries in the United Nations Economic Commission for Europe (UNECE) region agreed by the Gothenburg protocol in 1999 to reduce by 2020 their non-methane VOC emissions by more than a 50% from the emission levels of 1990, which brought up the need to reduce and treat VOC emissions worldwide [26].

1.2.2 Odours

The term odour usually refers to the perception associated to a stimulus of the olfactory cells caused by specific molecules. The physiological response to the olfactory

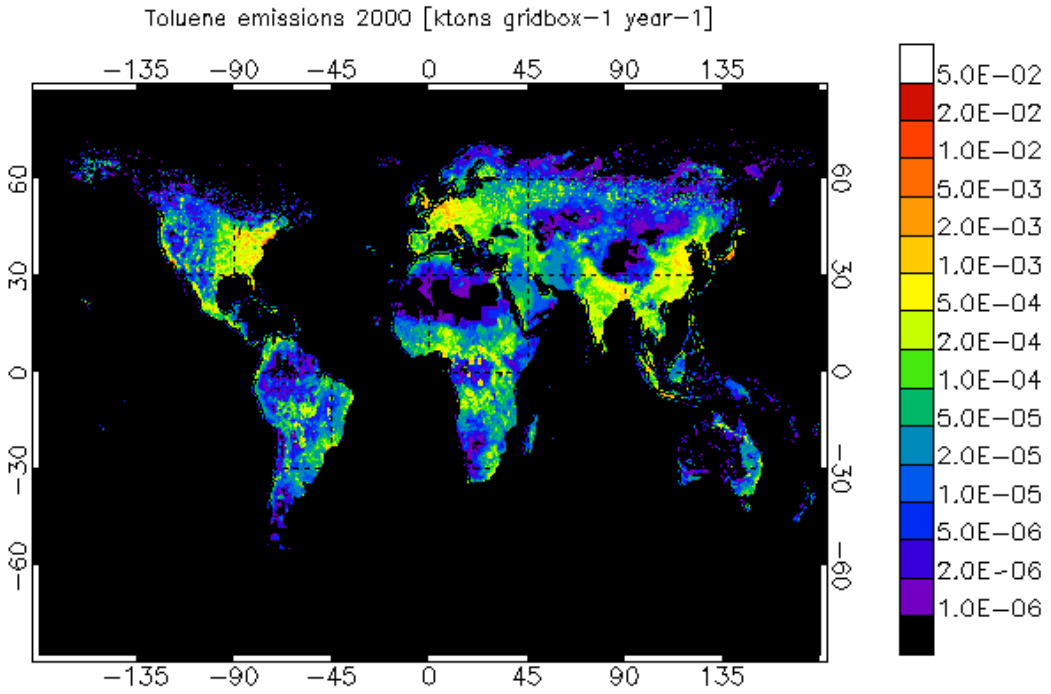


Figure 1.2 Worldwide toluene emissions in the year 2000. Source: RETRO project - REanalysis of the TROpospheric chemical composition over the past 40 years.

stimulus is highly variable among individuals (age, health or habits) and depends on many conditions such as temperature, pressure or humidity, which renders odour pollution a complex problem in terms of characterization and management. [27].

Based on the previous definition, odorants include a wide range of VOCs and volatile inorganic compounds (VICs) [28]. Most odorous emissions are complex mixtures of those compounds at trace level concentrations (ppm or ppb), which cause nuisance due to their low odour thresholds (Table 1.1) [29]. Malodorous emissions usually contain amines, aldehydes, ketones, alcohols, volatile fatty acids, hydrocarbons, ammonia and sulphur compounds (H_2S, methyl mercaptan or dimethyl sulphide). The activities originating unpleasant odours are included in sectors such as industry (chemical industry, fertilizer production, oil and gas refineries, etc.), agriculture and food production (livestock breeding, slaughterhouses, oil and wine industry, etc.) and waste management (waste treatment and recycling plants, composting plants, wastewater treatment, etc.) [30]. A complete characterization of the emission must include the emission flow rates, its composition and its sensorial properties [27].

Table 1.1. Some of the most important malodorous compounds and their olfactory detection threshold (Adapted from Muñoz 2010).

Compound	Detection threshold (ppm)
H_2S	0.0005
Dimethyl Sulphide	0.001
Dimethyl Disulphide	0.000026
Ammonia	0.038
Indole	0.0001
Trimethyl amine	0.0004
Propionic acid	0.028
Butyric acid	0.0003
Butanone	0.25
Acetaldehyde	0.0001
Toluene	2.1
Phenol	46

Odorous emissions have traditionally played a secondary role among environmental concerns, however, the long term exposure to malodours can cause nausea, headache, insomnia, loss of appetite, anger or depression [29, 31]. Despite not being a direct threat to human health, odours strongly impact the perception of the quality of life. The effects of an odorous emission depend on its hedonic tone, the intensity and frequency of the odour exposure, as well as on the sensitivity of the receptors: rural or heavily industrialized areas are considered "low sensitivity" areas, while residential, commercial or recreational areas are considered "high sensitivity" areas [30]. From an economic viewpoint, a recent study showed how housing location within one mile from a malodour source could reduce housing price up to 15% [32].

In the last decades, the encroachment of residential areas on odour sources has led to an increase in the number of odour-related public complaints, which has triggered the development of new and more stringent odour regulations [33]. In the US, more than one half of the total air quality complaints are associated to exposure to odours [34]. In Europe, odour annoyance affects between 13 and 20% of the population, and in some

Spanish cities like Madrid or Barcelona, even one quarter of the population is affected [35]. The enforcement of odour regulations faces several problems mainly related to the variable specific properties of these emissions and the subjective nature of their effects. However, countries such as Germany, the Netherlands, Denmark, Australia, New Zealand or Canada have already implemented odour regulations and policies. In most cases, these regulations consider not only the emission, but also its real nuisance impact on the surrounding population [33].

The development of new odour-related regulations is expected in many other countries, which will make malodours a mandatory issue to be considered in any industrial process, waste management activity or other potential odour source. In addition, companies are increasingly concerned about their public image and aware of the social benefits of being perceived as clean by their potential users or customers. All these factors have led to the need for odour management and treatment in a sustainable way, this need being expected to increase in the short to medium term [36].

1.2.3 Non-CO_2 Greenhouse Gases (GHGs): CH_4 and N_2O

Nowadays, CH_4 and N_2O represent 14% of the total GHG emission burden to the atmosphere and their release is expected to increase according to the latest forecasts. These gases are known to be key contributors to global warming with a potential which is 23 and 300 times higher than that of CO_2 for CH_4 and N_2O, respectively [37]. This high potential results from their chemical stability, leading to lifetimes in the atmosphere of 9.1 years for CH_4 and 131 years for N_2O. In addition, N_2O is considered the most important ozone depleting substance emitted to the atmosphere in the 21st century [37-39].

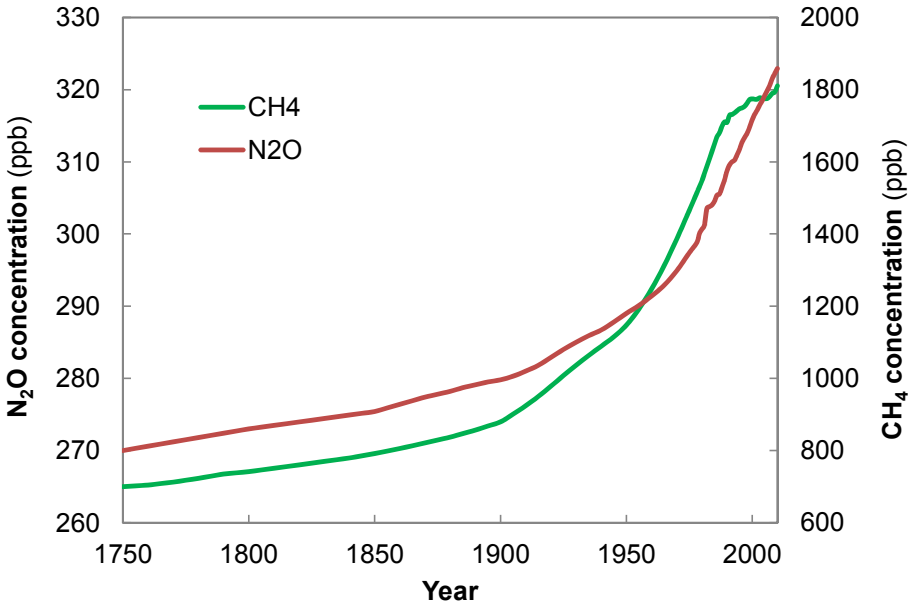


Figure 1.3 Time evolution of the global N_2O and CH_4 atmospheric concentrations between 1750 and 2011. Source: European Environmental Agency (EEA).

More than 60% of CH_4 emissions worldwide are due to human activities, including industry (natural gas and oil handling and storage, oil refining), cattle or agriculture (Intensive livestock, manure management, etc.) and waste management activities (wastewater, landfilling) [39]. In 2011, CH_4 emissions exceeded 580 and 380 million t CO_2-eq in the US and the EU-27, respectively, despite these emissions have followed a decreasing trend since 1990 [39, 40]. Atmospheric CH_4 concentration in 2011 exceeded pre-industrial values by 150% and after a period of relative stabilization between 1999 and 2006, its global concentration has started to increase again since 2007 (Figure 1.3) [38]. Emissions not suitable for energy recovery (under 30% CH_4 content) or treatment by incineration (under 20% CH_4 content) are difficult to treat and sometimes directly released to the atmosphere. Unfortunately, more than 50% of the anthropogenic emissions contain less than 3% of CH_4 [41, 42].

On the other hand, human activities are responsible for 40% of N_2O emissions worldwide, with agriculture (soil management by addition of synthetic fertilizers, livestock farming), transportation (motor vehicles fuel combustion) and industry (nitric acid production) as the main contributors [39]. The N_2O emission potential from wastewater (or waste) treatment is nowadays a key topic of debate and research [43].

In 2011, N_2O emissions accounted for 335 million t CO_2-eq in the EU-27 and more than 350 million t CO_2-eq in the US. N_2O concentration continues to increase in the atmosphere and in 2011 it exceeded pre-industrial levels by more than 20% (Figure 1.3) [44]. Despite not being directly studied in the present thesis, N_2O abatement presents a similar problematic to that of CH_4 due to its poor solubility in water and analogous emission sources.

According to most recent studies, global warming and climate change caused by the increase in GHG concentration in the atmosphere is unequivocal. The atmosphere and oceans have warmed, the Earth's ice content has decreased and the sea level has risen. The effects of this global atmospheric problem have already started to be perceived with increased frequency on extreme climatic events: higher frequency of hot days, heat waves, heavy precipitation events and increased incidence and intensity of extreme high sea level events [44]. These facts have been attributed to human contribution with at least *medium level of confidence*[1]. All these effects will *likely*[2] continue to further increase in the 21st century [44]. Even in the lowest emission scenario, temperatures worldwide are expected to continue increasing up to year 2100 [45]. Climate change is nowadays one of the greatest environmental concerns all over the world and governments in the developed countries are gradually implementing policies in order to limit its impacts. In this context, Europe is expecting a significant decrease in all its non-CO_2 GHG emissions during the next 20 years according to the application of current and future policies (Figure 1.4) [46]. In order to comply with the Kyoto Protocol or any other future International agreements, an active abatement of GHG emissions is mandatory and expected to become a general trend worldwide in the next decades.

[1] The IPCC 2013 Summary for Policymakers establishes a level of confidence to describe the available evidences of climate change, which is expressed using five qualifiers: very low, low, medium, high, and very high.

[2] In the IPCC 2013 Summary for Policymakers, the following terms are used to indicate the assessed likelihood of an outcome or a result: virtually certain 99–100% probability, very likely 90–100%, likely 66–100%, about as likely as not 33–66%, unlikely 0–33%, very unlikely 0–10%, exceptionally unlikely 0–1%.

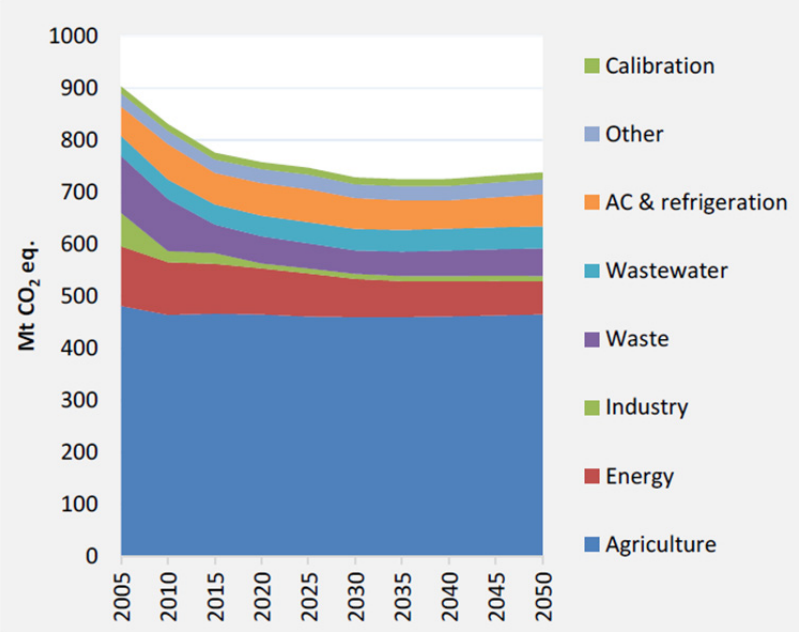


Figure 1.4 Non-CO_2 GHG emissions predicted in the EU-27 to 2050 by major sector. Source: European Commission, Directorate-General for Energy, Directorate-General for Climate Action and Directorate-General for Mobility and Transport.

1.3 End-of-the-pipe technologies for air pollution control[3]

End-of-the-pipe technologies for air pollution control are based on pollutant transfer from the gas emission towards a solid (adsorption) or a liquid phase (absorption), most of the times followed by a chemical or biological oxidation of the pollutant. In technologies such as incineration or gas-phase ozonation, the destruction of the pollutant takes place directly in the gas phase. Most of these techniques are not applicable for diffuse or mobile sources of pollution but can be employed in a wide range of waste gas streams: VOC emissions from solvent storage and industrial processes, malodourous emissions from the agricultural, food industry and waste management sectors and most of the non-CO_2 GHG emissions from farming, industry and waste management. As long as the emission is confined and a gas extraction system is implemented, the resulting polluted stream can be treated. End-of-the-pipe air pollution control technologies are nowadays classified in physical/chemical and

[3] Information in this section has been adapted from: Estrada J.M., Lebrero R., Quijano G., Kraakman N.J.R. and Muñoz R. Strategies for odour control (2012), in Odour Impact Assessment Handbook, pp. 85-124, John Wiley & Sons, Inc. [47].

biological [33]. The most important techniques belonging to each group are presented in this section.

1.3.1 Physical/chemical technologies

Chemical Scrubbers

Chemical scrubbers are based on the transfer of pollutants from the gas phase to an aqueous phase containing a chemical oxidant, where they react to be destroyed (Figure 1.5) [33]. In this technology, pollutants face a mass transfer resistance to be dissolved in the aqueous phase (and consequently to be oxidized). This resistance increases with the hydrophobicity of the compound being treated, often becoming the limiting step of chemical scrubbing performance. The mass transfer rate will be determined by the pollutant concentration in the gas phase and in the aqueous solution, its partition coefficient air/water (Henry's Law constant), the mass transfer resistance at the air/water interphase and the interfacial area available for transfer [47]. Chemical scrubbers present high removal efficiencies of up to 99.0% for low hydrophobicity gas pollutants (Henry's Law constant lower than 0.07 M atm^{-1}) such as H_2S, while the removal of highly hydrophobic compounds such as terpenes or hydrocarbons (Henry's Law constant higher than 20 M atm^{-1}) can be as low as 50%, thus limiting the implementation of this technology in certain applications [48].

Counter-current packed towers (gas in upwards flow, liquid in downwards flow) are the most common configuration (Figure 1.5), but co-flow or cross-flow scrubbers can also be found [49]. One or two-stage systems are also implemented depending on the nature of the emission and the degree of abatement needed. Another type of chemical scrubbers are mist chambers, where the oxidizing liquid solution is atomized (droplet size around 10 μm) in the polluted gas emission, which horizontally flows across the chamber. Although this configuration achieves higher removal efficiencies than packed towers due to the higher mass transfer area available, it is less commonly implemented since it requires complex maintenance and more space for its installation [50].

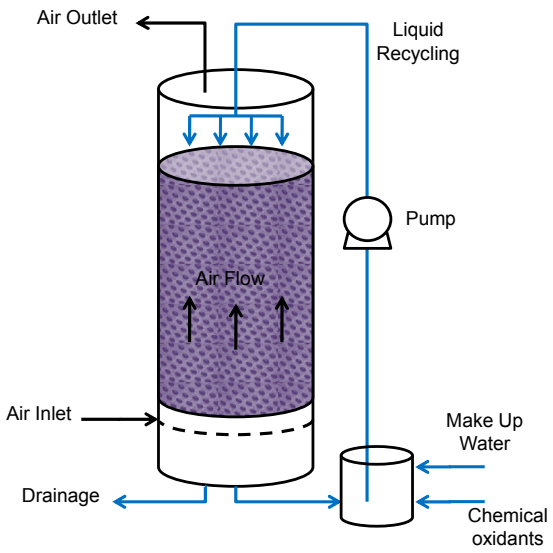


Figure 1.5 Schematic design of a chemical scrubber in counter-current configuration (Adapted from Estrada et al. 2012 [48].)

Chemical scrubbers have been traditionally employed in a wide range of gas treatment applications such as NO_x emission control [51], VOC and malodourous emissions abatement [47] or biogas upgrading [52].

Activated carbon adsorption

Adsorption is the process where pollutant molecules are removed from the gas emission by physically bonding with the surface of a solid adsorbent by weak intermolecular forces [47]. If the adsorbed compounds are stable and poorly reactive, they will remain trapped in the solid adsorbent. However, if the pollutants are reactive, they may chemically react with other compounds present in the system. For instance, reduced sulphur compounds are easily oxidized in presence of atmospheric oxygen when adsorbed into activated carbon. Adsorption systems usually consist of at least two towers that work alternatively in order to allow a continuous process operation: while adsorption is taking place in one of the towers, the saturated packing material is being regenerated in the other one (desorption of the adsorbed pollutants by temperature increase, pressure decrease, or carbon washing) [47, 49]. At the end of the carbon packing lifespan or when no regeneration of the activated carbon is possible or programmed, one of the towers will be in operation while the packing material is substituted in the other one [48].

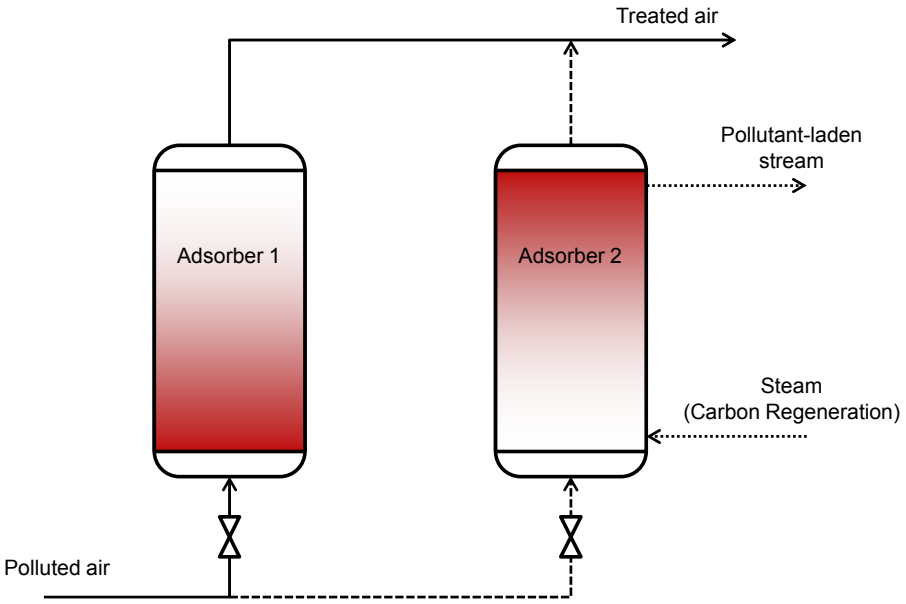


Figure 1.6 Schematic design of an activated carbon adsorption system equipped with two beds for continuous adsorption/steam regeneration (Adapted from Estrada et al. 2014 [54].)

Activated carbons are usually obtained by activation of organic materials such as wood or anthracite coal at high temperature. High performance materials like impregnated activated carbons have been recently developed for adsorption, which are available in the market at higher prices and provide increased removal efficiencies for specific applications. In impregnated activated carbons, a chemical reagent (such as NaOH, KOH, urea or $KMnO_4$) or a catalyst (heavy metals) is impregnated onto the surface of the activated carbon to adsorb a certain family of compounds or accelerate chemical reactions, respectively [53]. Although impregnation usually improves the removal of specific compounds, it does not always represent an advantage due to the notorious decrease of the available surface for adsorption and the reduction of the auto-ignition temperature of the material, increasing the risk of ignition during its storage in the presence of air.

Adsorption does not require the transfer of odorants to an aqueous phase, and the high affinity of the adsorbent for hydrophobic compounds allows achieving the highest efficiency for highly hydrophobic pollutants among gas treatment technologies (up to 99.9% for impregnated activated carbon). The adsorption capacity of a bed depends on several factors: the type of bed, the pollutant concentration in the gas

emission, the operation temperature and humidity, and the composition of the gas emission to be treated [48]. Activated carbon adsorption has been intensively applied in the field of odour treatment due to the low pollutant concentrations found in malodourous emissions, which entails reasonably high carbon bed lifespans. Adsorption can be also used in combination with another abatement technique, leading to hybrid technologies where the adsorption bed acts as a polishing step, which supports higher pollutant removal efficiencies. The combination of adsorption with chemical scrubbers or with biological techniques has been reported [54].

Incineration

Incineration is based on the complete oxidation of gas pollutants at high temperatures in the presence of air to form mainly CO_2, SO_2 and H_2O. It is a common technology for the control of industrial effluents where VOCs are present at high concentrations, including hazardous wastes. The performance of this method is strongly influenced by the flowrate of the emission and its pollutant concentration [47, 48].

Flares can be devised as the most simple incinerator configuration, where an open flame and the atmospheric air mediate pollutant oxidation. They are widely employed as emergency control systems but they can be also used as a regular VOC abatement method. For instance, flares are employed as the main method for VOC emission control in oil refining or in some chemical industries [47]. As mentioned earlier, flares are also a very common technique to treat CH_4 emissions not suitable for energy recovery [41].

Thermal incinerators typically operate heating the air emission up to 650-800ºC and mixing it with air in a combustion chamber for pollutant oxidation to occur. Depending on the concentration and the nature of the pollutants in the emission, an auxiliary fuel is often needed in order to maintain the combustion when the oxidation reaction cannot be self-maintained [47]. Incinerators are typically equipped with pre heaters using the hot combustion gases to heat the inlet polluted emission, thus reducing energy needs. More complex systems such as regenerative thermal incinerators include high efficiency ceramic beds which accumulate heat and allow to

recover 95% of the combustion energy [55]. Another alternative available is catalytic incineration, where the pre-heated pollutants are completely oxidized at moderate temperatures (250-500 ºC) by flowing through a solid catalyst bed. Typical catalysts are metals such as palladium or platinum coated on inert support materials [47]. The reduction in the oxidation temperature allows to decrease fuel needs and also reduces the construction material specifications in the incinerator [35]. Incineration technologies are applied in the control of gaseous VOC emissions in industrial processes such as automotive, paints, plastics, chemical and electronics industries [55].

1.3.2 Biological technologies

Biological techniques for air pollution control are based on the enzymatic oxidation of pollutants following their transfer from the gas emission to an aqueous phase, and then to the microorganisms responsible for this enzymatic oxidation. These oxidation mechanisms occur at ambient temperature and pressure, and in the absence of an external supply of chemicals (only water and nutrients). The absence of extreme operating conditions and hazardous chemicals constitutes an additional advantage from a safety viewpoint for on-site staff and operators.

The biological oxidation of organic pollutants is based on their use as a source of carbon and/or energy by microorganisms in a bioreactor [56]. Microorganisms can also use inorganic pollutants such as H_2S or NH_3 as an energy source to sustain cell maintenance and/or growth based on the assimilation of inorganic carbon from the environment. There are two main requirements for these processes to occur: the presence of an aqueous medium to support all the metabolic reactions, and the availability of macronutrients (such as phosphorus, nitrogen, sulphur or potassium) and micronutrients (generally heavy metals needed for enzymatic synthesis) [57]. In 2005, van Groenestijn and Kraakman estimated more than 7,500 biological waste gas treatment systems operating in Europe [58]. The most applied bioreactor configurations for biological air pollution control are discussed in the present section.

Biofiltration

Biofiltration is based on the biodegradation of pollutants by a microbial community attached onto a fixed packing material in the presence of a stagnant water layer surrounding the biofilm. The pollutant-laden air stream is forced through this packed bed that hosts the microorganisms forming a biofilm, while water is periodically supplied for moisture maintenance (Figure 1.7) [33]. Hence, pollutants must diffuse from the gas emission to the biofilm where degradation takes place, facing a mass transfer resistance to be transported first to the aqueous phase surrounding the biofilm and then into the biofilm itself. Common removal efficiencies reported in biofilters range from 75% for highly hydrophobic pollutants (Henry's Law constant higher than 20 M atm^{-1}) to 99% for hydrophilic odorants (Henry's Law constant lower than 0.07 M atm^{-1}), these removal efficiencies being similar to those achieved by chemical scrubbers [59].

The selection of biofilter packing media is a key parameter determining both the removal efficiency and the biofilter lifespan. Both inorganic and organic supports are available, the latter providing an extra carbon source to maintain microbial activity when dealing with low carbon concentrations in the gas emission (for instance in odour abatement scenarios) or during starvation periods. Among inorganic materials, ceramics, plastic, lava rock or activated carbon are the most commonly used packings. A recent compilation of data from biofilters at full scale odour abatement facilities revealed that 87% of the biofilters used organic packing materials (either alone or mixed with inorganic media). Among the organic materials, wood chips and compost were the most employed (37 and 33%, respectively) [48].

The main drawback of biofiltration, together with the frequent replacement of the packing media, is the extensive land needed for its installation. The high empty bed residence time requirements (from 20 s to 2 min, depending on the application), and the low packing heights to keep low pressure drops across the media, result in high design areas. Another limitation is the difficult control over operational parameters such as pH, temperature, humidity, nutrients or accumulation of degradation intermediates. However, the vast experience gained for almost 100 years from the first

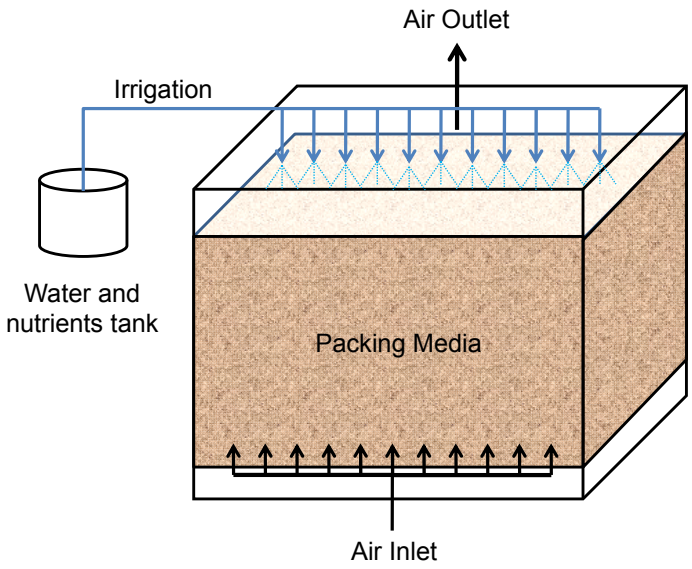


Figure 1.7 Schematic design of a biofilter (Adapted from Estrada et al. 2012 [48].)

full scale applications of biofilters provides an extensive knowledge background of both design and operation parameters, making biofiltration a reliable technology for air pollution control [60]. Biofiltration has been widely employed for the treatment of VOCs from industrial effluents and CH_4 emissions [41, 61]. Biofilters are also the most common biological technology for odour abatement, being intensively used in applications such as wastewater treatment plants (WWTPs) or composting facilities. For instance more than 300 biofilters operated in 2005 in WWTPs in the US [62].

Biotrickling filtration

A biotrickling filter consists of a column packed with an inert packing material (usually plastic rings, resins, ceramic material, rock, etc.) where microorganisms grow attached. The main difference with biofilters is the presence of a nutrient solution continuously recycling through the bed at rates typically ranging from 10-30 l min^{-1} $(m^3_{bed})^{-1}$ (Figure 1.8). These systems present high specific surface areas (between 100 and 400 $m^2\ m^{-3}$), which allow for an improved mass transfer in lower bed heights and thus entail low pressure drops (often ranging from 100-400 Pa m_{bed}^{-1}) [56]. Pollutants are initially absorbed in the aqueous film trickling over the biofilm and degraded afterwards by the microorganisms present in the biofilm. The high gas-liquid contact surfaces provided by the packed bed allow the operation at lower empty bed residence times (EBRTs) than in biofilters. Nevertheless, pollutant mass transfer to the aqueous

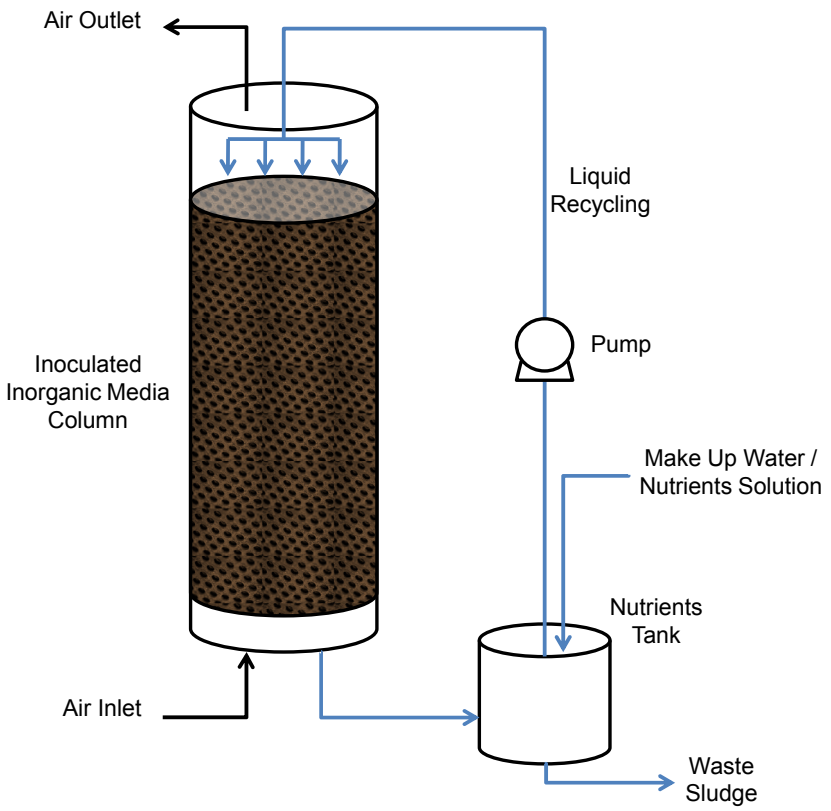


Figure 1.8 Schematic design of a biotrickling filter (Adapted from Estrada et al. 2012 [48].)

phase constitutes the main limitation for the removal of highly hydrophobic compounds, whose removal efficiencies account for ≈ 50%. The removal efficiencies reported for water soluble pollutants such as H_2S increase up to 99% [63]. Indeed, H_2S removals of ≈100% have been reported in biotrickling filters operated at EBRTs similar to those applied in physical/chemical technologies (1.6-2.2s) [64, 65].

The main advantage of biotrickling filtration systems over conventional biofilters is the ability to control key operational variables such as moisture content, pH, temperature and nutrients concentration. In addition, biotrickling filters allow the continuous wash-out of harmful bioreaction products from the system. Byproduct accumulation in biotechnologies mediates media acidification and microbial toxicity, problems that significantly reduce the lifespan of the packing material and biological activity in standard biofilters. Despite the recent advances in their design and operation, biotrickling filters are not as implemented as biofilters nowadays [48]. However, the number of industrial applications and research studies on this type of

bioreactor has increased drastically since the 1990s. Common applications include the treatment of VOCs, ammonia, H_2S and malodorous streams [33, 66]. Recently, there has been an increasing interest in the use of biotrickling filters for the treatment of CH_4, sometimes employing non aqueous phases to improve pollutant mass transfer [42, 67].

Hybrid technology (biotrickling filter + activated carbon adsorption)

Despite this technology has been particularly employed in odour abatement applications, it could be applied to the treatment of other complex gaseous emissions. It consists of a two-stage system combining a biotrickling filter and an activated carbon tower installed in series. In the first stage, the biotrickling filter efficiently removes most water soluble compounds from the polluted stream, this biological step being responsible for the depletion of the greatest part of the pollutant load. Following this biological stage, an activated carbon adsorption system acts as a polishing step, removing the pollutant fraction that non-treated in the biotrickling filter. This kind of systems provide three major benefits: first, the presence of this additional adsorption system increases the removal efficiency to high public protection levels (over 99.7% for any compound in odorous emissions, regardless of their hydrophobicity), thus overcoming the main limitation of conventional biotrickling filters. Second, the combination of both systems provides an extra robustness, as one technology can back up the other minimizing the effect of any potential malfunctioning in one of the systems. Third, the removal of most of the pollutant load in the biological stage increases the packing lifespan of the activated carbon and therefore reduces the most important cost associated to adsorption processes [35].

Activated sludge diffusion

Activated sludge diffusion systems are innovative technologies for odour control. Malodorous emissions are directly sparged into the aeration tank of a wastewater treatment plant together with the air to satisfy the oxygen needs of the reactor [68] (Figure 1.9). Obviously, this technology is only cost-effective in WWTPs where water treatment is carried out via air diffusion. Otherwise, it would imply the construction of a separate aeration tank and prohibitive operating costs associated to the energy to overcome the pressure of the water column height (4-5 m). Pollutants diffuse from the

gas emission to the aqueous phase together with the oxygen needed for the stabilization of the organic matter of the wastewater and are then destroyed by the microbial community present in the activated sludge [69, 70]. There are not many data available about this innovative technique, however experiences at large scale have reported odour removal efficiencies higher than 99% in WWTPs treating composting odours and H_2S [35, 71]. The application of this technology to other kinds of polluted emissions different from malodours must consider the potential influence of gas pollutant load on the wastewater treatment process.

Despite the lack of information available, it can be assumed that the investment costs of this technology are low because all the equipment required is already present in the wastewater treatment line (even the air compressor system might be used for the dispersion of the malodorous emission). Additional costs can derive from the installation of moisture traps to avoid corrosion problems in the pipeline caused by H_2S. The installation should also include dust and grease aerosols filters and corrosion resistant materials in blowers and air piping. Corrosion is a major issue for this kind of systems, nevertheless, a survey collecting data from 30 WWTPs located in the US where activated sludge diffusion was implemented showed that these concerns might be not well founded. The selection of adequate materials and protection equipment as described earlier can easily mitigate these problems [72].

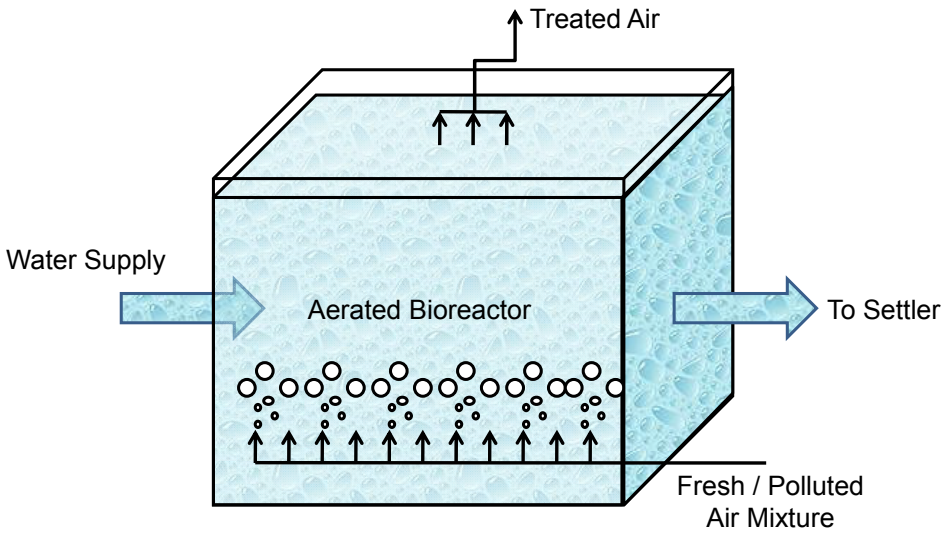


Figure 1.9 Schematic design of an activated sludge diffusion system (Adapted from Estrada et al. 2012 [48].)

1.4 Technology selection criteria

Traditionally, air pollution control technologies have been selected according to the flow rate and pollutant concentration of the emission. These selection criteria were based on previous experience in industrial applications of these technologies (Figure 1.10). However, in a world of increasing competitiveness and environmental awareness, selection criteria must shift from this simplistic economic approach towards a more detailed analysis including the three pillars of the sustainability concept: environmental, economic and social aspects.

In this context, a lack of data from air pollution control techniques in full scale facilities has been identified both at an economical and environmental level. The poor information sharing between private companies and academia is often the cause of discrepancies or inadequate research. A compilation of this kind of data would be extremely valuable in order to develop improved selection criteria and would allow for fairer comparisons by applying new tools such as Life Cycle Assessments or Sustainability evaluations. **Chapter 3** in the present thesis covered this research niche

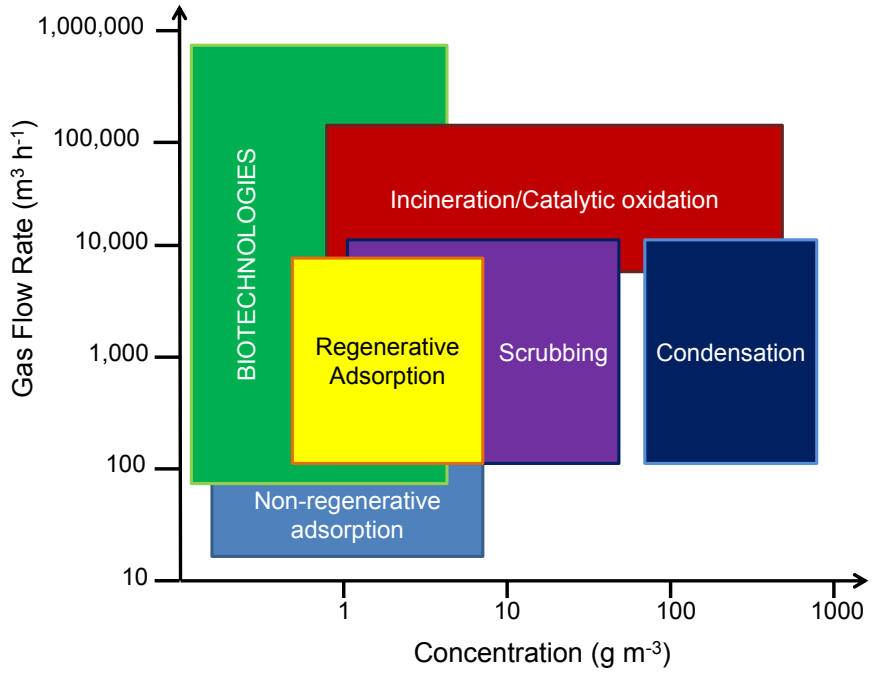


Figure 1.10 Comparative chart for the selection of air pollution control techniques traditionally employed since the 90s (Adapted from Detchanamurthy and Gostomsky, 2012 [61]).

by presenting a comparative evaluation of air pollution control technologies using sustainability metrics under a specific full scale scenario: odour pollution control in WWTPs. This reference scenario was chosen based on the complexity of malodorous emissions in order to perform a complete evaluation with different pollutants and data compiled from different private companies and public administrations in Spain and Australia[4]. Despite being the most difficult category to evaluate, a preliminary social impact assessment of the different technologies evaluated was also carried out taking into account health and safety issues.

The economic data collected were further analyzed in **Chapter 4**. The sensitivity of the process economics against different operational and design parameters was evaluated. In this sense, the economics of the most commonly used air pollution control technologies was assessed under different scenarios including different commodity prices or geographical locations worldwide. Robustness was also identified as a key parameter for technology selection, being here evaluated for the target air pollution control techniques based on field experience of facility operators.

The main implications of the previously cited sustainability and economic studies were discussed in **Chapter 5** along with the identification of the main technological research niches that supported the experimental part of the present thesis.

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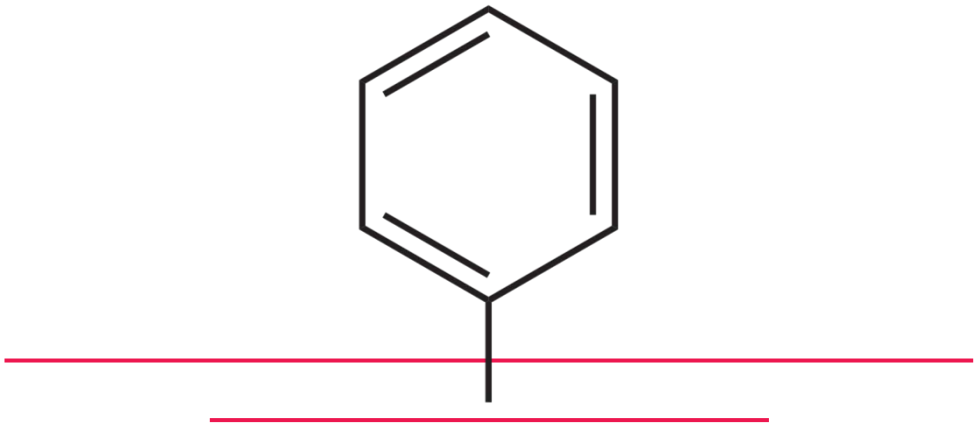
Aims and Scope of the Thesis

Chapter 2



2.1 Justification of the thesis

The severe environmental impact of different forms of anthropogenic air pollution, together with the increasing environmental awareness of our society, has led to the need to treat polluted gaseous effluents in a sustainable way. VOCs, odours and GHGs are responsible of three major air pollution problems worldwide. Biotechnologies have emerged in the past two decades as feasible alternatives to traditional physical/chemical technologies for air pollution control. They exhibit consistent removal efficiencies in most off-gas treatment applications while presenting reduced costs and a better environmental performance than their physical/chemical counterparts. However, there is a lack of comparative assessments based on real full scale data to show the actual potential and limitations of biotechniques. This would allow developing new technology selection guidelines under specific scenarios and identifying the main limitations of biotechnologies. Moreover, experimental research focused on overcoming these limitations is necessary to increase the range of application of biological air pollution treatments.

2.2 Main objectives

The overall objective of the present thesis was to develop innovative design and operational strategies in order to overcome the main limitations of biotechnologies for air pollution control, previously identified by state-of-the art comparative technology studies and prior research in the field. More specifically, individual goals to achieve this overall objective were:

1. Complete sustainability analysis based on data from full scale facilities under a reference odour treatment scenario of the most commonly employed air pollution control techniques: biofiltration, activated sludge diffusion, biotrickling filtration, chemical scrubbing, activated carbon adsorption, regenerative incineration and a hybrid technology (biotrickling filtration coupled with carbon adsorption).
2. In-depth economic analysis of the most commonly employed technologies focusing on the influence of design parameters and commodity prices on the overall costs.

3. Identification of the main advantages and limitations of biotechnologies for air pollution control.
4. Study of the influence of pollutant concentration on the microbial diversity of suitable inocula for air pollution control applications. Study of the implications of inocula on process performance.
5. Comparative experimental assessment of the advantages and disadvantages of fungal and bacterial biofiltration for VOC treatment.
6. Evaluation of a novel configuration to reduce the pressure drop and improve the lifespan of packing materials in traditional biofiltration. Analysis of its energetic and economic implications.
7. Evaluation of alternatives to overcome mass transfer limitations in traditional biotechnologies (mainly biotrickling filters) for the treatment of highly hydrophobic compounds taking into account energetic and economic considerations.

2.3 Development of the thesis

In the present thesis, technologies for air pollution control were analyzed from a theoretical and experimental viewpoint. In the first section, data from full-scale facilities were compiled, compared and analyzed. In the experimental section, the main limitations of biological technologies for air pollution control were addressed.

In order to fulfill the first and second objectives, the most widely employed technologies for air pollution control were comparatively evaluated from a sustainability viewpoint employing the IChemE sustainability metrics (Chapter 3). An effort was made to compile real economic and environmental data from full scale applications provided by private companies and public institutions. Data were further evaluated focusing on process economics and technology robustness (Chapter 4). These two works allowed identifying the strengths and main limitations of biotechnologies (third objective), which supported the basis for the experimental part of the thesis.

Different strategies and experimental set-ups were evaluated to accomplish the fourth objective: suspended growth bioreactors for microbial isolation (Chapter 6) and biofilters inoculated with fungi and activated sludge (Chapter 7). The enrichment of

specialized CH_4-degrading communities was addressed in Chapter 9. Fungal and bacterial biofilter performance (fifth objective) was studied in Chapter 7 by systematically comparing their performance in the abatement of a VOC mixture.

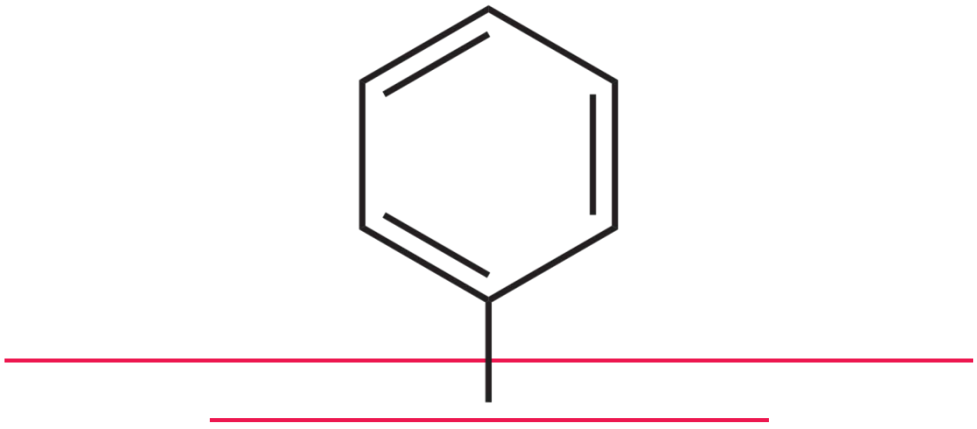
The main limitations of traditional biofiltration (pressure drop buildup due to biomass clogging and limited packing material lifespan) were addressed in Chapter 8. A step-feed design was evaluated to overcome these drawbacks and its energetic and economic implications were discussed.

Finally, the potential of new operational strategies to overcome mass transfer limitations in biotechnologies was studied. In Chapter 9, the performance of an innovative gas recycling strategy implemented in a biotrickling filter devoted to methane abatement was studied, and its economic and energetic implications were discussed. The novel application of bioactive latex coatings for air pollution control was investigated in Chapter 10 as a promising strategy to overcome the mass transfer limitations associated to the water layer covering biofilms in traditional bioreactors.

A comparative analysis of odour treatment technologies in wastewater treatment plants.

Estrada JM, Kraakman NJ, Muñoz R, Lebrero R. Environ Sci Technol. 2011 Feb 1;45(3):1100-6.

Chapter 3



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A Comparative Analysis of Odour Treatment Technologies in Wastewater Treatment Plants

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Biofiltration, activated sludge diffusion, biotrickling filtration, chemical scrubbing, activated carbon adsorption, regenerative incineration, and a hybrid technology (biotrickling filtration coupled with carbon adsorption) are comparatively evaluated in terms of environmental performance, process economics, and social impact by using the IChemE Sustainability Metrics in the context of odor treatment from wastewater treatment plants (WWTP). This comparative analysis showed that physical/chemical technologies presented higher environmental impacts than their biological counterparts in terms of energy, material and reagents consumption, and hazardous-waste production. Among biological techniques, the main impact was caused by the high water consumption to maintain biological activity (although the use of secondary effluent water can reduce both this environmental impact and operating costs), biofiltration additionally exhibiting high land and material requirements. From a process economics viewpoint, technologies with the highest investments presented the lowest operating costs (biofiltration and biotrickling filtration), which suggested that the Net Present Value should be used as selection criterion. In addition, a significant effect of the economy of scale on the investment costs and odorant concentration on operating cost was observed. The social benefits derived from odor abatement were linked to nuisance reductions in the nearby population and improvements in occupational health within the WWTP, with the hybrid technology exhibiting the highest benefits. On the basis of their low environmental impact, high deodorization performance, and low Net Present Value, biotrickling filtration and AS diffusion emerged as the most promising technologies for odor treatment in WWTP.

Introduction

Atmospheric pollution, and more specifically odor pollution, has been traditionally given less priority compared to solid or liquid wastes. However, this situation has changed over the past several years (*1*). With environmental legislations becoming more stringent and residential areas encroaching on odor sources, there is an increasing need for odor management. Malodors not only are a direct threat for human health and welfare but also represent a significant contribution to photochemical smog formation and particulate secondary contaminant emission (*2*). Thus, a cost-effective and environmentally friendly abatement of malodors from agro-industrial sources is crucial in a world increasingly concerned about sustainability and environmental preservation.

Odor treatment technologies can be classified into physical/chemical (chemical scrubbers, incinerators, adsorption systems, and so forth) and biological (biofilters, biotrickling filters, bioscrubbers, and activated sludge diffusion reactors) (*3*−*6*). Physical/chemical technologies have been widely used because of their low empty bed residence time (EBRT), extensive experience in design and operation, and rapid start-up (*7*). On the other hand, biotechnologies have been marketed as low-cost, environmentally friendly odor abatement methods (*8, 9*). Thirty years of intense lab and field R&D have finally resulted in the acceptance of biotechnologies as a robust and reliable alternative to conventional physical/chemical treatment methods (*4, 10, 11*).

All these technologies have been widely reviewed in the literature, and their optimal range of application and performance for industrial volatile organic, inorganic, and sulfur compounds treatment (VOCs, VICs, and VSCs, respectively) has been clearly established on the basis of laboratory and field experiences (*6*). The same criteria are often extrapolated for odor treatment despite the different nature of the emissions (complex mixtures of volatile compounds at trace level concentrations). These selection criteria, mainly driven by process economics, are based on the pollutant air flow and concentration to be treated. For instance, high concentrations of pollutants and high air-flow rates are most cost-efficiently treated by incineration or catalytic oxidation (*6*). However, environmental concerns are gradually shifting these selection criteria, with social and environmental issues becoming as important as process economics (*12, 13*). Despite the merits of this holistic approach, and to the best of our knowledge, no detailed comparative analyses have been published on the existing odor-abatement technologies.

The purpose of this work is to analyze and compare different odor-treatment technologies by using wastewater treatment plants (WWTPs) as model odor sources. Chemical scrubbing (CS), activated carbon (AC) filtration, regenerative incineration (RI), biofiltration (BF), biotrickling filtration (BTF), activated sludge (AS) diffusion, and biotrickling filtration coupled with AC (BTF+AC) were compared in terms of environmental performance, process economics, and social impact in order to provide basic guidelines for technology selection in odor management in WWTPs.

Materials and Methods

Methodology. This comparison was based upon the triple-bottom-line concept, which includes the assessment of environmental performance, social responsibility, and process economics. The IChemE Metrics are based on ratio indicators in order to provide a measure of impact independently of process scale. In this particular study, most ratios refer to the air-flow rate treated (*14*).

Model Malodorous Emission. An emission of 3000 m^3 h^{-1} (293 K, 1 bar, 40% relative humidity) with a composition based upon the characterization of odor pollution from a WWTP located at Stuttgart University was selected as model malodorous emission for the environmental evaluation (*15*).

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Flow-rate selection was arbitrary, but this choice does not influence the main conclusions extracted from the environmental assessment. Methyl mercaptan (1.96 mg m^{-3}) and hydrogen sulphide (20.9 mg m^{-3}) were also included in the above-mentioned model VOC- and VSC-containing emission (*16*). All target compounds present in this malodorous air were classified according to their Henry's law constant into highly (0.07 M atm^{-1}), moderately (0.07 − 20 M atm^{-1}), and poorly (20 M atm^{-1}) hydrophobic (*17*). The economic and social assessment was based on a compilation of real data from full-scale facilities and workshop information. Finally, it must be stressed that this comparative evaluation constitutes a preliminary assessment of odor-abatement technologies focused on constant model emissions, and the influence of fluctuations in odor loading was out of the scope of this publication. All the systems evaluated were based on designs capable of coping with daily and seasonal fluctuations in odor concentration, which often requires the oversizing of the reactor. This oversizing would finally impact both the environmental and economic indicators in a comparable percentage, but it will not significantly modify the main outcomes of this study.

Odor-Abatement Technologies. *Biofilter.* A system packed with a mixture of organic and inorganic material, densities ranging from 300 to 600 kg m^{-3}, and a lifespan of two years was used as model biofilter (*18*). The unit operated at EBRTs ranging from 50 to 70 s with a packed bed height of 1 m and a pressure drop ranging from 1000 to 1500 Pa (including the pressure drop in the humidifier) (*3, 9, 19*). A typical pressure drop of 50 kPa was considered for the liquid nozzle installed for biofilter irrigation.

AS Diffusion Tank. A typical activated sludge aerated tank equipped with fine-bubble diffusers (7000 Pa of pressure drop) located at a depth of 4−5 m was used as a model AS diffusion unit (*3, 19, 20*).

Biotrickling Filter. A two-stage biotrickling filter of 2 m height with an EBRT of 12−16 s was considered in this comparative analysis. The system was packed with inert plastic packing (density of 75−140 kg m^{-3}) with a lifespan of 10 years and a pressure drop of 400−500 Pa (*21*). Liquid nozzles were used for the recirculation of the aqueous medium at 5−8.3 L $m^{-3}_{reactor}$ min^{-1} (0.3−0.5 m h^{-1}). The renewal of the aqueous medium was calculated on the basis of the empirical derived design criteria ratio of 2.5 L/g H_2S removed.

Chemical Scrubber. A two-stage NaOH−NaClO scrubber packed with 2 m of Intalox Saddles (density = 550−650 kg m^{-3}; lifespan = 10 years; pressure drop = 400−500 Pa) and operated at an EBRT of 3−5 s (1.5 to 2.5 s per stage) was used as model scrubber (*19, 22*). The system was operated at a recirculation rate of 180 L m^{-3} min^{-1} (16 m h^{-1}) with a pressure drop of 50 kPa in the liquid nozzles (*7*). The first stage is typically operated at a pH range of 10−12, whereas the second stage is operated at a lower pH (8.5−10). At pH levels above 10, a significant absorption of CO_2 from ambient air occurs (which leads to higher NaOH consumptions), whereas at pH levels below 8.5, the NaClO may revert to free chlorine. Acid washing in chemical scrubbers' media is normally periodically done (typically once a year) to avoid scaling of the media. However, these costs are not included in the economic analysis because they are considered not significant.

AC Filter. A 0.5 m granular impregnated AC bed (density 320−430 kg m^{-3}) operated at an EBRTs ranging from 2 to 3 s and a pressure drop of 1400−1750 Pa was used as model adsorption filter (ECOTEC Ecología Técnica S.A) (*23, 24*). The most common practice in AC filtration involves two filters (one filter in operation and one in standby to allow bed replacement). Bed replacement is based on empirical experience because carbon manufacturers do not often guarantee carbon life in WWTP applications. A standard carbon life of 3−9 months was used for stand-alone applications. No regeneration of the AC was considered.

TABLE 1. Performance of the Target Treatment Technologies for Each Group of Compounds

class[a]	removal efficiency (RE)
Biofilter	
H	0.750
M	0.950
L	0.990
Activated Sludge	
H	0.500
M	0.900
L	0.990
Biotrickling Filter	
H	0.500
M	0.900
L	0.990
Chemical Scrubber	
H	0.500
M	0.900
L	0.990
Impregnated AC	
H	0.999
M	0.980
L	0.990
Incineration	
H	0.999
M	0.999
L	0.999
Hybrid Technology	
H	0.999
M	0.998
L	0.997

[a] High hydrophobicity (H), medium hydrophobicity (M), and low hydrophobicity (L).

Regenerative Incineration. Natural gas was used as support fuel in a system with a heat recovery of 90−95% and an EBRT of 1s (*25*). The system was operated at 850 °C, and a pressure drop of 1000−1200 Pa was considered in the burner (Powerflame Burners Inc. USA). An air heat capacity of 0.424 cal g^{-1} $°C^{1-}$ was used for energy calculation (*5, 26*). Despite being rarely considered nowadays for odor treatment in WWTPs, this odor-abatement technology was evaluated because it is still employed in landfill or industrial applications (*6, 19*).

Biotrickling Filtration + AC Filtration. This hybrid technology consists of a biotrickling filtration (EBRT = 7−10 s; packing height = 2 m; inert plastic packing of 75−140 kg m^{-3}; ΔP = 400−500 Pa) backed up by a conventional AC filtration (EBRT = 2−3 s; packing height = 0.5 m; ΔP = 1400−1750 Pa; 1.5−2.5 years of lifespan).

A biological oxidation yield of 0.5 g C−CO_2 (g-$C_{oxidized}$)$^{-1}$ and 0.5 g C-biomass (g-$C_{oxidized}$)$^{-1}$ was considered for all biotechnologies in the estimation of CO_2 emissions. Water consumption in the biotrickling filter, biofilter, and chemical scrubber considered the water supply needed to saturate the odorous emission. The energy consumption [kw] for gas circulation was calculated as Q [m^3 s^{-1}] × ΔP [kPa] × Blower efficiency (∼0.7). Table 1 summarizes the removal efficiency (RE) of the target VOCs and VICs for the evaluated technologies (*4, 6, 10, 19, 27*). The design parameters of these technologies are typical for H_2S removal of minimal 99% and odor removals of ∼95%.

Results and Discussion

Environmental Indicators. *Resource Usage.* A comparative resource usage evaluation, involving material, energy, land,

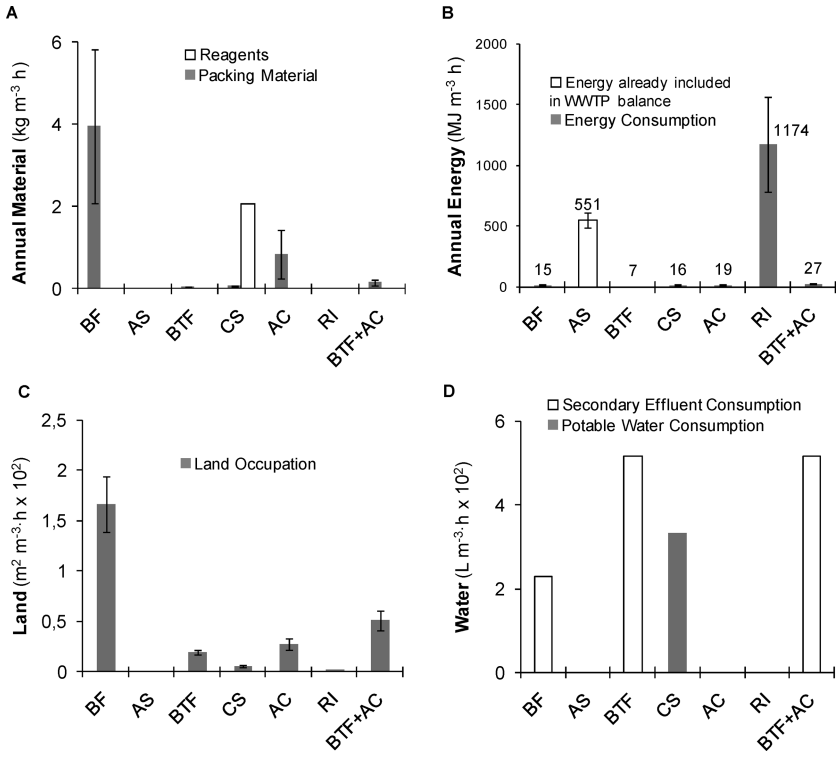

FIGURE 1. Environmental performance of the target odor-abatement technologies evaluated according to IChemE sustainability metrics. (A) Annual material usage, where gray bars represent the packing bed material and white bars represent the reagents. (B) Annual net primary energy usage. White bars represent energy already spent in regular WWTP operation. (C) Land occupation. (D) Annual water consumption. White bars represent water that can be substituted by secondary effluent.

and water requirements, was initially performed among the seven target odor-abatement technologies. Material usage accounted for both packing material and reagent requirements. Biofiltration and AC filtration presented the highest annual packed-bed-material requirements (4 ± 2 kg $(m^3/h)^{-1}_{air\ treated}$ and 0.8 ± 0.6 kg $(m^3/h)^{-1}_{air\ treated}$, respectively) because of the short lifespan of AC (six months replacement) and packing-material deterioration and because of the high EBRT in biofiltration (Figure 1A). Biofilter-material requirements depended on the type of packing material, which itself determines the density and structural resistance of the packed bed. Commercial biofilter packing materials exhibit typical durabilities ranging from 1 to 15 years, with the highest lifespans found for nutrient-enriched inert materials, and 1−3 years for conventional organic materials (*28*). All this variability is reflected in the error shown in the figure. In biotrickling filters and chemical scrubbers, the high durabilities of their packing materials (often inerts) together with their low EBRTs (∼4−15 s) resulted in very low materials usage ($4 \pm 2 \times 10^{-2}$ and $7 \pm 2 \times 10^{-2}$ kg $(m^3/h)^{-1}_{air\ treated}$, respectively). However, chemical scrubbing (2 kg $(m^3/h)^{-1}_{air\ treated}$) required the largest annual amounts of chemical reagents (Figure 1A). The hybrid technology presented 60% of the material requirements of a stand-alone biotrickling filter and one-fourth of the packing material requirements of AC filtration, while exhibiting a superior odorant-abatement performance than any of them separately.

Incineration presented the highest annual energy consumption (1170 ± 400 MJ $(m^3/h)^{-1}_{air\ treated}$) because of the low concentration of VOCs and VICs in the odorous emission and because of the need to heat this emission to combustion temperature (Figure 1B). These energy requirements, estimated by using natural gas as support fuel and by assuming a 90−95% heat recovery, were comparable to those reported by odor treatment technology manufacturers (820 MJ $(m^3/h)^{-1}_{air\ treated}$ in full-scale regenerative incinerators treating 2500 $m^3\ h^{-1}$ with odor-abatement efficiencies of 99% (*25*). The high energy use in activated sludge diffusion processes resulted from the compression of the odorous emission at a pressure similar to that of the water tank height (∼4 m). However, this energy is often included in the energy requirements of the wastewater treatment line in plants operated with air-diffusion aeration. This assumption is only valid when the amount of odorous air does not exceed the amount of air needed to satisfy the biological oxygen demand of the wastewater. The energy in AC filtration (19 ± 2 MJ $(m^3/h)^{-1}_{air\ treated}$) was exclusively due to the compression of the odorous emission and was lower than the requirements of the hybrid technology (that incorporated the energy needs of the preliminary biotrickling filter). Biofilters and chemical scrubbers presented comparable annual energy consumptions (15 ± 3 MJ $(m^3/h)^{-1}_{air\ treated}$) followed by biotrickling filters (7 ± 1 MJ $(m^3/h)^{-1}_{air\ treated}$). These annual energy requirements were estimated in the best and worst case scenarios and were lower than those in incinerators or AC filters. Biofilter operation at pressure drops of 2 cm H_2O would result in energy consumptions of 2.5 MJ $(m^3/h)^{-1}_{air\ treated}$, which are comparable to the requirements estimated by Prado et al. (2009) in a compost biofilter treating 3000 $m^3\ h^{-1}$ of an odorous emission (1.7 MJ $(m^3/h)^{-1}_{air\ treated}$ where P(kW) $= 3.64 \times 10^{-4} \times$ [odorous emission flow rate]).

Because of the need to maintain low pressure drops across the packing bed and because of the high EBRT needed for efficient odor treatment, biofiltration presented the highest land requirements ($1.7 \pm 0.3 \times 10^{-2}\ m^2\ (m^3/h)^{-1}_{air\ treated}$, Figure 1C). This footprint was 7 and 25 times higher than that of the biotrickling filter and chemical scrubber, respectively, and can limit the application of this biotechnology during plant upgrading in WWTPs facing land limitations. In practice, the footprint ratio between biotrickling filters and chemical scrubbers is slightly lower than $25/7 = 3.6$ because of the land required for chemical storage, this ratio ranging from 1 to 3. It is thus noticeable that biotrickling-filter footprints are comparable to those of physical/chemical technologies

partly as a result of their relative higher media depth. The hybrid technology ranked the second highest in land usage.

Biotrickling filters and the hybrid technology exhibited the highest water consumptions (5.2×10^{-2} L $(m^3/h)^{-1}_{air\ treated}$, Figure 1D). This high water consumption exceeded the critical water supply of 2.3×10^{-2} L $(m^3/h)^{-1}_{air\ treated}$ needed to compensate media moisture evaporation. This water supply to restore evaporation water losses from the packing media was also required for efficient biofilter operation. At this point, it must be stressed that the fact that water requirements in BTF were calculated on the basis of practical design guidelines (related to the inlet H_2S load) rather than evaporation looses resulted in slightly higher water requirements in the former technology. Apart from this critical water supply, an extra 1.0×10^{-2} L $(m^3/h)^{-1}_{air\ treated}$ must be added together with the chemical reagents (50% NaOH + 12.5% NaOCl) in the chemical scrubber in order to keep the salt concentration lower than 2% (total water requirements of 3.3×10^{-2} L $(m^3/h)^{-1}$). Although in BF, BTF, and BTF + AC low cost water is available from the secondary effluent and water requirements are negligible compared to the water available in a WWTP, chemical scrubber requires higher quality (preferably softened) potable water.

Waste, Effluent, and Emission Impacts. The land, aquatic, and atmospheric impact of the selected odor-abatement technologies as a result of process operation was also assessed. The deposition of the residual packing material was the main responsible for the impacts on land, and therefore, biofiltration and AC filtration generated the greatest impacts. However, although waste packing materials from biofilters can be considered as nonhazardous wastes (the packing can be mixed up with other disposal materials and/or the pH neutralized), AC must be treated as a hazardous waste (*29*). The hybrid technology composed of biotrickling + AC filtration generated one fourth of the impact of conventional AC filtration as a result of the biannual carbon replacement. Aquatic impact only concerned technologies where odor removal involves the transfer of the odorous compounds from the gas to an aqueous phase where oxidation takes place (biofiltration, hybrid technology, biotrickling filtration, AS diffusion, and chemical scrubbing) and was mainly due to H_2S oxidation. Because H_2S was almost completely removed regardless of the technology evaluated because of its rapid mass transfer from the gas phase (low hydrophobicity) and oxidation, the aquatic impact was comparable for the above referred technologies, this impact being zero for incineration and AC filtration (no water-based regeneration). However, because drain water from chemical scrubbers and biological technologies is sent back to the wastewater treatment line and these flow rates are relatively low compared to the total wastewater stream flowing through the WWTP, this impact is often negligible. For instance, in the WWTP used for the characterization of the model emission used here (Zarra et al. 2008), 2−3 L/day of sulphuric acid are produced per 2000−3000 m^3 of wastewater treated.

The release of gaseous emissions from odor-abatement units can cause an impact on human health, ozone depletion, and global warming. The human-health burden is due to the emission of toxic hydrocarbons such as benzene, xylene, and so forth, and was quantified in terms of benzene equivalents per year (Figure 2). Photochemical-ozone burden indicates the capacity of the emissions to form photochemical ozone or smog and was expressed in terms of ethylene equivalent per year, hydrocarbons being also the main contributors to this effect. These two impacts were due to the untreated fraction of the odorous emission, and therefore, their values were linked to process RE (Table 1). On the other hand, the global warming impact in odor treatment was mainly due to the emission of CO_2 from VOC oxidation, despite the fact

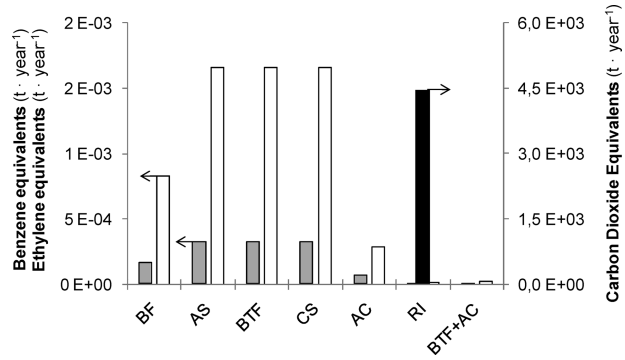

FIGURE 2. Atmospheric impact burdens according to IChemE sustainability metrics. Black bars represent the global-warming burden (in tons per year of CO_2 equivalents), gray bars represent the human-health burden (in tons of benzene equivalents per year), and white bars represent the photochemical-ozone burden (in tons of ethylene equivalents per year).

that the potency factor of the nontreated VOCs was ∼11-fold higher than that of CO_2. At this point, it must be highlighted that the emissions caused by trucking of AC or packing materials were not considered, and a further lifecycle analysis should be used to account for these additional issues. AS diffusion, chemical scrubbing, and biotrickling filtration, the technologies exhibiting the lower VOC REs as a result of their limited hydrophobic VOC mass transfer from the gas to the aqueous phase, showed the highest human-health and photochemical-ozone effects. Conversely, the hybrid technology, AC adsorption and incineration supported the highest VOC REs and therefore the lowest human burdens. However, these burdens can be considered negligible from an environmental viewpoint on the basis of the high REs of the seven technologies evaluated and the low odorant concentrations typically found in WWTP emissions. Overall, the global warming burden in odor treatment was negligible as a result of the low CO_2 and VOC concentrations in the treated odorous emission. However, regenerative incineration presented a global warming burden four orders of magnitude higher than the rest of the technologies because of the oxidation of the support fuel, despite exhibiting heat recoveries of 90−95%. In addition, the emission of NO_x and SO_x derived from fossil-fuel combustion negatively contributes to acid-rain formation and is currently under strict regulation (*6*).

Economic Indicators. A compilation of recent (2007−2010) investment and operating costs of full-scale systems obtained from odor-treatment technologies manufacturers in Spain and Australia was analyzed (Regional Government of Andalucía, ECOTEC-Ecología Técnica S.A., TECNIUM and BIOWAY™). Because of the reticence of manufacturers to provide economic data of their processes to academic researchers, this issue has been traditionally poorly discussed in the literature. In fact, most of the recent reports addressing these issues (*6, 30, 31*) based their data in turn on papers dated from 1992−1996 (*32*−*35*). Data obtained in this survey confirm that operating and investment costs are strongly dependent on the design removal efficiencies and scale of the project. In this data compilation, removal efficiencies for H_2S ranged from 90 to 99%, and odor removal ranged from 70 to 95%, which resulted in an increased variability of both investment and operating costs.

The investment costs per unit of flow rate treated decreased exponentially with increasing design flow rates regardless of the technology evaluated, which highlights the relevance of the economies of scale in odor treatment (Figure 3A). In the low flow rate range (<20000 $m^3\ h^{-1}$), AC adsorption exhibited the lowest investment costs (4−14 € $(m^3/h)^{-1}$, 5.5−20 $ $(m^3/h)^{-1}$; currency: 1€ = 1.40$), the effect of the

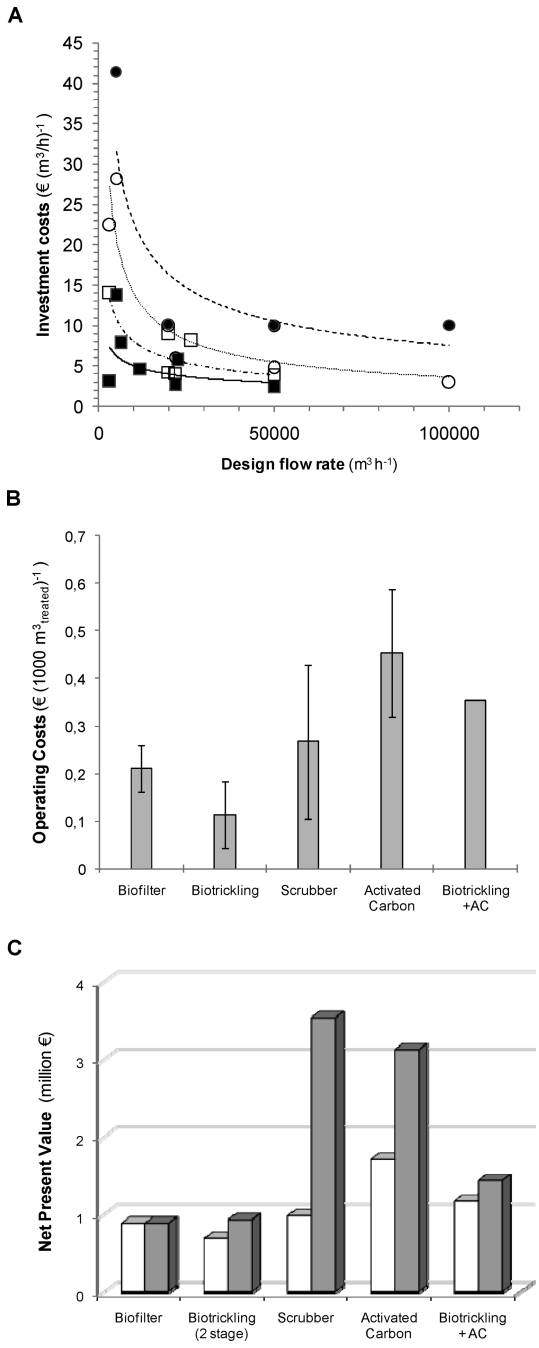

FIGURE 3. (A) Influence of design flow rate on the investment costs for AC (■/solid line), chemical scrubber (□/dash-dotted line), biofilter (○/dotted line), and biotrickling filters (●/dash line). (B) Operating costs obtained from odor-abatement technologies manufacturers. Vertical bars represent the standard deviation when enough data were available. (C) net present value (NPV) evaluated for a 5000 m^3 h^{-1} odorous emission containing 20.9 mg H_2S m^{-3} (white bars) and 105 mg H_2S m^{-3} (gray bars), a design useful life of 20 years, and an interest rate of 5%.

economy of scale being less important. Capital costs ranged from 3 to 12 € $(m^3/h)^{-1}$ (4 to 17 $(m^3/h)^{-1}$) and from 5 to 28 € $(m^3/h)^{-1}$ (7 to 40 $ $(m^3/h)^{-1}$) in chemical scrubbers and biofilters, respectively, whereas these costs accounted for 10−41 € $(m^3/h)^{-1}$ (14−57$ $(m^3/h)^{-1}$) for biotrickling filters (one and two stages). The investment costs in AS diffusion would be minimal because all equipment required is already present in the wastewater treatment line. Additional investments would derive from the installation of moisture traps and dust and grease aerosols filters and from the use of corrosion-resistant materials in blowers and air piping. In this context, a survey from 30 WWTPs in the U.S.A. showed that these corrosion concerns were not well founded; but in most cases, corrosion-resistant materials must be installed in filters and moisture traps (*20*). The EBRT and the cost of the packing material often constitute the key parameters determining the initial investment cost in biofiltration (*28*). When the land available is limited or the price of land relatively high, biofiltration will come with an additional cost. The configuration selected in chemical scrubbing (one stage versus two stages) significantly influenced the investment costs, two-stage chemical scrubbers presenting higher costs than their one-stage counterparts. Nowadays, odor removal at typical design efficiencies of 90−95% demands at least two stages whereas one stage is enough when only H_2S abatement (99%) is required. Although no economic data were available for incineration, the most recent information available in the literature reports capital costs ranging from 8 to 52 € $(m^3/h)^{-1}$ (11−73 $ $(m^3/h)^{-1}$) (*36*), which supports the general belief that incineration exhibits the highest investment costs within odor-abatement systems. Overall, the capital costs presented were comparable to those previously reported in the literature (*32, 34*−*36*). Finally, it is important to notice that, apart from the scale and design efficiencies of the odor-abatement systems, the final investment costs are strongly influenced by the installation costs (approximately 25% of the cost), currency, instrumentation and ductwork, project management, manufacturing location, sales commission, and so forth.

AC adsorption presented the highest operating costs among the evaluated technologies (0.45 € $(1000\ m^3_{treated})^{-1}$; 0.63 $ $(1000\ m^3_{treated})^{-1}$) because of the short lifespan of the packing material and the needs for specific management procedures (regeneration or disposal as hazardous waste, Figure 3B). These operating costs were within the lower range of the cost interval reported by the European Commission in its Waste Water and Gas Treatment/Management Systems in the Chemical Section BREF: 5−200 € $(m^3/h)^{-1}$ (7−280 $ $(m^3/h)^{-1}$). The cost of the hybrid technology was 0.35 € $(1000\ m^3_{treated})^{-1}$ (0.49 $ $(1000\ m^3_{treated})^{-1}$). Biofiltration and chemical scrubbing presented similar operating costs: 0.21 and 0.27 € $(1000\ m^3_{treated})^{-1}$ (0.29 and 0.38 $ $(1000\ m^3_{treated})^{-1}$), respectively. The operating costs in chemical scrubbing are mainly determined by reagent requirements (which itself are a function of odorant concentrations and flow rates). For instance, the operating cost of a two-stage chemical scrubber increases by a factor of four when the H_2S concentration increased from 21 mg m^{-3} to 105 mg m^{-3}. The average operating costs obtained in our survey for biofiltration agreed with those published by Prado et al. (2009), who reported a cost of 0.20 € $(1000\ m^3_{treated})^{-1}$ (0.28 $ $(1000\ m^3_{treated})^{-1}$) for 20000 m^3 h^{-1} facilities including annualized bed-replacing costs. Biotrickling filters exhibited the lowest costs (0.11 € $(1000\ m^{-3}_{treated})^{-1}$ or 0.15 $ $(1000\ m^{-3}_{treated})^{-1}$) mainly because of the higher lifespan of its packing material (which did not result in costly periodic packing replacements), lower liquid recirculation rates compared to those of chemical scrubbers, and absence of chemical requirements. This former cost was comparable to that reported by Gabriel and Deshusses (2004) for a retrofitted biotrickling filter in California treating 15800 m^3 h^{-1} of a headworks odor emission (0.1 € $(1000\ m^{-3}_{treated})^{-1}$ or 0.14 $ $(1000\ m^{-3}_{treated})^{-1}$), which confirmed the lower operating costs of this technology.

During technology evaluation, the NPV rather than the initial investment cost must be used as economic selection criterion. A simplified NPV calculation considering the capital and operating costs for a 5000 m^3 h^{-1} odorous emission containing 20.9 mg H_2S m^{-3}, a design useful life of 20 years, and an interest rate of 5% revealed that AC filtration and the

hybrid technology are the most expensive technology (1 703 384 €/2 384 737 $ and 1 172 019 €/1 640 826 $, respectively), followed by chemical scrubbing (979 126 €/1 370 776 $), biofiltration (883 348 €/1 236 687 $), and biotrickling filtration (687 493 €/962 490 $), see Figure 3C. To illustrate the key role of H_2S concentration on the technology evaluation, the NPV was recalculated for a new scenario at 105 mg H_2S m^{-3}. In this particular case, although the NPV of the biotechnologies increased by a factor of 1−1.3, the NPV increased by a factor of 1.8 and 3.6 for AC filtration and chemical scrubbing, respectively, the latter clearly becoming the most expensive technology (Figure 3C).

Social Indicators. Social indicators in odor abatement aim at estimating the impact of technology implementation on society at large, which is relevant to ensure WWTP licensing to operate over the long-term. The social benefits derived from odor treatment involve improvements in both occupational safety within the WWTPs (employees' welfare) and life quality of the nearby population. In our particular comparative analysis, incineration and the hybrid technology (if properly operated) would provide the largest social benefits in terms of mitigation of odors nuisance, because of their highest REs for all target odorants. However, the environmental impact caused by fossil fuels and CO_2 emission should be considered in the case of incineration. All technologies evaluated are capable of providing satisfactory levels of H_2S treatment when maintained and operated properly. The risks associated to equipment operation for the WWTP operators must be also considered in technology selection. Risk workshops always reveal that chemical scrubbers (transport, storage, and handling of hazardous chemicals) are less safe than carbon filters (risk for smoldering when loaded with and not ventilated), and both are less safe than biological techniques. The social benefits in the nearby population are the most difficult to quantify because of the high subjectivity and variability of human odor perception (and therefore odor nuisance), which involves aesthetic and emotional associations. A reduction in plant odorous emissions mitigates a large number of negative effects on the nearby population such as insomnia, headaches, loss of appetite, respiratory problems, and so forth (*2*). In this context, odor-dispersion modeling shows significant advantages of abatement technologies with relatively high emission points (e.g., biotrickling filters as well as chemical scrubbers). For instance, 500 OU m^{-3} from a surface area source like a biofilter often cause a higher degree of annoyance than 1000 OU m^{-3} from a point source at 10−15 m high such as a biotrickling filter. The current working practice nowadays recommends the implementation of 10 m high stacks in biofilters and AC filter to increase odor dispersion and therefore minimize odor annoyance.

Some authors estimated the social benefits of odor treatment by evaluating the reduction in house prices as a function of the distance to the odor source. Price reductions of up to 30% were recorded in properties situated 1 mile away (*37*). A more recent study conducted by VanBroek et al. (2009) (*38*) estimated to 60−120 € $year^{-1}$ the price that a family would be willing to pay for a 80% odor nuisance reduction. Conversely, Garrod and Willis (1998) found that people living near odor sources became used to the nuisance and had little incentive to pay for additional measures to reduce the level of disamenity (*39*). However, it should be noted that, over the past decade, people have notably increased their life quality and environmental standards, and the results obtained in 1998 might not apply in today's society any longer.

In summary, despite not providing a reference for acceptable process performance, the IChemE sustainability metrics constituted a valuable tool for odor-abatement technology selection in WWTPs, allowing for an intercomparative evaluation in terms of environmental performance, process economics, and social impact. The atmospheric impact of the physical/chemical technologies evaluated was negligible because of the combination of high odor-abatement efficiencies and low odorant concentrations in WWTP emissions, with the exception of the large CO_2, SO_x, and NO_x, footprint of incineration. However, the high energy consumption, the generation of hazardous wastes, the higher risk for operators, and their high reagent/material consumption continue to be their major drawbacks. The large water consumption in biological treatment technologies constitutes their main disadvantage, although the use of treated wastewater can help mitigating their environmental impact while reducing their operating costs. Among them, biofiltration presents the highest environmental impacts because of its high EBRT, low design height, and short lifespan of its packing material. These limitations explain the sustained effort in the development of novel high-performance packing materials supporting both high odorant transfers at low pressure drops and high structural stabilities. On the basis of their high odor abatement efficiencies and low environmental impact, activated sludge diffusion, biotrickling filtration, and the hybrid biotrickling + AC filtration emerged as the most promising technologies in modern WWTPs. In spite of their high capital costs, biofiltration and biotrickling filtration exhibited the lowest operating costs. In this context, the NPV represents the most appropriate economic criterion during technology evaluation because technologies with the lowest investment costs often presents the highest operating costs. Hence, the expected operational lifespan, the scale of the project, the odorant concentration, and the degree of odor reduction required constitute the most important parameters influencing process economics. From a social viewpoint, biotrickling + AC filtration entails the largest social benefits because of its highest odor-abatement performance and low environmental impacts.

Acknowledgments

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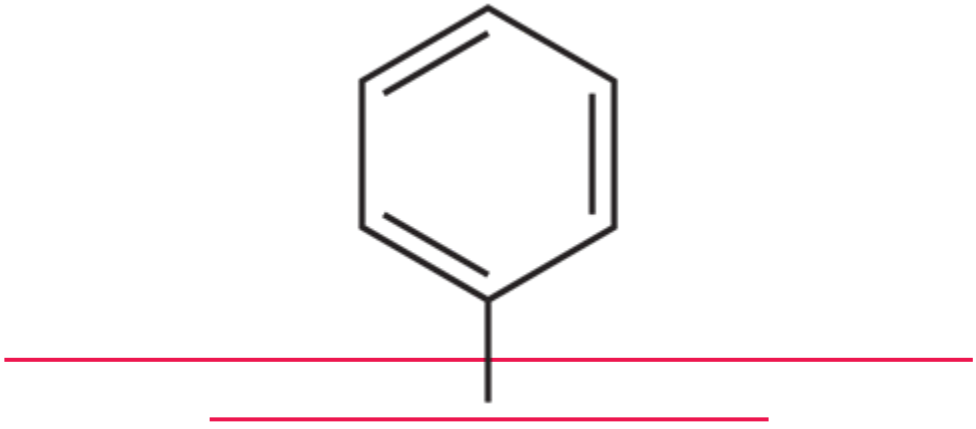
Chapter 4



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Research review paper

A sensitivity analysis of process design parameters, commodity prices and robustness on the economics of odour abatement technologies

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ABSTRACT

The sensitivity of the economics of the five most commonly applied odour abatement technologies (biofiltration, biotrickling filtration, activated carbon adsorption, chemical scrubbing and a hybrid technology consisting of a biotrickling filter coupled with carbon adsorption) towards design parameters and commodity prices was evaluated. Besides, the influence of the geographical location on the Net Present Value calculated for a 20 years lifespan (NPV20) of each technology and its robustness towards typical process fluctuations and operational upsets were also assessed. This comparative analysis showed that biological techniques present lower operating costs (up to 6 times) and lower sensitivity than their physical/chemical counterparts, with the packing material being the key parameter affecting their operating costs (40–50% of the total operating costs). The use of recycled or partially treated water (e.g. secondary effluent in wastewater treatment plants) offers an opportunity to significantly reduce costs in biological techniques. Physical/chemical technologies present a high sensitivity towards H_2S concentration, which is an important drawback due to the fluctuating nature of malodorous emissions. The geographical analysis evidenced high NPV20 variations around the world for all the technologies evaluated, but despite the differences in wage and price levels, biofiltration and biotrickling filtration are always the most cost-efficient alternatives (NPV20). When, in an economical evaluation, the robustness is as relevant as the overall costs (NPV20), the hybrid technology would move up next to BTF as the most preferred technologies.

Contents

Abbreviations: AC, activated carbon adsorption; BF, biofiltration; BTF, biotrickling filtration; BTF + AC, hybrid technology consisting of a biotrickling filter coupled with an activated carbon adsorption unit; CS, chemical scrubbing; EBRT, empty bed residence time; NPV20, Net Present Value calculated for a 20 years lifespan; WWTP, wastewater treatment plant.

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1. Introduction

The expansion and encroachment of urban residential areas on potential malodor sources such as wastewater treatment plants (WWTP), chemical and food industries or solid waste treatment facilities has resulted in an increasing number of public complaints (Lebrero et al. , 2011). This, together with the fact that a frequent exposure to malodors involves a direct threat to human welfare and health, has lead to tighter environmental regulations (Sucker et al., 2008). For instance, densely populated countries like Germany or the Netherlands have recently approved stricter odour regulations, which will be likely adapted by other countries in a near future (Nicolay, 2006). Thus, odour management has become a major environmental and economical issue, not only due to the enforcement of odour-related regulations, but also because most companies are increasingly aware of their public image.

Odour treatment technologies can be classified as physical/chemical (incinerators chemical scrubbers, adsorption systems, etc.) and biological (biofilters, biotrickling filters, bioscrubbers and activated sludge diffusion reactors) (Lebrero et al., 2011; Revah and Morgan-Sagastume, 2005). Despite these techniques are not different from those generally used in industrial off-gas treatment, their selection criteria have not been fully validated in the field of odour treatment (often characterized by high volumetric flow rates of complex pollutant mixtures at trace level concentrations) (Delhoménie and Heitz, 2005). A recent sustainability analysis carried out by the authors quantified the environmental and social impacts, and the net present value (NPV20), of the most commonly used odour abatement technologies, confirming the more sustainable performance of biological technologies and the key relevance of the operating costs in the overall process economics (Estrada et al., 2011). However, this study also revealed the high uncertainty in the evaluation of the operating costs due to their high dependence on process design parameters, and wage and commodity prices (which are time and location dependant). Unfortunately, there is a lack of systematic studies assessing the influence of these variables on process economics, which today still constitutes the main selection criterion despite the recent increased attention on sustainability.

A detailed sensitivity analysis should also include the quantification of the operational risks for each technology by quantifying process robustness. In this context, detractors of biological treatment technologies have pointed out process robustness as their main drawback, although recent studies suggest that state-of-the-art biotechnologies can be as robust as their physical/chemical counterparts (Kraakman, 2003; Lebrero et al., 2010). However, the number of case studies systematically assessing the robustness of biological odour treatment techniques is scarce.

This study was thus designed to evaluate the influence of the cost of the energy, chemicals, water, packing material and labour along with reactor design parameters such as the size (=empty bed residence time, EBRT), packing lifespan, pressure drop and H_2S concentration on the process economics (operating costs and NPV20) of the five most commonly applied odour abatement technologies: biotrickling filtration, biofiltration, activated carbon adsorption, chemical scrubbing and a hybrid technology (biotrickling filtration coupled with activated carbon adsorption). Additionally, a comparative analysis of the net present value (NPV20) of the five technologies evaluated as a function of their geographical location was carried out for thirteen representative cities in the world. Finally, a semi-quantitative analysis of the robustness of each technology towards typical process fluctuations and operational upsets was undertaken. This sensitivity analysis, together with the previous paper by the authors (Estrada et al., 2011), provides up-to-date guidelines for odour abatement technology selection.

2. Materials and methods

2.1. Model malodorous emission

An emission of 50,000 $m^3\,h^{-1}$ (293 K, 1 bar, 40% relative humidity) with a composition based upon the characterization of the odour pollution from a WWTP located at Stuttgart University (Germany) was selected as model malodorous emission (Zarra et al., 2008). Methyl mercaptan (2 $mg\,m^{-3}$) and hydrogen sulphide (21 $mg\,m^{-3}$) were also included in the above mentioned emission (Barbosa et al., 2002; Estrada et al., 2011). This emission was used as a reference in the sensitivity and geographical analysis here developed and all the cases mentioned.

2.2. Odour abatement technologies

The technologies here evaluated rank among the most commonly applied methods in the field of odour treatment: biofiltration, biotrickling filtration, chemical scrubbing, activated carbon adsorption and a hybrid technology composed of biotrickling filtration backed up by activated carbon filtration. These technologies, and therefore this sensitivity analysis, can be applied in different industries: WWTPs, food industries, livestock farms and municipal solid waste treatment facilities. The reference design parameters for each of the evaluated technologies are described below (Estrada et al., 2011) for abatement efficiencies higher than 99% for H_2S and higher 95% for odour:

2.2.1. Biofilter (BF)

A system packed with a mixture of compost (75%) and perlite (25%), a pressure drop (ΔP) of 1000 Pa m^{-1} (including the pressure drop in the humidifier) and a lifespan of 2 years with a packing material cost of 72 € m^{-3} was used as model biofilter (Lebrero et al., 2010). The unit operated at an EBRT of 60 s with a packed bed height of 1 m (Burgess et al., 2001; Harreveld, 2007; Revah and Morgan-Sagastume, 2005). A cost of 42 € m^{-3} (non-hazardous waste) and 30 € m^{-3} $year^{-1}$ were considered for disposal, and transport and handling, respectively, of the packing material.

2.2.2. Biotrickling filter (BTF)

A 2-stage biotrickling filter of 2 m packed bed height (1 m per stage) with a total EBRT of 15 s was here selected. The first stage operates at low pH (around 2), optimal for acidophilic H_2S oxidizing bacteria and the second one operates at a more neutral pH eliminating the rest of odorants. The system was packed with inert plastic packing with a lifespan of 10 years, a cost of 1200 € m^{-3} and a total ΔP of 500 Pa (Dorado et al., 2009). The relatively high cost of the packing material is based on previous experience in full-scale applications to guarantee the lifespan and removal efficiencies higher than 99% for H_2S and 95% for odour. Liquid nozzles were used for the dispersion of the recycling aqueous medium at 7.2 $L\,m^{-3}_{reactor}\,min^{-1}$ (0.9 $m\,h^{-1}$). The renewal of the aqueous medium was calculated based on the empirical design criterion of 2.5 L/g H_2S removed (Estrada et al., 2011). A cost of 120 € m^{-3} or 320

€ m^{-3} was considered for disposal of the packing material as hazardous waste for landfilling and incineration, respectively (depending on the legislation of the country selected) and 2500 € $year^{-1}$ as labour costs.

2.2.3. Chemical scrubber (CS)

A two-stage NaOH–NaClO scrubber packed with 2 m of scrubber packing (lifespan = 10 years; total pressure drop = 500 Pa; cost = 1200 € m^{-3}) operated at an EBRT of 4 s (2 s per stage) was used as model scrubber (Burgess et al., 2001; Tchobanoglous et al., 2003). A recirculation rate of 180 L m^{-3} min^{-1} was selected (21.6 m h^{-1}) (Gabriel and Deshusses, 2004) with a pressure drop of 50 kPa in the liquid nozzles. A cost of 120 € m^{-3} or 320 € m^{-3} was considered for disposal of the packing material as hazardous waste for landfilling and incineration, respectively and 2500 € $year^{-1}$ as labour costs.

2.2.4. Activated carbon filter (AC)

A 0.5 m granular activated carbon bed (density 450 kg m^{-3}) operated at an EBRT of 2.5 s with a total pressure drop of 1750 Pa and a cost of 4.8 € kg^{-1} was used as model adsorption filter (Turk and Bandosz, 2001). The bed replacement was based on empirical experience since carbon manufacturers do not often guarantee carbon life in odour applications. A standard carbon lifespan of 6 months was used for stand-alone applications (no regeneration of the activated carbon was considered). Disposal costs as hazardous waste of 120 € m^{-3} and 320 € m^{-3} were used for landfill and incineration, respectively. Transport and handling costs were of 432 € m^{-3} $year^{-1}$.

2.2.5. Biotrickling filtration + AC filtration

This hybrid technology consists of a biotrickling filter (EBRT = 9 s, packing height = 2 m, inert plastic packing cost of 1200 € m^{-3}, total ΔP = 500 Pa) backed up by an AC filtration using impregnated activated carbon (EBRT = 2.5 s, packing height = 0.5 m, ΔP = 1750 Pa, 2 years of lifespan, cost = 7.2 € kg^{-1}). The disposal costs above mentioned for the AC and BTF were applied to each packing material.

The packing material of the BTF and CS was here considered as hazardous waste because of the extreme pH, but when these packing materials are neutralized before disposal they might be considered as non-hazardous. This potential cost–benefit will depend on the media volume and will be site specific as additional costs for media neutralisation like chemicals and labour should be taken into account.

The investment costs here used were selected from a compilation of recent (2007–2010) investment costs in full-scale applications obtained from odour treatment technologies manufacturers in Spain, USA and Australia (Regional Government of Andalucía, ECOTEC-Ecología Técnica S.A., TECNIUM®, BIOWAY™ and OdorTool.com™. The investment costs for 50,000 m^3 h^{-1} units are as follows: biofilter — 225,000€, biotrickling filter — 330,000€, activated carbon filter — 90,000€, chemical scrubber 150,000€, hybrid technology — 354,000€. Maintenance costs were considered to be a 2% of the investment cost per year regardless of the technology evaluated. The maintenance costs for a more complex system (CS) using rotating items like pumps and valves might be slightly higher than for other systems like AC, however, this was not considered in the present study. Australia was selected as a reference scenario in this study for evaluating the costs and geographical location around the world (landfill disposal for hazardous waste, energy cost of 0.09 € kW h^{-1} and water cost of 1.12 € m^{-3}).

2.3. Sensitivity analysis for process design parameters and commodity prices

The sensitivity of the costs for the energy, water, chemicals, packing materials and work (the latter including labour, transport and handling, disposal and maintenance) on both the operating costs and the net present values at 20 years (NPV20) (interest rate of 5%) were evaluated for all five technologies using the above described malodorous emission. The NPV20 was defined as a simplified net present value calculation of the overall spent money in a technology during 20 years of operation according to Estrada et al., 2011 (Eq. (1)):

$$NPV = \sum_{i=1}^{20} \frac{F_i}{(1+r)^i} \quad (1)$$

where F_i is the operating costs plus the investment costs corresponding to year "i" and r is the interest rate applied (5% was chosen in the present study).

A cost increase by 25% from the reference values described above was chosen to analyze and illustrate the sensitivity of commodity prices.

The sensitivity of the EBRT on the NPV20 was analysed when increased by 50% from the above mentioned design values due to the large differences observed in the design volume provided by technology suppliers for full-scale facilities. The sensitivity of the H_2S concentration was evaluated when increased by 250% (from 21 to 73 mg m^{-3}) according to the high variability in concentration of this odorant reported in different odour treatment scenarios (Dincer and Muezzinoglu, 2008; Iranpour et al., 2005). The sensitivity of the packing material lifespan was evaluated (when reduced by 50%) in order to account for the variability found for the different packing materials available in the market (Prado et al., 2009). Also the sensitivity of the pressure drop (when increased by 100%) was calculated in order to cover the realistic range of pressure drops provided by packing material manufacturers.

2.4. Geographical analysis

Based on the large variability in wages and commodity prices all around the world (UBS, 2009), the net present value at 20 years (interest rate of 5%) for the five technologies was evaluated in 13 cities representative of all global regions using the previously described malodorous emission: Sydney (Australia, Asia-Pacific), Madrid (Spain, Southern-Mediterranean Europe), Copenhagen (Denmark, Central and Northern Europe), Sofía (Bulgaria, Eastern Europe), Toronto (Canada, North America East Coast), Los Angeles (USA, West Coast), Sao Paulo (Brazil, South America), Johannesburg (South Africa, African emerging countries), Doha (Qatar, Middle East), New Delhi (India, Asian emerging countries), Shanghai (China, Asian emerging countries), Tokyo (Japan, Asia), Singapore (Singapore, Asia).

Maintenance, transport, handling and labour costs were considered to be "wage dependant" parameters, while investment costs, packing material and disposal were considered "price dependant" parameters (UBS, 2009). Water and electricity prices were compiled from local suppliers and governmental databases in the period 2008–2011. Industrial prices were selected when available. The Global Water Intelligence 2010 Tariff Survey was used as comparative database to assess the quality of the data (GWI, 2010).

2.5. Robustness analysis

A way to evaluate the operational risk of a target odour abatement technology is to quantify the robustness of its performance, which can be defined as the ability of a technology to deal with process fluctuations and the ability to recover after operational upsets (Kraakman, 2003). The robustness of a technology (R) can be quantified by determining the risk of negative effects on the performance of the technology for each possible disorder (process fluctuation or operational upset), multiplied by its frequency of occurrence, and summing over all possible disorders as show in Eq. (2):

$$R = \sum (p \; x \; E) \quad (2)$$

where p is the probability of occurrence of a disorder and E the effect of a disorder. The probability of occurrence of any disorder can be expressed as the expected number of occurrences per year (number/year) or as the

percentage of operating time during which it is likely to occur. The effect of the disorder (E), or so called severity, can be expressed as the loss of the removal efficiency (%), the loss of the total removal (kg day^{-1} or kg $year^{-1}$), or the impact on the people living near the installation (e.g. the number of occurrences during which the concentration of the emitted air stream exceeded the odour threshold in the neighbourhood). In this study both the probability of occurrence and the effect are semi-quantified on a scale of 1 to 5 based on operator field experiences and other studies (Barona et al., 2004; Choi et al., 2004; Kraakman, 2003; Lebrero et al., 2010; Marek et al., 2000; Morales et al., 2003; Muñoz et al., 2008; Vergara-Fernández et al., 2007).

3. Results and discussion

3.1. Operating costs

Operating costs are expressed as the yearly costs in euros (€) per unit of flow rate treated ($m^3\ h^{-1}$). This unit provides figures which do not depend on the process scale and allow for fare comparison between technologies and scenarios. Thus, for example, the yearly operating costs of 2.0 € $(m^3/h)^{-1}$ for biofiltration, would be translated into 100,000 € $year^{-1}$ for the flowrate of 50,000 $m^3\ h^{-1}$.

AC adsorption presented the highest operating costs as a result of the high cost and frequent replacement of the activated carbon, while the high durability and low pressure drop of the packing material in BTFs rendered this technology the most economical in terms of operating costs followed by BFs (Fig. 1).

The cost of the packing materials constituted the main expense for the biotechnologies and the hybrid technology. Although the cost of the BF packing material was 17 times lower than that of the BTF, the larger packing volume needed for the BF due to its higher EBRT and its lower lifespan increased the contribution of the packing costs up to a 47% of the total operating costs (compared to 41% and 44% in the hybrid technology and BTF, respectively). The contribution of work (labour, transport and handling, disposal and maintenance) to the overall operating costs depended mainly on the need for packing media replacement. Hence, this parameter accounted for 29% and 17% in the BF and the hybrid technology, respectively, due to the short lifespan of the organic media and the AC. The relative contribution of energy consumption was comparable in both biotechnologies (18 and 22% for the BF and the BTF, respectively) and lower than in the hybrid technology (32%) due to the high pressure drop across the two beds in series (BTF + AC). Despite water consumption was comparable (0.02 l $m^{-3}_{air\ treated}$ in the BF and 0.05 l $m^{-3}_{air\ treated}$ in the BTF and hybrid technology), it only represented a small percentage of the operating costs in the BF and hybrid technology (6–9%) compared to the 21% in the BTF as a result of its lower operating costs. In the case of biofiltration, the yearly operating costs herein estimated for a gas flow rate of 50,000 $m^3\ h^{-1}$ were of 2.0 € $(m^3\ h^{-1})^{-1}$ and agreed well with those published by Prado et al. (2009), who reported yearly operating costs of 1.8 € $(m^3\ h^{-1})^{-1}$ for a compost biofilter treating 20,000 $m^3\ h^{-1}$ at an EBRT of 60 s. The yearly operating costs for BTF here obtained (1.2 € $(m^3\ h^{-1})^{-1}$) were slightly higher, but in the same range of values, than those reported by Gabriel and Deshusses (2004): 0.93 € $(m^3\ h^{-1})^{-1}$ for a BTF treating 15,800 $m^3\ h^{-1}$, this difference probably due to the fact that make-up water cost was considered negligible in that previous study, and to the introduction of up-to-date prices in the present work.

The highest contribution to the CS operating cost was the purchase of chemicals (69%) followed by the energy consumption (22%) as a result of the high liquid recycling rates. The contribution of water consumption, packing material and work were marginal for this technology (3, 4 and 4%, respectively). The monthly costs of 20 € $(kg\ H_2S_{removed})^{-1}$ here estimated, which results in 169,200 € $year^{-1}$ (based on 705 kg $H_2S_{removed}$ $month^{-1}$) for the CS, agreed with those published by Gabriel and Deshusses (2004). These authors reported average monthly costs of 22 € $(kg\ H_2S_{removed})^{-1}$ and 60 € $(kg\ H_2S_{removed})^{-1}$ in chemical scrubbers treating, respectively, 16,000 and 41,000 $m^3\ h^{-1}$ of polluted air with 10 ppm of H_2S and located at the trunkline and headworks of a WWTP in California. The yearly costs for those cases would be of 30,096 € $year^{-1}$ and 82,080 € $year^{-1}$ (based on 114 kg $H_2S_{removed}$ $month^{-1}$), respectively. The fact that higher amounts of H_2S were eliminated per month in our study (705 kg $month^{-1}$ *vs* 114 kg $month^{-1}$ in the study mentioned) might explain the slightly lower operating cost per kg $H_2S_{removed}$ here calculated (Gabriel and Deshusses, 2004).

The relatively short lifespan of the activated carbon can explain the large contribution (66%) of the packing material to the overall operating costs in the AC filter. This frequent packing replacement, together with the high costs of disposal as a hazardous material and transport and handling, also increased the contribution of the work costs up to 25% of the total operating costs. Despite the AC filter exhibited the largest pressure drops, the energy consumption only accounted for a 9% of the total operating costs. There was no cost associated to

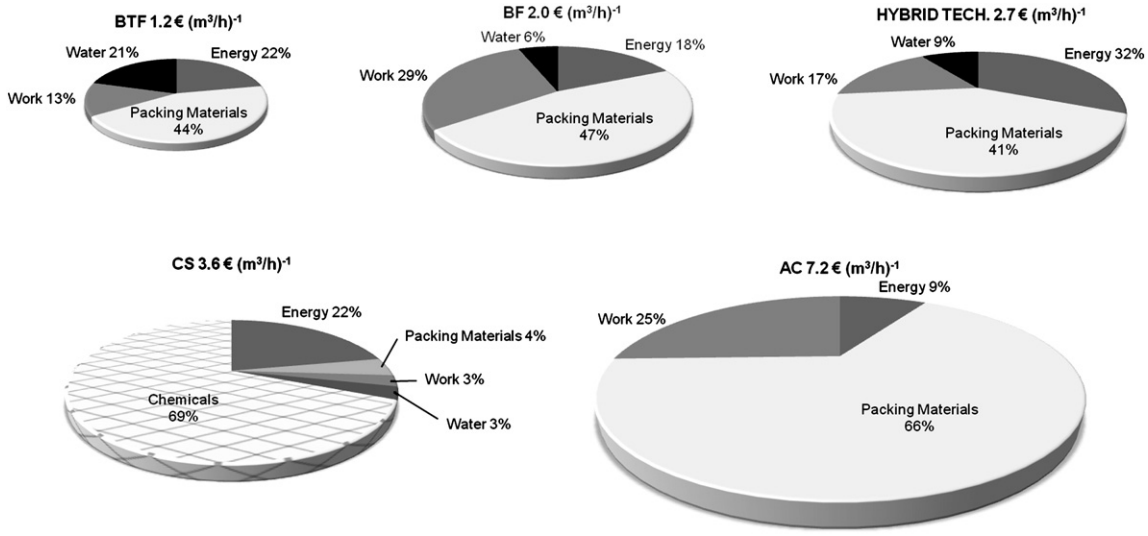

Fig. 1. Detailed composition of the operating costs for the 5 odour treatment technologies evaluated. The size of the graphs is proportional to the total yearly operating costs of each technology in the reference scenario (BTF = 1.2 € $(m^3/h)^{-1}$, BF = 2.0 € $(m^3/h)^{-1}$, hybrid tech. = 2.7 € $(m^3/h)^{-1}$, CS = 3.6 € $(m^3/h)^{-1}$, AC = 7.2 € $(m^3/h)^{-1}$.

water or chemicals consumption as no regeneration of the saturated activated carbon was considered. Finally, the yearly operating costs of the AC filter (7.2€ $(m^3/h)^{-1}$, 360,000€ $year^{-1}$ for a 50,000 $m^3\,h^{-1}$ flowrate) were low compared to the cost interval reported for AC systems by the European Commission in its Waste Water and Gas Treatment/Management Systems in the Chemical Sector BREF report (5–200€ $(m^3/h)^{-1}$, 250,000€ $year^{-1}$–10 million€ $year^{-1}$ for a 50,000 $m^3\,h^{-1}$ flowrate) (EC, 2003), probably due to the relatively low pollutant concentration and the relatively high airflow of the model emission used in this study.

3.2. Sensitivity analysis of process design parameters

A 50% variation in the EBRT was herein selected to account for the high variations in the current design practices among technologies suppliers (Fig. 2a). The hybrid technology was the most sensitive towards EBRT variations, with an overall costs (NPV20) increase of ≈350,000€ (15%) mainly caused by the increased cost of the extra activated carbon. The BF ranked second in sensitivity towards variation in the EBRT, with a NPV20 increase of ≈320,000€ (20%) when the design EBRT was increased from 60 to 90 s. This high sensitivity was previously reported by Prado et al. (2009), who estimated an increase in the total annual costs of 50,000€ $year^{-1}$ for a BF treating 50,000 $m^3\,h^{-1}$ when the EBRT was varied from 60 to 120 s (Prado et al., 2009). The BTF and CS showed a lower sensitivity of the EBRT to the overall costs NPV20). The NPV20 of the AC adsorption, somewhat surprisingly at a first sight, was not affected by an EBRT increase, as packing bed replacement is in practice load-related. Hence, due to the high operating cost of adsorption technologies, the inlet loads and abatement efficiencies are often carefully monitored and beds are only replaced at the saturation point. An increase in the design EBRT of the AC filter resulted in an increase in the unit's lifespan and the operating costs were therefore not affected. However, this operation practice is not currently applied for the hybrid technology, where the AC filter is devised as a polishing step following the treatment in the BTF. Under this particular configuration, the AC performance is not as critical as in stand-alone units and carbon replacement is conducted on a time basis (≈every 2 years).

The most sensitive technology to an increase of 250% in the concentration of H_2S (from 20.9 to 73.1 mg m^{-3}) was the AC, which would increase its NPV20 in 10 million€ (220%) (Fig. 2b). This dependence of AC filtration on H_2S concentration is due to the direct relation between loading rate and packing material lifespan. At concentrations commonly found in odour treatment facilities, the adsorption takes place in monomolecular layers on the activated carbon active sites, which means adsorption takes place in the linear region of the Langmuir isotherm. This fact explains the proportional decrease of the bed's lifespan when the H_2S load is increased and thus, the increase in the operating costs which at the same time cause an increase in the NP20. The CS was also severely impacted by the variation in H_2S concentration, increasing its NPV20 in more than 4 million€ (161%) due to the higher operating costs derived from the increased chemicals needs. The hybrid technology (17%), the BTF (30%) and the BF (18%) exhibited a low sensitivity towards H_2S concentrations, with NPV20 increases approximately 10 times lower than that of the CS. In the hybrid technology and the BTF, an increase in H_2S concentration resulted in higher water consumption

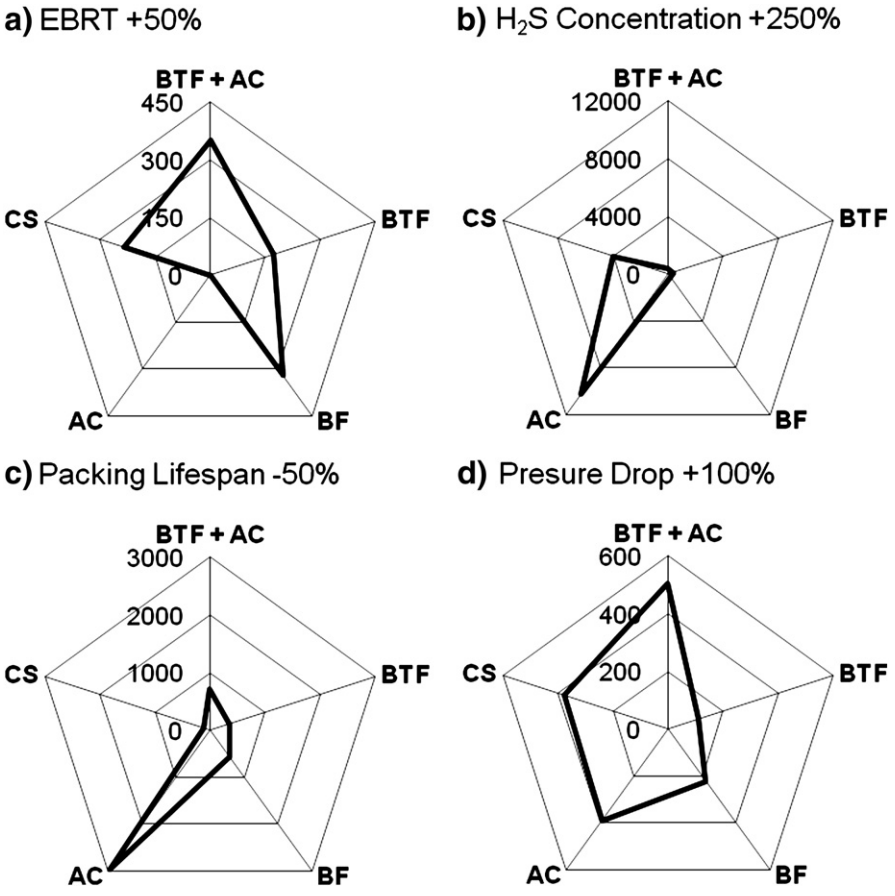

Fig. 2. Influence of the process design parameters on the NPV20 of the 5 odour abatement technologies evaluated. The results are shown as k€ increase of the NPV20 when the EBRT (a), the H_2S concentration (b), the packing lifespan (c) and the pressure drop were increased by 50%, 250%, −50% and 100%, respectively.

(complete H_2S degradation in the BTF stage was assumed for the hybrid technology), while the effect in the BF of such an increase in H_2S concentration is often a lifespan reduction by a factor of 1.5 due to a increased acidification of the BF media (which reduces its abatement performance).

The high contribution of the packing material lifespan to the operating cost of AC adsorption resulted in the highest increase in NPV20 (3 million€, 64%) when reduced by 50% (Fig. 2c). The hybrid technology (0.7 million€ NPV20 increase, 32%) ranked second in NPV20 increase despite the lower contribution of the packing material compared to the BF (because of the higher total operating costs of the former). The high sensitivity of the BF towards packing lifespan (0.6 million€, 36% increase in NPV20 when lifespan was reduced to 1 year) was due to the high packing material needs of this technology. The sensitivity of the BF towards this parameter is of particular interest considering the wide range of materials available in the market with lifespans ranging from 1 to more than 10 years (Prado et al., 2009).

When the pressure drop increased by 100%, the hybrid technology increased its NPV20 in ≈0.5 million€ (22%) due to its high pressure drop and therefore high contribution of energy to the operating costs (Fig. 2d). The CS and AC underwent a similar increase in NPV20 absolute terms of ≈0.4 million€ (15 and 8%, respectively) this significant NPV20 increase explained by the high total operating costs of AC, and the important contribution of energy costs (22%) to CS operating costs. The BF and the BTF showed a low sensitivity towards variations in the pressure drop, with a rise in NPV20 of 0.2 and 0.1 million€ (14 and 8% respectively), respectively, which suggests that these technologies will not be highly affected in economic terms by increased pressure drop due to unexpected packing media decomposition or biomass growth. However, despite the assumption that pressure drop increases do not significantly affect the performance of the technology, this might not always be the case and will depend on the type of technology and manufacturer.

3.3. Sensitivity analysis of commodity prices

The influence of an increase in the packing materials cost on the NPV20 was similar to that found for a decrease in the packing lifespan (Fig. 3a). A 25% increase in the price of activated carbon increased the NPV20 of the AC in more than 0.7 million€ (15%). The short lifespan of activated carbon and its high cost also impacted the hybrid technology, which would register a NPV20 increase of 0.16 million€ (7%). Biofiltration was not significantly affected as a result of the low price of its packing material (organic and inorganic, and often natural material mixture), which increased its NPV20 in only 90,000€ (6%). Finally, the BTF and CS were the least sensitive technologies towards this cost due to the high lifespan and the relatively low volumes of the inert packing materials required, with NPV20 increases of 78,000 (6%) and 21,000€ (0.8%), respectively.

The work costs (which included labour, transport, handling, disposal and maintenance) were directly correlated to the packing material replacement and maintenance, AC adsorption being the most sensitive technology with a 0.3 million€ increase (7%) followed by BF with 0.14 million€ (9%) (Fig. 3b). The contribution of the work cost to the NPV20 in these two technologies was significant as a result of their relatively low investment costs. The hybrid technology ranked third with a 0.08 million€ increase (4%), while the high packing material lifespan in the BTF and CS resulted in a low impact of this parameter. An additional analysis was performed to identify the

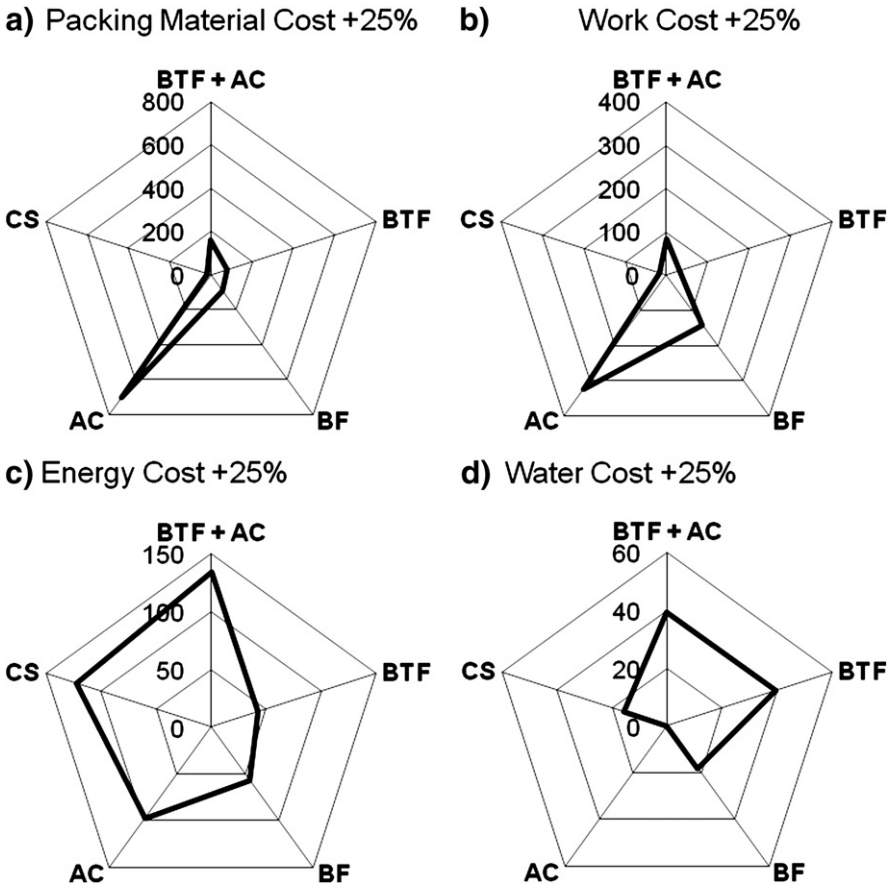

Fig. 3. Influence of the commodity prices on the NPV20 of the 5 odour abatement technologies evaluated. The results are shown as k€ increase of the NPV20 when the packing materials cost (a), work cost (b), energy cost (c) and water cost (d) were increased by 25%.

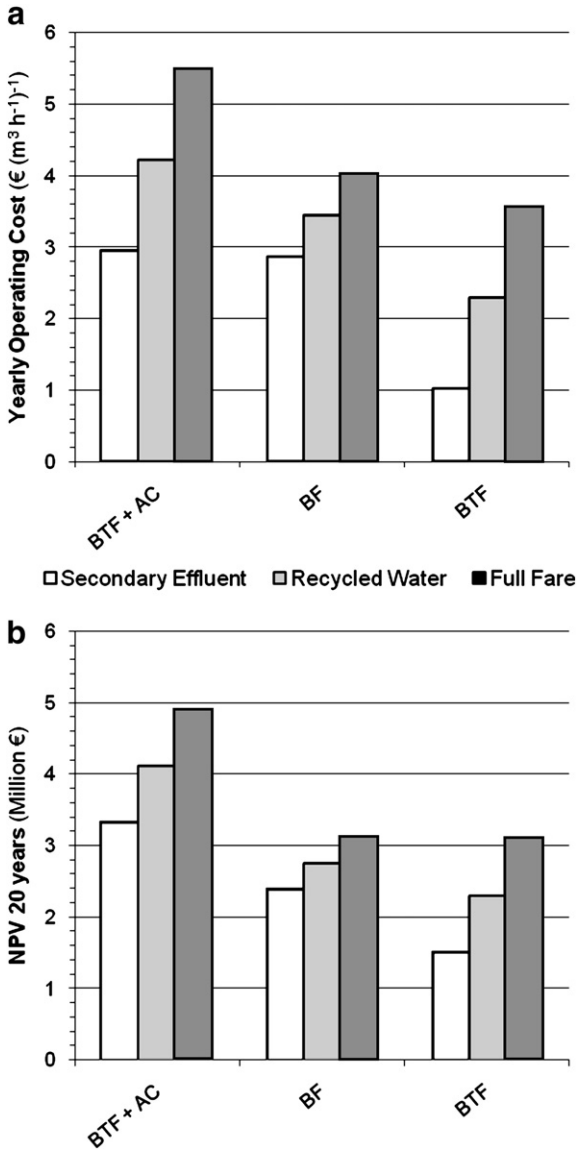

Fig. 4. Operating costs and NPV20 for the 3 biotechnologies (BF, BTF, BTF + AC) operating in Copenhagen with full fare tap water (5.6 € m^{-3}), recycled water (2.8 € m^{-3}) and secondary effluent from a WWTP (0 € m^{-3}).

importance of considering packing material hazardous or non-hazardous, and this parameter showed very low influence on the NPV20: if the packing material is disposed as non-hazardous NPV20 would decrease 1.5% for the hybrid technology, 1.8% for the BTF, 2.6% for the AC and 0.3% for the CS.

The hybrid technology and the CS presented a comparable increase in NPV20 (0.13 million (6%) and 0.12 million € (5%), respectively) when increasing the energy cost by 25% (Fig. 3c). Thus, in the case of the hybrid technology the pressure drop was the main responsible for this extra energy cost. However, in the CS the high energy demand as a result of inherently high liquid recycle rate mediated this increase in NPV20 at this 25% higher energy costs. The AC ranked third with a 97,000 € increase (2%), despite its energy costs only accounted for 9% of the total operating costs. The biological technologies, due to their low total operating costs, low pressure drops and low liquid recycling rates were the least sensitive to energy price variations, which is in accordance with the economic evaluation carried out by Prado et al. (2009) for a BF.

Finally, the increase in water prices by 25% highly impacted on the most water demanding technologies: the BTF and the hybrid technology (Fig. 3d). Both NPV20 increased by 40,000 € (3 and 2%, respectively) due to their comparable water consumption (estimated on a H_2S load basis and complete H_2S removal). On the other hand, the NPV20 for the BF and CS only increased by 18,000 € (1%) and 16,000 € (0.6%), respectively, while AC adsorption was not sensitive to water price variations since it did not present any water consumption.

Water is an increasingly scarce and valuable resource, whose price is expected to experience high variations in a near future. The increasing pollution of natural water reservoirs, demographic expansion, global warming and failures in water governance could eventually result in a global "water crisis" (Lopez-Gunn and Ramón Llamas, 2008). In the past several years, many governments and water institutions are revising their water policies in an attempt to reduce the overuse and inefficiencies in water consumption, especially in emerging economies like China (Cominelli et al., 2009; Zhong and Mol, 2010).

Thus, in an expensive water price scenario like Copenhagen (the country with the most expensive tap water in the world in 2010 according to GWI survey 2010) (GWI, 2010), water cost would significantly influence the process economics. The yearly operating costs of the hybrid technology decreased by more than 46% (from 5.5 to 2.9 € $(m^3\ h^{-1})^{-1}$) when using secondary effluent (available at zero cost in a WWTP) instead of full fare tap water (Fig. 4a), excluding the savings on nutrients. When using secondary effluent as water source, the operating costs of the hybrid technology were comparable to those of biofiltration under the same scenario. Likewise, the operating costs of the BTF can be drastically reduced by 71% (from 3.6 to 1.0 € $(m^3\ h^{-1})^{-1}$) when using secondary effluent instead of full fare water. Thus, while the BTF was the technology most economically benefited from an optimization in water use and price, biofiltration was the least sensitive as expected from its lower water consumption compared to the other biotechnologies. This finding is in agreement with the low influence of the water supply rate on the total annual costs estimated by Prado et al. (2009) in their recent economical assessment of biofiltration.

Table 1
Wage and price levels referred to Sydney (Australia) used in the geographical analysis for work, packing and disposal costs adapted from UBS (2009). Electricity prices and water prices were obtained from national suppliers. AC disposal refers to the activated carbon disposal method as hazardous waste according to the national regulations.

Reference cities	Wage level	Price level	Energy price (€ $kW\ h^{-1}$)	Water price (€ m^{-3})	AC disposal method
Sydney (AUSTRALIA)	1.00	1.00	0.110	1.120	Landfill
Madrid (SPAIN)	0.79	1.18	0.118	1.000	Incineration
Toronto (CANADA)	0.93	1.15	0.048	1.384	Landfill
Los Angeles (USA)	1.30	1.29	0.047	0.736	Landfill
Sao Paulo (BRAZIL)	0.35	0.92	0.083	2.500	Landfill
Johannesburg (SOUTH AFRICA)	0.37	0.71	0.050	0.936	Landfill
Doha (QATAR)	0.29	0.99	0.013	0.986	Landfill
New Delhi (INDIA)	0.10	0.55	0.006	0.076	Landfill
Shanghai (CHINA)	0.22	0.94	0.060	0.177	Landfill
Tokyo (JAPAN)	1.06	1.49	0.150	3.434	Incineration
Singapore (SINGAPORE)	0.38	1.20	0.098	0.299	Incineration
Sofía (BULGARIA)	0.19	0.77	0.063	1.000	Landfill
Copenhagen (DENMARK)	1.79	1.58	0.101	5.600	Incineration

The BTF exhibited the highest increase in NPV20, which doubled its value when using tap water instead of secondary effluent. Although the BTF and the BF presented a similar NPV20 when using full fare water (≈3.1 million€), the BTF became clearly the most economic alternative when secondary effluent was used as the water source, which is usually feasible in wastewater treatment facilities. Under all scenarios evaluated, the hybrid technology exhibited the largest NPV20 among the biotechnologies analyzed.

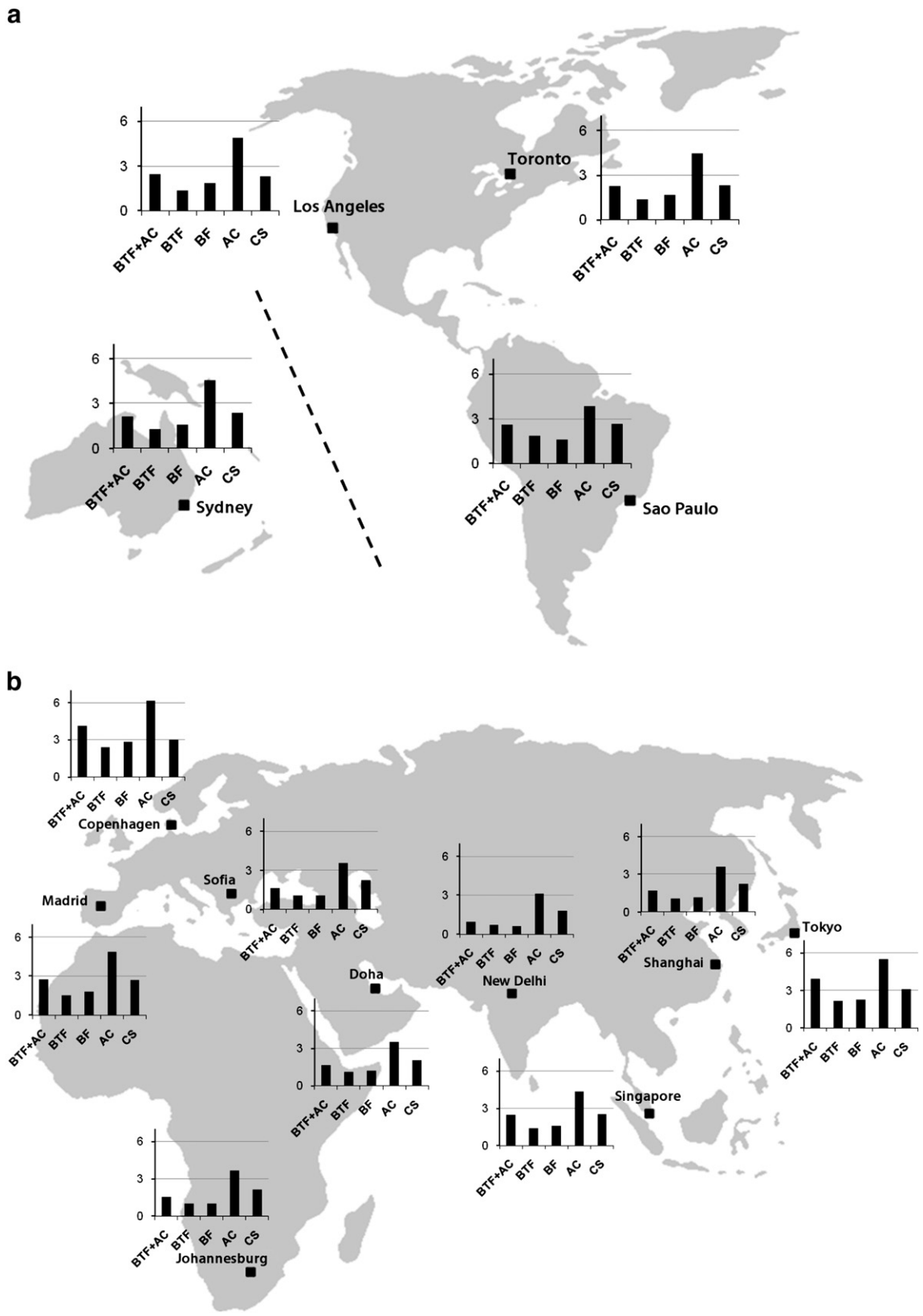

Fig. 5. a and b. Influence of the geographic location of the plant on the NPV20 of the five technologies evaluated in the 13 representative cities of the world.

3.4. Geographical analysis of economics

This analysis was based on a compilation of worldwide economic data involving both wage and price levels and commodity prices. While the variation in wage level modified the maintenance, labour, transport and handling costs, the price levels modified the investment, disposal and BF packing material costs according to the methodology used (plastic inert packing materials and activated carbon prices were considered to be constant and dependent on a global market, with no shipping costs considered). Despite water and energy costs did not result in large NPV20 variations when increased by 25% (Fig. 3b), the variations within the regions evaluated can be as high as 3000% in the real market (0.177 to 5.6 € m^{-3} for water and 0.006 to 0.150 € $kW\,h^{-1}$ for energy) (Table 1).

Chemical scrubbing showed the lowest geographic dependence in the present study, showing variations in its NPV20 from −22% (New Delhi) to +32% (Tokyo) compared to the reference scenario (Sydney) (Fig. 5). This high stability is derived from the fact that chemicals, which constitute the main cost in these systems, were considered to be part of a global market and their prices did not depend on the geographical location. On the other hand, the BF and the hybrid technology were the most sensitive technologies to the geographical location. The large variations in the NPV20 of the BF (−60% New Delhi, +83% Copenhagen) can be attributed to the high influence of packing material costs in the operating cost, which depended on the price level of the country considered. A combination of the water cost in the BTF unit and work costs in the AC unit determined the high NPV20 variability in the hybrid technology (−55% New Delhi, +94% Copenhagen).

BTFs are the most economic alternative in terms of 20 years NPV20 in developed countries, while BFs became the most economic technology in emerging countries closely followed by BTFs. These results might be explained by the key role of the packing material in BF operating costs, which mediated a significant decrease of the NPV20 in low prices scenarios (Fig. 1). On the other hand, activated carbon adsorption was always the most expensive technology regardless of the disposal method (landfill or incineration) and city. However, the difference in NPV20 between the AC and the rest of technologies increased when incineration must be applied (Table 1, Fig. 5). Chemical scrubbers became significantly more expensive than the biological and hybrid technologies in low water cost scenarios (e.g. Sofía, New Delhi), due to the low influence of the water costs on CSs. On the other hand, in high water price scenarios like Tokyo or Copenhagen the hybrid technology was the second most expensive in NPV20 terms, as a result also of their high wage levels (high work costs).

Cities in industrialized countries (e.g. Los Angeles, Copenhagen, Tokyo) exhibited larger differences among technologies in terms of NPV20, while emerging countries (e.g. New Delhi, Johannesburg), showed a more homogeneous NPV20 distribution (excluding AC adsorption). Brazil, and especially Sao Paulo, is an exception due to its high water prices and low wage levels. This scenario benefited technologies with high work needs and penalized those with high water consumptions, making Sao Paulo the city with the lowest differences among technologies in NPV20 terms. However, these conclusions should not be extrapolated to the rest of Brazil or South America due to the high variations in water prices found throughout the country, and more specifically between rural and urban areas.

Among the cities evaluated in this study, Copenhagen presented the highest 20 year NPV20 for all the technologies except for chemical scrubbing due to its high water, wage and price levels. The most expensive CSs were located in Tokyo due to its higher energy price. On the contrary, New Delhi presented the lowest NPV20 for all technologies because of its low commodity prices, price and wage levels.

3.5. Robustness analysis

The robustness of BF, BTF and CS is about half of the robustness of AC and the hybrid technology (Table 2). Based on the fact that process robustness might be as relevant as the net present value in an economical evaluation, the hybrid technology would move up next to BFT as the preferred technology. However, it is important to remark that operational failures that entail loss of biological activity will cause a decrease in the AC bed lifespan of the hybrid technology due to the higher odorants load to be treated in this step. The methodology to quantify the robustness also identifies and analyzes how to avoid failures, and/or mitigate the effects of the operational risks inherent to the system. It can be used to set requirements for control, monitoring and backup equipment, criteria for performance testing as well as maintenance schedules (Table 2). Please note that the effect of an interruption in the electricity supply causes a lower effect compared to a water supply disorder, despite water supply and recirculation pumps use electricity. This robustness evaluation considered that the times to repair an electrical breakdown are lower than those for

Table 2
Semi-quantitative robustness evaluation for the 5 target odour abatement technologies evaluated according to the methodology proposed.

Technology		BF			BTF			CS			AC			Hybrid			
Disorder/upset	Possible cause	p	E	p·E	p	E	p·E	p	E	p·E	p	E	p·E	p	E	p·E	Recommended control (protection/detection)
Water supply disorder	failure of supply or recirculation pumps. control failures (e.g. valves). changing conditions inlet air (Temp.. Rel Humidity)	4	−3	−12	3	−3	−9	3	−4	−12	1	−1	−1	3	−1	−3	duty-standby pumps/back-up water supply/spare parts/flow and level transmitter alarms
Electricity supply interruption	power outage	2	−2	−4	2	−3	−6	2	−3	−6	2	−1	−2	2	−1	−2	back-up/alarms
Chemical dosing disorder	pump or control failures. empty chemical storage tank	1	−1	−1	1	−1	−1	2	−4	−8	1	−1	−1	1	−1	−1	duty-standby pumps/spare parts/flow and level transmitter alarms
Foul air supply interruption	fan failure. blockage extraction ductwork. production stops	2	−2	−4	2	−2	−4	2	−1	−2	2	−1	−2	2	−1	−2	duty-standby fans/flow and pressure transmitter alarms
Fluctuation of inlet concentrations	changing or discontinuous production. diurnal or seasonal changes. production stops	4	−2	−8	4	−2	−8	4	−2	−8	4	−1	−4	4	−1	−4	combine foul air from sources/ continuous pollutant detection transmitter alarms
Fluctuation of inlet temperature	changing or discontinuous production. diurnal or seasonal changes. production stops	3	−2	−6	3	−1	−3	3	−1	−3	3	−1	−3	3	−1	−3	combine foul air from sources/ temperature transmitter alarms
Robustness of performance (R)			−35			−31			−39			−13			−15		

Probability (P): 1. Very unlikely or not possible. 2. Low. 3. Occasional. 4. Probable. 5. Frequent (it is certain that it will happen).
Effect (E): 1. Minor. 2. Marginal. 3. Moderate. 4. Critical. 5. Catastrophic.

problems commonly affecting water supply systems (usually pumps mechanical failures), since electrical failures are nowadays easy to identify, isolate and have a minimal effect on the operation of the rest of the elements of the plant. In addition, most full-scale facilities have auxiliary power supply systems. These facts lower the effect of electrical issues on odour abatement.

For odour abatement systems the operational risk is critical because, firstly, operational experience shows that when a technology is not critical for the main objectives of a facility (e.g. treat wastewater, production…) a lower priority is often given in terms of operator and maintenance attention. For example, a study by Sivret and Stuetz (2011) showed that the key indicator for many operator monitoring odour abatement systems is through odour complaints. Secondly, a reduced performance of the odour abatement system is usually not noticed first by the operators but by the surrounding population, as emission points are located at high positions and odour detection equipment is expensive or often not highly reliable (e.g. electrical sensors like H_2S and non-specific sensor array analyzers like electrical noses are subject to inaccuracy and corrosion in the usually humid airflow).

4. Conclusions

In summary, this analysis constitutes a valuable tool to assess the sensitivity of the 5 most commonly applied odour abatement technologies to design parameters and commodity prices. The biological technologies showed the lowest sensitivity and operating costs, biotrickling filtration exhibiting the lowest among them. Water price also played a key role on the yearly operating costs. Thus, the use of recycled water or secondary effluents can reduce the NPV20 of biotrickling filtration up to 50% and below that of conventional biofiltration. The physical/chemical technologies were highly impacted in economic terms by the concentration of H_2S, which constitutes an important drawback for these technologies as malodorous emissions are known to be highly variable in H_2S concentration. In AC adsorption, the cost and lifespan of the activated carbon was the main contributor to the overall operational costs (66%), while chemical consumption was the key contributor to the CS operation costs (69%).

The geographical analysis highlighted the relevance of location on the process economics and showed that local market conditions must be always carefully analyzed when evaluating the economic viability of a technology. Thus, while chemical scrubbing was the less sensitive technology to the geographic location, biofiltration and the hybrid technology were highly impacted. However, despite the large differences in wage and price levels and commodity prices worldwide, biofiltration and biotrickling filtration exhibited the lowest NPV on a 20 year basis while AC adsorption was always the most expensive technology on overall costs (NPV20).

The robustness evaluation here conducted showed that activated carbon and the hybrid technology were the most reliable technologies, while biotechnologies exhibited robustness comparable to that of chemical scrubbers.

Acknowledgements

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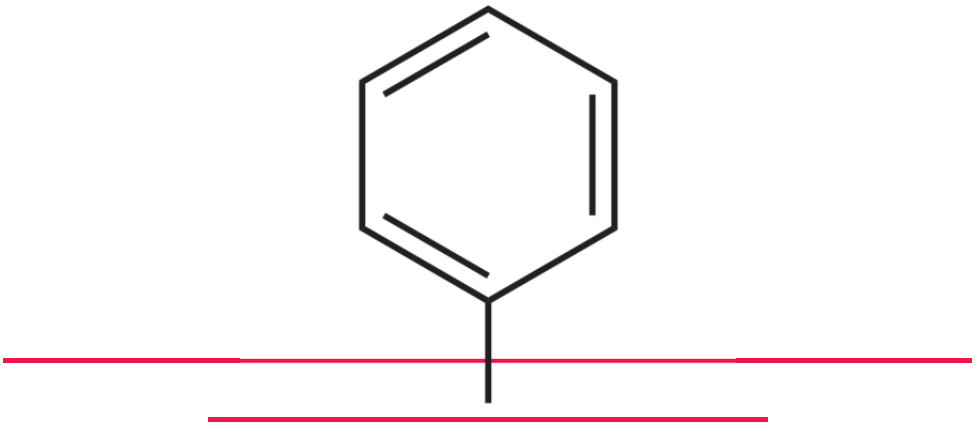
Implications of technology evaluation. Strategies to improve biological air pollution control technologies.

Chapter 5



5.1 Biotechnologies for air pollution control: merits and limitations

Biotechnologies have emerged as sustainable technologies for air pollution control exhibiting lower costs and environmental impacts compared to their physical/chemical counterparts [1]. They are already becoming more widespread and will progressively substitute traditional technologies in many applications in a near future. However, there are still technical issues to be improved in order to extend their application to a wider range of pollutants and scenarios [2].

Biofilters continue to be the most applied biological air pollution technique based on their proficient performance and low operating costs despite their operational limitations. They are often the preferred option under scenarios with high land availability or low economic and technological resources [1]. The analysis of the costs associated to biofiltration revealed that the most important expenses are related to packing material purchase and substitution. The loss of structural stability along with biomass accumulation (inducing a pressure drop build-up) result in low packing material lifespans in the most commonly employed materials, requiring frequent substitution and entailing significant maintenance and operating costs [3]. Research in the development of new packing materials or operational strategies leading to enhanced packing lifespan is crucial to reduce operating costs in biofiltration processes.

Biotrickling filters provide outstanding economic savings together with a low environmental impact and efficient abatement performance [1]. However, one of the main problems of this bioreactor configuration originates from the fact that pollutants have to diffuse from the gas phase to the aqueous phase in order to be degraded by the microorganisms (Figure 5.1). This fact results in situations where a merely physical mass transfer phenomena limits the overall process performance. Mass transfer coefficients are a function of the diffusivity of the pollutant in water (often surrounding the biofilm), the gas/water partition coefficient for the target pollutant, the gas turbulence in the bioreactor and the area available for mass transfer in the system (size and type of packing in packed systems). In addition, the transport (and hence biodegradation) of hydrophobic pollutants is often limited by the low concentration

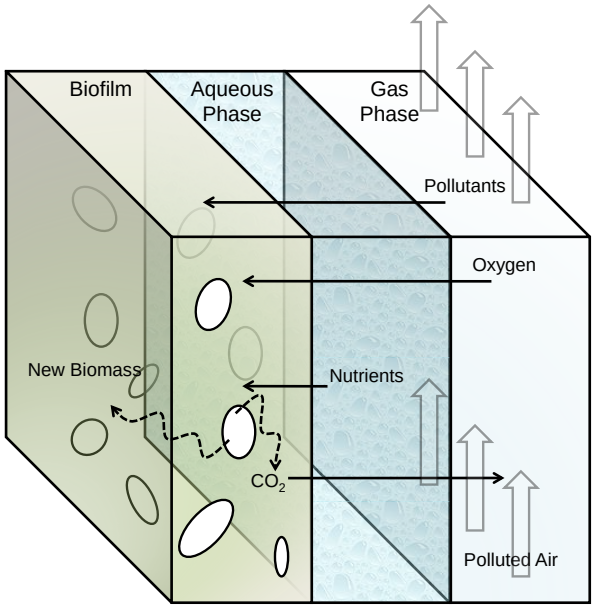


Figure 5.1 Mass transfer and biodegradation mechanisms in a biological process for air pollution control. Estrada et al. 2012 [1].

gradients available for transport (low driving forces) due to their high partition coefficients [4]. In this context, mass transfer limitations hinder the application of biotrickling filters for the removal of highly hydrophobic compounds such as certain volatile organic compounds, CH_4 or N_2O. Therefore, further improvements in their design and operation are mandatory to overcome mass transfer limitations [5-7].

Finally, biological processes for air pollution control are still perceived as unreliable despite exhibiting a similar robustness to traditional technologies such as scrubbers, probably due to the overall lack of knowledge on the microbial aspects governing the biodegradation process inside the reactors. For many years, bioreactors for air pollution control have been treated as "black boxes" and only in the past two decades there has been an increasing interest in shedding light over the microbial community structure and functionality responsible for pollutant biodegradation [8]. A deep understanding of process microbiology will help to overcome the long start-up periods often reported for these technologies and to improve their process robustness.

5.2 Overcoming design and operational limitations in biotechnologies for air pollution control

In the present thesis, the enhancement of the applicability of biotechnologies for air pollution control was addressed using two different approaches: biological and

design/operational. The biological approach focused on exploring microbial diversity and fungal biofiltration, providing key insights on the microscopic characteristic of the microbial community and its relationship to the macroscopic process performance. On the other hand, novel design and operational strategies based on traditional engineering concepts such as step-feed (applied in wastewater treatment), internal recirculation and on novel bioactive coatings were evaluated in order to overcome the inherent problems of biotechnologies above mentioned.

From a biological point of view, the influence of pollutant concentration on the diversity, structure of microbial communities and biodegradation kinetics during continuous gas-phase culture enrichment was investigated in **chapter 6** using toluene as a model VOC. The study discussed the implications of a proper acclimation of the microbial inoculum to a certain air pollution control application and revealed the limitations of traditional microbial enrichment techniques. A comparative analysis between fungal and bacterial biofiltration was carried out in order to provide fair performance data of both microbial approaches for the treatment of a VOC mixture (**chapter 7**). This work discussed the potential of fungi for the treatment of hydrophobic compounds and explored the concept of multi-stage biofilter using microbial stratification as an alternative for the treatment of complex hydrophobic pollutant mixtures [9].

Three different design and operational strategies were evaluated in order to improve the performance of biotechniques for air pollution control: in **Chapter 8**, an innovative step-feed biofiltration design was operated in order to overcome the excessive pressure drop build-up and limited packing material lifespan due to biomass overgrowth and media deterioration in standard biofilters. This design was evaluated using both an organic and an inorganic packing material, and toluene as the model pollutant. The economic and energetic implications of this strategy were discussed. **Chapter 9** reported an innovative gas recycling strategy to improve pollutant mass transfer in a biotrickling filter treating CH_4. The influence of liquid recycling rates was also evaluated from an economic and environmental viewpoint. Finally, the potential of

bioactive latex coatings (compared to water-based biofilms) to enhance the mass transport and further biodegradation of toluene was investigated in **chapter 10**.

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Influence of gaseous VOC concentration on the diversity and biodegradation performance of microbial communities.

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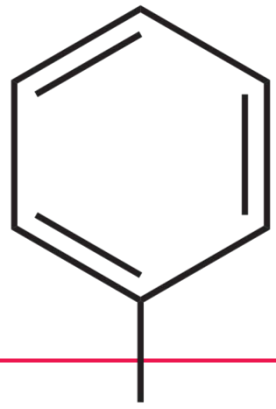
Chapter 6



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ORIGINAL PAPER

Influence of gaseous VOC concentration on the diversity and biodegradation performance of microbial communities

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Abstract In this work, the influence of toluene gas concentration on the isolation of toluene degrading microbial communities from activated sludge was studied. Toluene biodegradation at gas phase concentration of 10 g m^{-3} (R1) resulted in process instability with removal efficiencies (RE) lesser than 33 %, while operation at toluene gas phase concentrations of 300 mg m^{-3} (R2) and 11 mg m^{-3} (R3) was stable with RE ranging from 74 to 94 %. The consortium isolated in R1 exhibited the highest tolerance toward toluene but the lowest biodegradation performance at trace level VOC concentrations. Despite R2 and R3 showed a similar sensitivity toward toluene toxicity, the microbial community from R2 supported the most efficient toluene biodegradation at trace level VOC concentrations. The Shannon-Wiener index showed an initial biodiversity decrease from 3.2 to 2.0, 1.9 and 2.7 in R1, R2 and R3, respectively. However, while R2 and R3 were able to recover their initial diversity levels by day 48, this loss in diversity was permanent in R1. These results showed that traditional inoculum isolation/acclimation techniques based on the exposure of the inoculum to high VOC concentrations, where toxicity tolerance plays a key role, may result in a poor abatement performance when the off-gas stream is diluted.

Keywords Biodiversity · Biofiltration · Isolation · Odor abatement · VOC

Introduction

Volatile organic compounds (VOC) present in industrial and odorous off-gases can cause harmful effects on both human health and natural ecosystems. Thus, while VOC in industrial emissions are associated with toxicity and carcinogenicity problems, in agro-industrial and waste treatment activities, VOC are often responsible for odor nuisance in the nearby population [1]. This has caused an increasing public concern about atmospheric pollution, which has finally resulted in stricter environmental regulations. These tighter legislations, together with the recent quest for sustainability in human activities, have entailed the need to develop cost-effective and environmentally friendly VOC abatement methods. Among the available technologies for odor abatement, biological methods constitute the most sustainable and cost-effective technology, exhibiting the lowest operating costs and environmental impacts [2].

In the last 20 years, there has been wide interest in the characterization of the microbiology governing the removal of VOC, which is of paramount importance since biodegradation is the main VOC destruction mechanism [3]. Microbial acclimation is often based on the previous exposure of a microbial community to high gas VOC concentrations (>100 g m^{-3}) [4, 5]. Under these conditions, tolerance toward pollutant toxicity constitutes the main driving force for microbial selection and often results in the isolation of few but very specialized microorganisms. However, little is known about the nature and characteristics of the microbial communities governing the

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biodegradation of VOC at trace level concentrations. This is of key relevance to guarantee a rapid start-up and robust long-term operation in biological-based odor abatement facilities, where typical VOC concentrations are in the range of mg m^{-3} and μg m^{-3} [6]. Therefore, microbial consortia isolated and acclimated at high gaseous VOC concentrations often require long start-up periods when inoculated in systems treating VOC at trace level concentrations as recorded by several authors [4, 5, 7]. Consequently, inoculum production under trace level VOC concentrations might lead to high performance inocula, able to reduce the start-up period and improve the stability of biological processes dealing with diluted off-gases.

This work aimed at studying the influence of gaseous VOC concentration at trace level on the microbial diversity and their macroscopic biodegradation performance (VOC removal efficiency, biomass growth, metabolite accumulation and specific ATP content) using toluene as model VOC. In addition, the inoculum isolated under VOC trace level concentrations was compared with inocula obtained at higher pollutant concentrations in terms of biodegradation kinetics and toxicity tolerance.

Materials and methods

Chemicals

Toluene was purchased from Fisher Scientific® (United Kingdom) with purity higher than 99.9 %. All other chemicals and reagents were purchased from Panreac® (Barcelona, Spain) with a purity of +99 %. The mineral salt medium (MSM) was prepared according to Muñoz et al. [8].

Experimental setup

Three sterile jacketed 500-mL glass reactors containing 400 mL of sterile MSM were inoculated with fresh aerobic activated sludge (Valladolid Wastewater Treatment Plant, Spain) to an initial concentration of 300 mg l^{-1} of dry weight (DW). The bioreactors were stirred magnetically at 200 rpm and operated at 25 °C. Inert polyurethane polymers (1 g) were introduced in the reactors to act as an abrasive in order to avoid biofilm formation on the bioreactor walls. Toluene was continuously supplied via aeration (400 ml min^{-1}) at 9.5 ± 1.1 g m^{-3}, 306 ± 38 mg m^{-3} and 11.5 ± 1.7 mg m^{-3} into reactors 1, 2 and 3 (namely R1, R2 and R3, respectively) using 2 μm porous stainless steel diffusers (Supelco®, USA). Toluene concentrations were regulated using mass flow controllers (Aalborg™, Denmark) by mixing a toluene-free air stream with toluene saturated streams (Fig. 1). Sterile conditions were maintained in the bioreactors by 0.2 μm PTFE filters (Millipore™ Corporation, USA) located at the gas inlet and outlet. Water-cooled condensers were installed at the bioreactor's gas outlets to avoid water losses by evaporation and clogging of the sterile filters by condensation.

The cultures were operated batch wise for 48 days with a periodic MSM exchange under sterile conditions in order to maintain the pH above 5.5 as well as to avoid nutrient limitation and metabolite accumulation. Hence, 200 ml of cultivation medium were exchanged twice a day in R1. The cultivation medium was centrifuged at 2,800×*g* for 10 min under sterile conditions and the biomass pellet re-suspended in fresh sterile MSM and returned to the bioreactor. This procedure was also carried out in R2 and R3 when pH levels decreased to 5.5. Despite the differences that may arise from the culture growth in suspension rather than as a biofilm, suspended reactors were here selected in order to allow for a better control of sterile conditions, pH, temperature, and to avoid VOC or O_2 concentration gradients or preferential flow paths.

Liquid samples (10 ml) were periodically drawn under sterile conditions from the bioreactors to determine pH, biomass concentration, the specific ATP content, and to perform the toxicity and trace level concentration kinetic tests. Samples for DGGE analysis and further sequencing were also periodically taken to monitor the dynamics of microbial populations. Sterile fresh MSM was periodically added to minimize water losses by evaporation and sampling. Toluene gas concentration was monitored by GC-FID using gas-tight syringes or solid phase micro extraction (SPME) fibers at the inlet and outlet of reactors. The aqueous concentration of toluene in the reactors under steady state conditions was also analyzed. Liquid samples (5, 10 and 10 ml from R1, R2 and R3, respectively) were drawn and injected in 16, 120 and 120 ml air-tight bottles containing 0.5, 2 and 2 ml of H_3PO_4, respectively, to stop biological activity. The bottles were maintained overnight under magnetic stirring at 25 °C (to ensure phase equilibrium) and toluene headspace concentration was analyzed by GC-FID using gas-tight syringes (R1) and SPME (R2 and R3). The concentration of toluene in the aqueous phase of the reactors was determined from mass balance and equilibrium calculations (Henry's law) corrected linearly for the biomass concentration present in the sample according to Lin et al. 2005 [9].

Toxicity tests

Respirometric toxicity assays for the microbial consortia established in each bioreactor were periodically performed according to Muñoz et al. [8] (days 16, 28 and 48) in a Strathkelvin Strathox respirometer (Strathkelvin Instruments Ltd., Glasgow, UK). Reaction vessels filled with

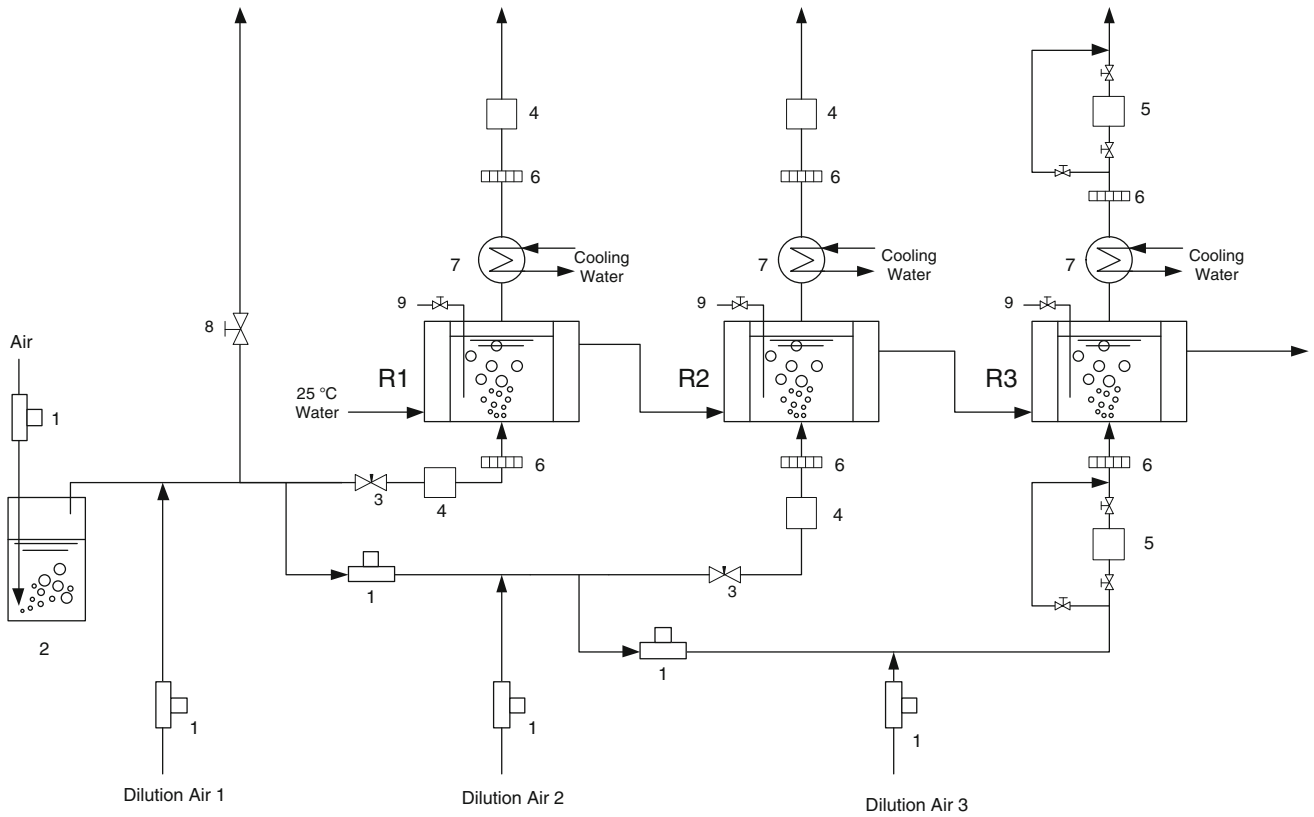

Fig. 1 Schematic representation of the experimental set up. *1* Mass flow controllers, *2* toluene saturation vessel, *3* needle valves, *4* gas sampling points, *5* sampling bulbs SPME sampling, *6* sterile filters, *7* water-cooled condensers, *8* pressure relieve safety valve, *9* liquid sampling points

18 ml of fresh O_2-saturated MSM were inoculated with 2 ml of the corresponding diluted microbial consortium to an initial concentration of 0.27 mg DW l^{-1} and supplied with toluene from a toluene saturated water stock solution to 5, 10, 20, 35 and 50 mg l^{-1}. This low biomass concentration supported a test duration of 15 min, which enabled to record consistent oxygen consumption rates. The saturated stock solution consisted of a two phase water–toluene mixture in equilibrium. All experiments were carried out in duplicate including a control test deprived of toluene in order to determine the endogenous respiration rate of the microbial culture.

Toluene biodegradation kinetic assays at trace level concentration

The kinetics of toluene biodegradation at trace level concentration of the microbial populations established in each bioreactor were periodically assessed (days 21, 36 and 45) in 120 ml glass bottles containing 20 ml of MSM and inoculated with 0.12 mg DW l^{-1} from the corresponding bioreactor. The bottles were closed with butyl septa, sealed with aluminum caps and supplied with toluene at a headspace concentration of 4.4 ± 0.2 mg m^{-3} (corresponding to an initial toluene aqueous concentration of 16.0 ± 0.6 μg l^{-1}). The systems were incubated at 25 °C and 300 rpm for 8–10 h. The toluene headspace concentration was periodically measured by SPME-GC-FID (5 min of adsorption). Due to the destructive nature of the SPME technique, 5–6 bottles were initially prepared for each microbial population evaluated and removed from the tests following analysis. Abiotic controls acidified with 0.1 ml of 96 % H_2SO_4 were also prepared to avoid any biological activity. External standards prepared in 120 ml bottles were used for toluene quantification.

Analytical procedures

Toluene gas concentration in R1 and R2 was analysed by GC-FID according to Bordel et al. [10]. Toluene gas concentration in R3 was determined by SPME-GC-FID according to Lebrero et al. [3]. The metabolites present in the cultivation medium of R1 were concentrated by eluting 150 ml of the cultivation medium (prior centrifugation at 5,000 rpm for 10 min) in a SupelClean™ Envi-Carb™ SPE cartridge (Supelco®, USA) and further analysed by GC–MS according to Bordel et al. [10]. ATP was measured using a Microbial ATP Kit HS (BioThema®, Stockholm, Sweden) and a Microtox® 500 luminometer (Azur Environmental®, Carlsbad, Germany). Biomass concentration

was determined using dry weight measurements according to Bordel et al. [10].

Sample collection, DNA extraction, PCR amplification and denaturant gradient gel electrophoresis (DGGE)

Aliquots of 20 ml of well-homogenized microbial cultures from R1, R2 and R3 were collected at days 0, 15 and 48 for DGGE analysis. The samples were immediately concentrated by centrifugation and stored at −20 °C. DNA extraction and PCR amplification using the universal bacterial primers 968-F-GC and 1401-R (Sigma-Aldrich, St. Louis, MO, USA) and DGGE analysis of the amplicons were carried out according to Lebrero et al. [3].

Analysis of DGGE data

DGGE profiles were compared using the GelCompar IITM software (Applied Maths BVBA, Sint-Martens-Latem, Belgium). For normalization of the DGGE, reference markers composed of an equal mixture of the PCR products from each sample were loaded into the DGGE gel every third sample. After image normalization, bands were defined for each sample using the software bands search algorithm. Similarity indices between the bacterial populations in each sample were calculated from the densitometric curves of the scanned DGGE profiles using the Pearson product–moment correlation coefficient [11] and were then used to construct a dendrogram using UPGMA clustering. The peak heights in the densitometric curves were also used to determine the diversity indices based on the Shannon–Wiener diversity index.

Sequencing and phylogenetic analysis

Individual bands were excised from the DGGE gel with a sterile blade, resuspended in 50 µl of ultrapure water, and maintained at 60 °C for 1 h to allow DNA extraction from the gel. A volume of 5 µl of the supernatant was used for reamplification with the original primer sets. Before sequencing, PCR products were purified with the GenElute PCR DNA Purification Kit (Sigma-Aldrich, St. Louis, MO, USA). The sequences obtained from excised bands were assigned to the different taxonomic ranges using the RDP classifier tool [12]. The sequences were also compared with public sequences in GenBank by BLAST search tool at the NCBI (National Centre for Biotechnology Information) [13]. Good quality sequences were deposited at GenBank under accession numbers JN606092-JN606095 (bands 1–4), JN606096-JN606102 (bands 8–14), JN606103 (band 16), JN606104 (band 17) and JN606105-JN606107 (bands 20–22).

Results and discussion

Toluene biodegradation performance and stability

Toluene biodegradation in the reactor operated at 10 g toluene m^{-3} was initially characterized by a lag phase of 3 days, a constant pH value ($\approx$7) and a severe decrease in the cell energy content from 0.7 to 0.1 nmol ATP mg DW^{-1} (Fig. 2a, b). This decrease in the specific ATP content could be attributed to the permeabilization effect of toluene over bacterial cell walls as shown by Heipieper et al. [14]. At day 3, toluene removal efficiency (RE) rapidly increased up to 23 % concomitantly with a sharp decrease in the cultivation pH down to 4.1. Despite the frequent medium exchange (dilution rate of approximately 1 day^{-1}), which restored pH levels to 6.6 ± 0.2 after fresh MSM addition, the process was characterized by a pattern of high amplitude pH variations and oscillating REs from days 3 to 10. From day 10 onwards, REs ranged from 1.8 to 33 %, this poor biodegradation performance being likely due to a microbial inhibition mediated by the exposure of the microbial community to high toluene concentrations and metabolites causing a loss of toluene degrading activity [15]. The average toluene elimination capacity (EC) achieved was 74.6 ± 7.5 g m^{-3} h^{-1} in R1, this value lying in the range commonly reported (28–250 g m^{-3} h^{-1}) for different types of bioreactors treating toluene at inlet loads of 29–255 g m^{-3} h^{-1} [16].

Aqueous toluene concentrations of 37.2 mg l^{-1} were detected in the cultivation medium of R1. The exposure at concentrations over 20–40 mg l^{-1} is known to inhibit bacterial consortia and non-acclimated strains such as *Pseudomonas putida* 54G [17]. During this period (day 10 onwards), the pH remained relatively constant (6.3 ± 0.1) while the specific ATP content steadily increased up to a stable content of 2.1 ± 0.2 nmol ATP mg DW^{-1}. The biomass concentration also increased from 0.3 (day 0) to 11.2 g DW l^{-1} at day 48 as a result of the biomass return after centrifugation.

At day 1 only benzyl alcohol was detected, while at day 6 benzyl alcohol and *o*-cresol were identified. However, only *o*-cresol was detected by days 23 and 37 (corresponding to the period of stable operation). While benzyl alcohol and *o*-cresol are known intermediates of aerobic toluene biodegradation pathways in species such as *P. putida*, *P. mendocina and Burkholderia cepacia*, these metabolites are also often excreted by *P. putida* F1 under oxygen limiting conditions [18]. Toluene biodegradation under oxygen limiting conditions was likely to occur in R1 based on a previous study by Bordel et al. [19], who recorded steady state oxygen concentrations of 0.7 mg/l when operating a similar bioreactor at 11.7 g toluene m^{-3}. Despite the DO was not measured directly in the

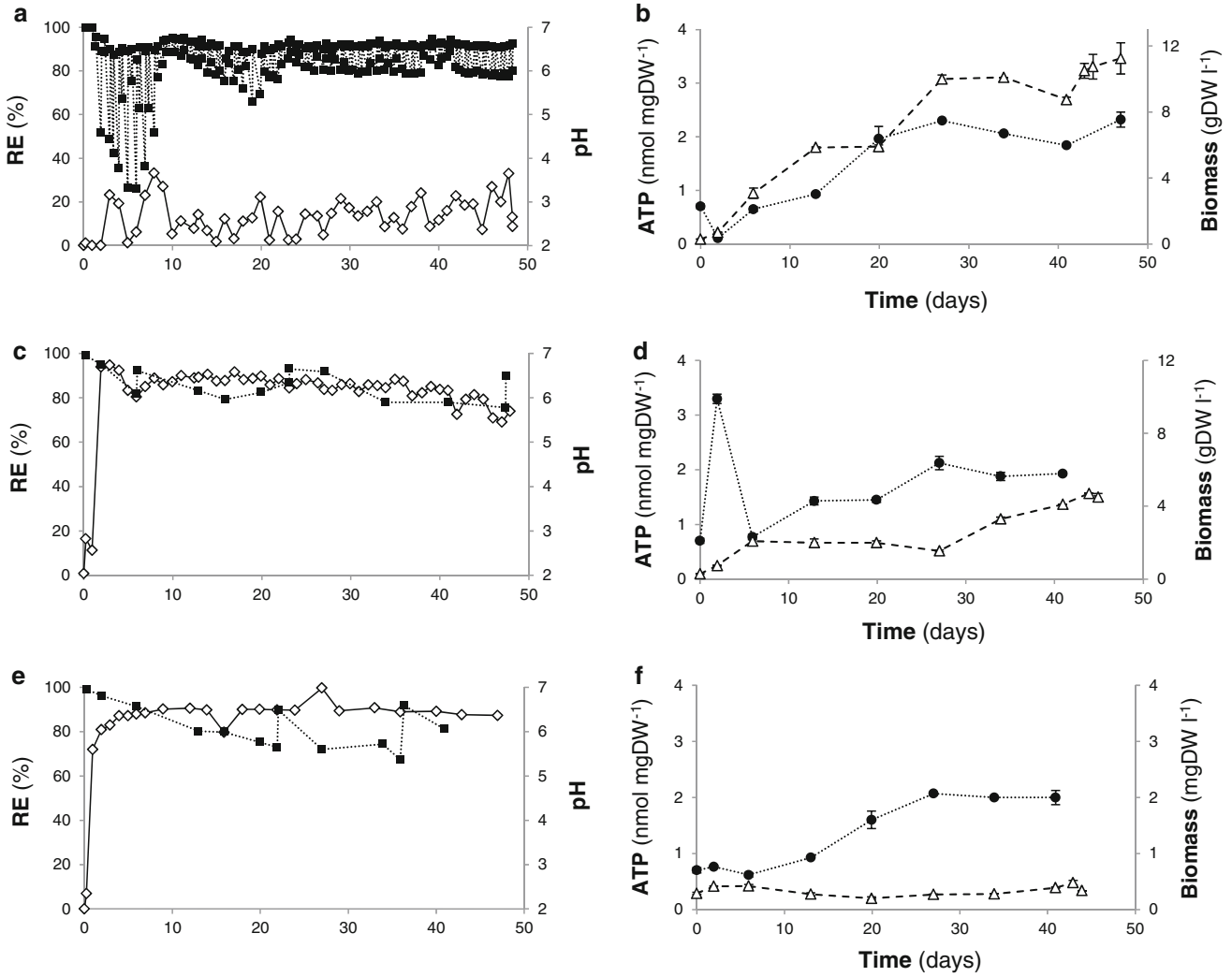

Fig. 2 Time course of pH (*filled square*) and toluene removal efficiency (*open diamond*) in R1 (**a**), R2 (**c**) and R3 (**e**), and biomass concentration (*open triangle*) and specific ATP content (*filled circle*) in R1 (**b**), R2 (**d**) and R3 (**f**)

bioreactors, theoretical calculations based on empirical $K_L a$ and concentration gradients for toluene and oxygen in R1, R2 and R3 clearly show that R2 and R3 were not operated under O_2 limiting conditions (see Supplementary Material).

No lag phase in toluene biodegradation was observed in the reactor operated at 300 mg m^{-3}, where the RE reached a value of 94 % within the first 2 days of cultivation (Fig. 2c). In this case, the average EC during the operation was 14.8 ± 1.7 g m^{-3} h^{-1}, corresponding to a 90 % of RE, which agrees well with the values reported for suspended biomass airlift systems where complete biodegradation has been reported at inlet loads below 35 g m^{-3} h^{-1} [20]. The ATP content rapidly increased from 0.7 to 3.3 nmol mg DW^{-1} concomitant with a sudden increase in RE, but decreased again to 0.8 nmol mg DW^{-1} by day 6 (Fig. 2d). In this context, Bordel et al. [10] reported a significant correlation between toluene degradation rates and the specific ATP concentration during process start-up. A slight decrease in both RE and pH occurred from days 4 to 6 but MSM exchange rapidly restored process performance to steady state values (90 %). From day 20 onwards, REs gradually decreased to 74 % at the end of the experiment, while the cell energy content steadily increased from day 6 to final level off at 1.99 ± 0.13 nmol mg DW^{-1} by day 27. Measurements of the aqueous toluene concentration revealed that biomass in R2 was exposed to negligible concentrations (0.80 μg l^{-1} compared to theoretical 110 μg l^{-1} in equilibrium with the outlet gaseous stream), which suggests that the process was limited by toluene mass transport (See supplementary material). Biomass concentration also increased from 0.3 to 4.7 g DW l^{-1} at day 44.

In the reactor operated at 11 mg m^{-3}, toluene RE rapidly increased up to 72 % within the first 24 h of experimentation and gradually leveled off to steady state values of 90 ± 4 %

by day 12 (Fig. 2e). The average EC recorded for R3 was 0.6 ± 0.1 g m^{-3} h^{-1}. Although this value might seem low at first sight, it is important to remark that the EC values are closely related to the toluene inlet loading rate used. Thus, for instance, EC of 1.2 mg m^{-3} h^{-1} was recorded in a real scale biofilter treating toluene at concentrations of 0.15 mg m^{-3} in a real odorous complex stream [21]. The specific microbial ATP content remained constant at 0.70 ± 0.07 nmol mg DW^{-1} within the first 13 days and increased afterward up to 2.02 ± 0.04 nmol mg DW^{-1} (Fig. 2f). Despite the differences in toluene concentration (up to 3 orders of magnitude), comparable specific ATP contents were recorded in R1, R2 and R3. These results are in agreement with Martinez-Lavanchy et al. [22] who reported ATP levels of 1.2 nmol mg DW^{-1} for *Pseudomonas putida* mt-2 during toluene degradation under oxygen limiting conditions. The slow variation of the ATP levels over the time course of the experiment together with its final convergence suggest that most of the specific ATP values reported in short-term experiments [10] should be carefully considered. More detailed studies should be conducted to elucidate the reasons underlying the similar ATP content recorded under such varied growth conditions. In this context, measurements of the specific ADP content and the ADP/ATP ratio would help clarifying this convergence. Interestingly, the rate of pH decrease in R3 (0.060 units day^{-1}) was comparable to that in R2 (0.064 units day^{-1}), which suggests that R3 community was less efficient assimilating toluene into biomass than R2 (assuming a pH decrease caused by metabolite excretion), despite its lower carbon source availability. This finding is in agreement with the observations of Subba-Rao et al. [23] who reported that at trace level concentrations the aromatics pollutants were not assimilated into cell constituents. According to estimations based on Henry's Law, gas phase concentrations and diffusivities, no oxygen limitation occurs in R2 or R3 (see Supplementary Material).

Despite being obvious that inoculum acclimation for VOC biodegradation processes should match as close as possible the conditions expected during the process, there are several examples in literature where the inoculum was acclimated to gaseous VOC concentrations much higher than those present during the subsequent biodegradation process. In these studies, the long start-up periods reported might be likely due to a re-acclimation stage at the beginning of the process. For instance, Hori et al. [4] acclimated a toluene degrading inoculum at gas phase concentrations of 100 g m^{-3}, while the VOC gas phase concentration during the biodegradation process was 0.8 g m^{-3} ($\approx$100 times lower). Likewise, Mathur et al. [5] acclimated a BTEX degrading inoculum at a gas phase concentration of 460 g m^{-3} prior to inoculation in a biofilter treating BTEX at 4 g m^{-3}, with a start-up period of 30 days after inoculation. Moreover, Jin et al. [7] acclimated an alpha-pinene degrading inoculum at gas phase concentrations of 41 g m^{-3} in glass bottles. Afterwards, the inoculum was tested in a biofilter treating 0.3 g m^{-3} leading to a lag period of 28 days. Interestingly, such long start-up periods are similar to those recorded for non-acclimated inocula [24]. Therefore, several experiments were here performed to assess key differences in the consortia isolated at several gas VOC concentrations.

Toxicity tests

Toluene severely inhibited the specific O_2 consumption rates of the microbial communities from R2 and R3 compared to R1, regardless of the toluene concentration and day of cultivation (Fig. 3a, b, c). The microbial communities from R1 always showed a higher degree of tolerance, with a slight decrease from 56 to 48 mgO_2 g DW h^{-1} when toluene was increased from 5 to 50 mg l^{-1} at day 16. These findings clearly confirmed that the exposure of a microbial community to a harsh environment (high toluene concentration, low pH and presence of significant amounts of toxic metabolites) resulted in the isolation of bacteria highly tolerant to pollutant toxicity. In this context, the modification of the membrane structure and the presence of VOCs extrusion via efflux pumps are among the most common mechanisms underlying VOCs tolerance [25]. The respiration rates here obtained were $\sim$20 times lesser than those found by Muñoz et al. [8] for the highly tolerant *P. putida* F1 at 25 mg toluene l^{-1}. Thus, despite the high selective pressure imposed by the high toluene concentration supplied to R1, which ranked among the highest found in real industrial emissions, the microbial consortium here isolated presented a lower tolerance toward toluene than highly specialized bacterial strains such as *P. putida* MTCC 1194 [26].

Toluene concentrations of 35 and 50 mg l^{-1} severely inhibited R2 and R3 consortia, which exhibited specific O_2 consumption rates below the endogenous metabolisms at these concentrations, regardless of the cultivation day. The results here obtained also showed a lower degree of tolerance in both consortia with the time course of the experiment, although both consortia were still capable of slowly degrading toluene at 5 and 10 mg l^{-1}. It is noteworthy that R2 and R3 consortia showed a similar response to toluene toxicity, which can be explained by the fact that both communities were exposed to comparable and extremely low aqueous toluene concentration of 0.80 μg l^{-1} in R2 and 0.09 μg l^{-1} in R3.

Surprisingly, despite the above-described differences, all microbial communities presented similar endogenous respiration rates, which correlate with the converging specific energetic values. Finally, the decreasing specific toluene respiration rates recorded for all consortia with the time course of the experiment were likely due to microbial

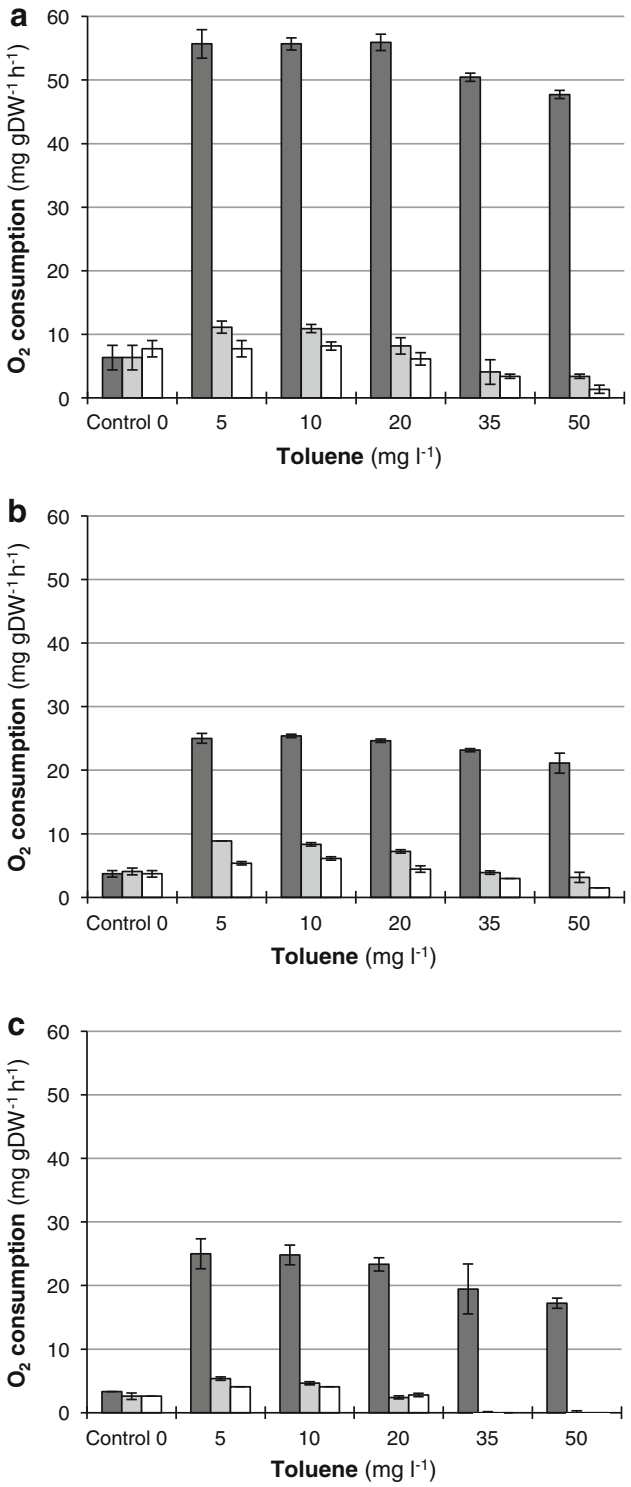

Fig. 3 Influence of toluene concentration on the oxygen consumption rate by the microbial consortia established in R1 (*dark gray bar*), R2 (*light gray bar*) and R3 (*open bar*) at days 16 (**a**), 28 (**b**) and 48 (**c**). *Error bars* represent the standard deviation from duplicate experiments

aging increasing the presence of inactive cells as a result of biomass retention in the systems (MSM exchange routines included the return of the biomass drawn prior centrifugation and cell re-suspension in fresh sterile medium).

Toluene biodegradation kinetic assays at trace level concentration

The abiotic control tests showed that toluene degradation from an initial aqueous concentration of 16.0 ± 0.6 μg l^{-1} was entirely due to microbial activity (Fig. 4). After 21 days of cultivation (Fig. 4a), R2 showed the most efficient toluene degradation at trace level concentrations, degrading 58 % of the initial toluene in 8 h. Toluene biodegradation by R1 and R3 consortia leveled off by the 2nd hour of assay to finally resume at the 4th hour and reach toluene consumptions of 46 and 41 %, respectively, by the 8th hour of experimentation. Likewise, on day 35 (Fig. 4b), R2 exhibited the best toluene degradation performance (89 % toluene removal in 8 h), followed by R3 (62 % in 8 h) and R1 (32 % in 8 h). After 45 days of cultivation (Fig. 4c), the community adapted to the lowest toluene concentrations (R3) showed the highest biodegradation rates (71 % in 4 h) despite the slightly longer lag phase. The community from R2 gradually degraded 60 % of the initial toluene in 8 h, while R1 supported the lowest removal efficiency in 8 h (46 %). Despite R1 consortium supported the poorest toluene biodegradation performance at trace level concentrations, it was still capable of degrading toluene at concentration 2,500 times lower than those present in R1 during its isolation. In addition, the community adapted to low toluene concentrations seems to gradually improve its biodegradation performance with the time course of the isolation experiment.

This constitutes, to the best of our knowledge, one of the few studies assessing toluene biodegradation at trace level concentrations [27]. Most of the research carried out to date on the microbiology of toluene biodegradation was performed at concentrations far from those found in full-scale facilities treating industrial and odorous off-gases, probably due to the analytical difficulties entailed. In this regard, these novel insights showed that traditional isolation techniques based on toxicity tolerance (using high VOC concentrations) may be inefficient when the VOC is present at trace level concentrations.

Molecular profiling of the bacterial communities

The Shannon-Wiener diversity index (*H*) takes into account both the number (richness) and the evenness of the

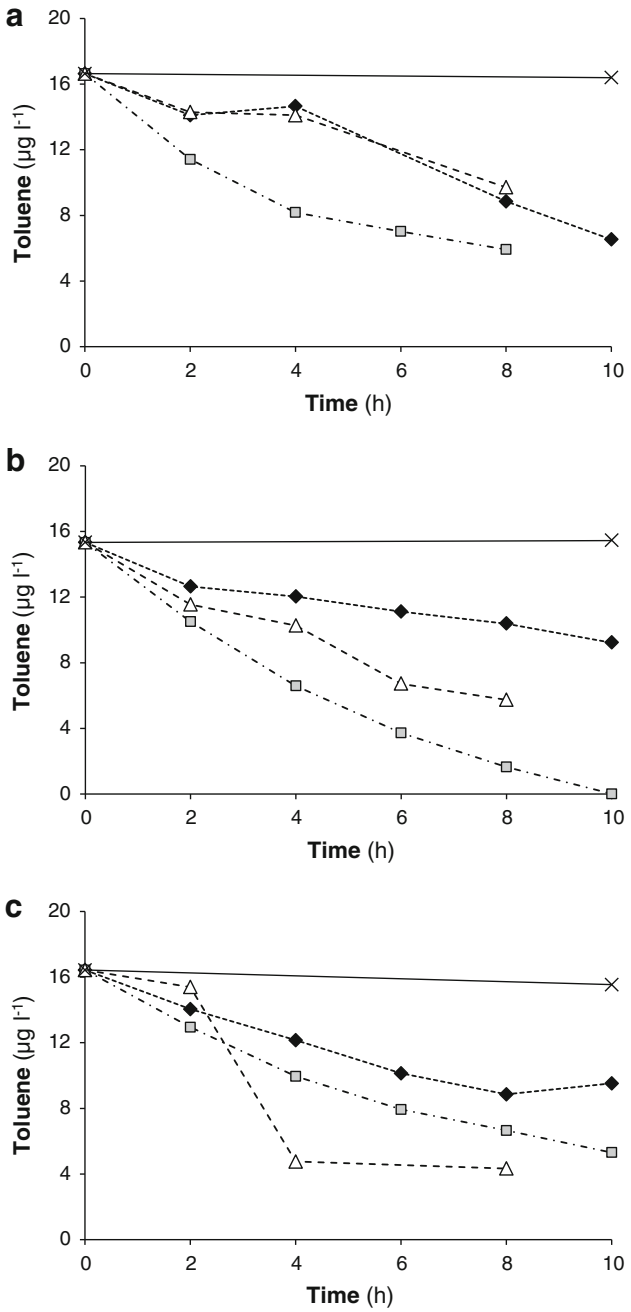

Fig. 4 Time course of the aqueous toluene concentration during the kinetic assays carried out on days 21 (**a**), 36 (**b**) and 45 (**c**) with the microbial communities established in R1 (*filled diamond*), R2 (*filled square*) and R3 (*open triangle*). Abiotic control tests were also performed (*times symbol*)

species (evaluating and comparing the intensity of the bands), allowing to obtain semi-quantitative results from the DGGE analysis. Typical *H* values range from 1.5 (low species evenness and richness) to 3.5 (high species evenness and richness). Toluene supply to the reactors brought about a deterioration in the microbial diversity at day 14, with the *H* indices decreasing from 3.22 (day 0) to 2.05, 1.93 and 2.74 in R1, R2 and R3, respectively (Fig. 5a). This deterioration has been previously observed in microbial communities exposed to aromatic hydrocarbons and was likely due to the high toxicity of these compounds [28]. It is noteworthy that despite the difference in toluene inlet concentration, R1 and R2 underwent a comparable biodiversity loss, since the number of bands decreased from 30 (at day 0) to 8 and 9, respectively, while 21 bands were detected in R3 by day 14. Despite the initial diversity reduction, R2 and R3 were able to recover high *H* values (3.06 and 3.23, respectively) by day 48 of operation. However, this recovery was not observed in R1, whose *H* index further decreased to 1.85 (7 bands recorded at day 48). In addition to the harsh conditions above mentioned (pH decreases and metabolites accumulation), the oxygen limitation suffered by microorganisms in R1 probably contributed to this diversity simplification. These results are in agreement with the findings of Bayle et al. [29], who reported a simplification of the bacterial community structure at high VOC loading rates and a high diversity at low loading rates. Likewise, Lebrero et al. [30] also observed a high bacterial diversity in an activated sludge diffusion system treating a complex mixture of VOCs at trace level concentrations.

The DGGE profiles (Fig. 5b) showed consistent results with the Shannon diversity indices. The seed sample (day 0) and the samples collected in R2 and R3 at day 48, which showed the highest diversity values, clustered separately and presented a similarity lesser than 40 % with the rest of the samples analysed. The DGGE profiles of samples R1, R2, R3 at day 14 and R1 at day 48 clustered together with a similarity of 66 %.

Finally, it must be stressed that it is hard to separate the influence of O_2 and toluene during the bacterial enrichment process in R1. Oxygen limitations probably resulted in the isolation of k_s strategic microorganisms for O_2, but also highly resistant to high toluene and metabolites concentrations, as showed by toxicity tests. On the other hand, the fact that oxygen was not limiting in R2 and R3 but the consortia obtained exhibited different characteristics (toxicity tolerance and ability to degrade toluene at trace level concentrations) suggests that toluene concentration was the main driving force on bacterial selection in R2 and R3.

Molecular composition of the bacterial communities

A total of 22 bands were sequenced from the DGGE gel. Taxonomic assignments of the DGGE bands were determined using the RDP Classifier tool [12] with 80 % confidence level and BLAST search tool (retrieving the closest cultured and uncultured relatives). A similarity cut off of 97 % was used for the defined genera using the public database Genbank. This information is summarized in

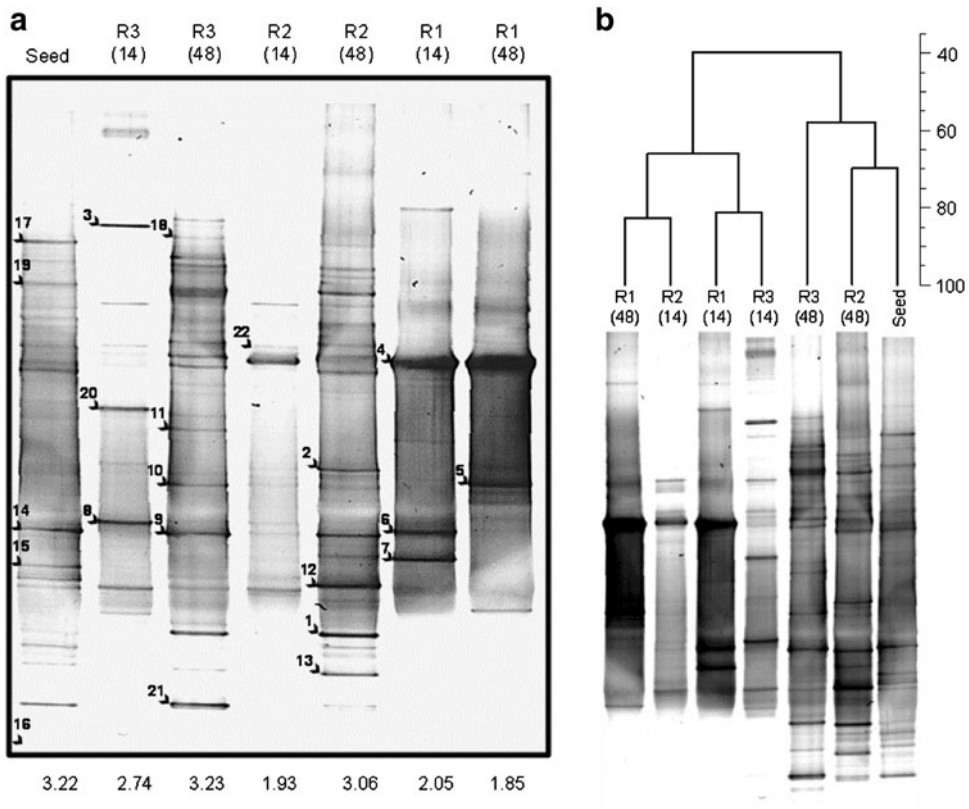

Fig. 5 **a** Bacterial community fingerprint. Sequenced bands are indicated by *arrows* with their corresponding number. Shannon-Wiener diversity values are indicated in the *lower part* of the gel. The samples analyzed and each corresponding sampling day are shown in the *upper part* of the gel. **b** Similarity dendrogram (UPGMA clustering)

Table 1 along with the presence of the sequenced bands in each reactor and the environment where their closest relatives were found. The results from the RDP tool were consistent with those obtained from the NCBI database, except for bands 14, 15, 21 and 22, which were differently classified by these two approaches. None of the sequences remained unclassified with an 80 % confidence level using the RDP classifier tool, which showed confidences values between 100 and 97 % at the phylum level. Representatives of the *Proteobacteria*, *Actinobacteria*, *Verrucomicrobia*, *Nitrospira* and *Chlamydiae* phyla were retrieved from the different samples analysed.

All bands sequenced from R1 (those of higher intensity) were only affiliated to the phylum *Proteobacteria*, specifically the *Gammaproteobacteria* class. The dominant bacteria in R1 (band 4) was classified in the *Acinetobacter* genus (100 % confidence level based on the RDP classifier). Fragments 6, 7 and 5 were also affiliated to the *Moraxellaceae* family of the order *Pseudomonadales* (70–100 % confidence level). Members of *Acinetobacter* are well-known solvent-tolerant microorganisms capable of growing at the high toluene concentrations present in R1 [25]. At day 14, *Proteobacteria* (band 4) was also dominant in R2, while *Proteobacteria* (band 3 and 8) and *Verrucomicrobia* (band 20) were the dominant phyla in R3. Members of the *Parachlamydia* genus, also present in the inoculum, were detected in R2 and R3. At day 48, *Actinobacteria* was the most abundant phylum in R2 and R3, with most of the bands belonging to the *Pseudonocardia* genus (bands 9, 10, 11 and 12) and *Mycobacterium* (fragment 13, 97 % confidence level). Moreover, members of the nitrifying *Nitrospira* genus (band 21, 100 % confidence level) within the *Nitrospira* phylum were also detected in R2 and R3. It has been shown that nitrifying consortia are capable of oxidizing a broad range of aromatic and non-aromatic hydrocarbons [31]. Lebrero et al. [30] also reported the presence of *Nitrospira* members in an activated sludge diffusion system treating toluene, butanone and α-pinene at trace level concentrations.

The differences observed in the structure and composition of the bacterial communities at the end of the experiment suggest two well-differentiated bacterial growth patterns. Some members of the genus *Acinetobacter*, like the few and dominant microorganisms in R1, have been proven to be fast-growing bacteria capable of growing at high toluene concentrations [32]. On the other hand, microorganisms belonging to the *Pseudonocardia*, *Mycobacterium, Nitrospira* genus and members of the *Rhodocyclales* and *Rhodospirillales* orders (found in R2 and R3) are slow-growing bacteria, which are also capable of degrading toluene at low concentrations [33]. These differences could be explained in terms of the μ_{max}/K_s selection theory: μ_{max}-strategic microorganisms (fast growing) will establish in resource-abundant environments

Table 1 Classification of the DGGE sequences (from phylum to genus ranks) determined using the RDP Classifier tool (bootstrap value of 80 %)

Phyla	Class	Order	Family	Genus	Band no	In	R3 48	R2	R1	R3 14	R2	R1	Closest relatives in Blast	Similarity (%)	Environment
Proteobacteria	α	Rhodospirillale	Rhodospirillaceae 39 %	*Oceanibaculum* 20 %	1	X	X	X					Uncultured clone (FJ433554)	99	Soil environment
													Uncultured α-Proteobacteria	98	Rizosphere soil
	β	Rhodocyclales	Rhodocyclaceae 46 %	*Sterolibacterium* 15 %	2			X					Uncultured clone (FJ375379)	96	Fuel cells (domestic wastewater)
													Uncultured Zooglea sp. (HQ184339)	93	Aerobic granules (fish canning effluents)
	δ	Bdellovibrionales	Bdellovibrionaceae **100 %**	*Bdellovibrio* **100 %**	3					X			Uncultured clone (DQ984624)	99	Oil-contaminated soil
													Bdellovibrio bacteriovorus (T) (NR027553)	96	Culture collection (gut of mammals)
	γ	Pseudomonadales	Moraxellaceae **100 %**	*Acinetobacter* **100 %**	4	X			X		X	X	*Acinetobacter gerneri* (T) (HQ180188)	99	Culture collection
			Moraxellaceae **100 %**	*Acinetobacter* 48 %	5				X					94	
			Moraxellaceae **97 %**	*Acinetobacter* 54 %	6							X		96	
			Moraxellaceae **70 %**	*Enhydrobacter* 51 %	7							X		92	
		Xanthomonadales	Sinobacteraceae **100 %**	*Hydrocarboniphaga* **80 %**	8					X			Uncultured clone (EU088414)	100	Microbial fuel cells
													Hydrocarboniphaga effusa (T) (NR029102)	99	Culture collection (alkane degrader)
Actinobacteria	*Actinobacteria*	Actinomycetales	Pseudonocardiaceae **100 %**	*Pseudonocardia* **100 %**	9		X	X					*Pseudonocardia aurantica* (T) (FR749916)	98	Culture collection
				Pseudonocardia **90 %**	10		X							97	
				Pseudonocardia 60 %	11		X							96	
				Pseudonocardia **100 %**	12	X	X	X					*Pseudonocardia sulfidoxydans* (T) (NR029324)	99	Culture collection ($(CH_3)_2S$ degrader)
			Mycobacteriaceae **97 %**	*Mycobacterium* **97 %**	13	X	X	X					Uncultured clone (HM269900)	99	Intramural skin microbiome consortium
													Mycobacterium novocastrense (T) (NR029208)	98	Culture collection
		Acidimicrobiales	Lamiaceae **90 %**	*Iamia* **90 %**	14	X							Uncultured Actinobacteria (CU924480)	100	Anaerobic digester (municipal sludge)
													Microthrix parvicella (×89774)	99	Activated sludge
		Acidimicrobiales	Lamiaceae **88 %**	*Iamia* **88 %**	15	X							*Microthrix parvicella* (×89774)	97	Activated sludge
		Bifidobacteriales	Bifidobacteriaceae **100 %**	*Bifidobacterium* **100 %**	16	X							*Bifidobacterium adolescentis* (T) (AB437354)	99	Culture collection
													Uncultured *Bifidobacterium* sp. (AF275884)	99	Human faeces
Verrucomicrobia	*Verrucomicrobiae*	Verrucomicrobiales	Verrucomicrobiaceae **100 %**	*Prosthecobacter* **100 %**	17	X							Uncultured clone (FJ873605)	100	Aged PHA-contaminated soil
													Prosthecobacter fluviatilis (T) (AB305640)	98	Culture collection
					18		X							98	Lake mesocosms
					19	X								96	Culture collection
				Verrucomicrobium **100 %**	20					X			*Verrucomicrobium spinosum* (T) (NR026266)	99	Culture collection

Table 1 continued

Phyla	Class	Order	Family	Genus	Band no	In	R3 48	R2	R1	R3 14	R2	R1	Closest relatives in Blast	Similarity (%)	Environment
Nitrospira	*Nitrospira*	Nitrospirales	Nitrospiraceae **100 %**	*Nitrospira* **100 %**	21	X	X	X					"*Candidatus* Nitrospira defluvii" (FP929003)	99	Activated sludge enrichment culture
													Nitrospira moscoviensis (T) (NR029287)	94	Culture collection
Chlmydiae	*Chlmydiae*	Chlamydiales	Parachlamydiaceae **100 %**	*Parachlamydia* **89 %**	22	X		X		X	X		*Protochlamydia naegleriophila* (T) DQ632609	95	Culture collection
													Parachlamydia acanthamoebae (T) NR026357	94	Culture collection

Bootstrap values for the phylum, class and order taxa were always ≥80 % (except for the Rhodocyclales order). Bootstrap values ≥80 % for the rest of the taxa are in bold face. The cultured (T) and uncultured closet relatives (with their respective similarity percentages) are also shown in the right part of the Table

(e.g. high toluene concentration), whereas K_s strategic will dominate in resource-limited environments (e.g. aqueous toluene concentrations in R2 and R3 of 0.63 and 0.09 μg l^{-1}, respectively) [33]. At this point, it must be highlighted that the operation mode here selected (periodical medium exchange with biomass return) did not cause bacterial dominance by dilution rate.

In addition, the low K_s attributed to the highly diverse communities established in R2 and R3 might be confirmed by the higher biodegradation rates recorded when toluene was present at low concentrations (Fig. 4). This suggests that the recent practices of some manufacturers of biological odor treatment technologies of inoculating their 3rd generation biofilters with specialized microorganisms might be inefficient, if these microorganisms have been isolated and cultured at high pollutant concentrations.

Conclusions

The results obtained showed that inocula for biological off-gas treatment based on toxicity tolerance (exposure at high VOC concentrations) are inefficient when the VOC in the off-gas to be treated is present at trace level concentrations. Despite the traditional isolation technique resulted in a highly tolerant consortium, this inoculum exhibited a poor diversity and low abatement performance when facing low VOC concentration scenarios. On the contrary, the inoculum isolated at low VOC concentrations was constituted by diverse microbial communities, highly sensitive toward toluene toxicity but highly efficient degrading VOC at trace level concentrations. These results bring new insights on the development of novel inoculum production protocols in order to optimize both the start-up period and the stability of bioreactors devoted to treat diluted off-gas streams. As future work, further experiments using biofilm reactors should be performed in order to confirm the same behavior here recorded for suspended biomass, as biofilm reactors are the most common systems applied for polluted gas and odor treatment.

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A comparative study of fungal and bacterial biofiltration treating a VOC mixture.

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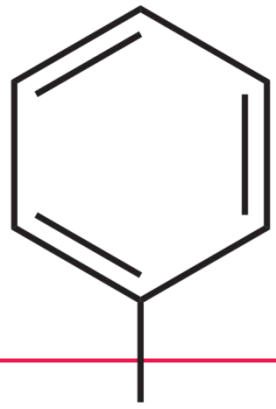
Chapter 7



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A comparative study of fungal and bacterial biofiltration treating a VOC mixture

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HIGHLIGHTS

- Bacterial biofilter showed better EC and ΔP than fungal biofilter.
- The preferential biodegradation order was: propanal > hexanol > MIBK > toluene.
- Propanal partially inhibited the biodegradation of the rest of VOCs.
- The two-stage biofilter showed a higher stability than the individual units.

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ABSTRACT

Bacterial biofilters usually exhibit a high microbial diversity and robustness, while fungal biofilters have been claimed to better withstand low moisture contents and pH values, and to be more efficient coping with hydrophobic volatile organic compounds (VOCs). However, there are only few systematic evaluations of both biofiltration technologies. The present study compared fungal and bacterial biofiltration for the treatment of a VOC mixture (propanal, methyl isobutyl ketone-MIBK, toluene and hexanol) under the same operating conditions. Overall, fungal biofiltration supported lower elimination capacities than its bacterial counterpart (27.7 ± 8.9 vs 40.2 ± 5.4 g C m^{-3} reactor h^{-1}), which exhibited a final pressure drop 60% higher than that of the bacterial biofilter due to mycelial growth. The VOC mineralization ratio was also higher in the bacterial bed (≈63% vs ≈43%). However, the substrate biodegradation preference order was similar for both biofilters (propanal > hexanol > MIBK > toluene) with propanal partially inhibiting the consumption of the rest of the VOCs. Both systems supported an excellent robustness versus 24 h VOC starvation episodes. The implementation of a fungal/bacterial coupled system did not significantly improve the VOC removal performance compared to the individual biofilter performances.

1. Introduction

The increasing need for economic and sustainable technologies to abate odors and industrial gaseous pollutants has gradually shifted the attention to biological waste gas treatment technologies [1,2]. Despite the wide range of bioreactor configurations, (e.g. biofilter, biotrickling filters, bioscrubbers, activated sludge diffusion, two-phase partitioning systems, etc.), conventional biofilters still constitute the most commonly implemented technology at full scale due to their relative simplicity, lower investment costs and the extensive experience gained in their design and operation over the last 20 years [3].

In general, biofiltration activity is mainly due to bacteria which exhibit a high diversity and versatility when treating VOC mixtures [4–6]. However, despite that bacterial biofilters have been reported as a robust technology for off-gas treatment, their performance rapidly deteriorates at low moisture contents, low pH values and nutrients limiting scenarios [7]. On the other hand, fungal biofiltration has been claimed to better withstand these harsh environmental conditions [8,9]. In addition, the fungal composition and its mycelial aerial growth can significantly increase the mass transfer of hydrophobic VOCs from the gas phase to the biomass [10]. Hence, VOCs such as toluene, styrene, α-pinene or hexane have been successfully treated in fungal biofilters at unprecedentedly high elimination capacities [11–14] despite the fact that fungi have lower specific degradation rates [15]. However, extensive fungal biomass growth, may lead to packing media clogging which has been reported to be the most important drawback in fungal biofiltration, particularly at high VOC concentrations [11,16]. Despite the recent advances on the understanding of both fungal and bacterial biofiltration, there is a lack of systematic comparative studies assessing their performance under identical operational conditions, especially when a mixture of pollutants is studied.

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The present work compared the performance of a fungal and a bacterial biofilter for the treatment of a complex VOC mixture. Their response was compared to that of a hybrid system, consisting of a bacterial biofilter in series with a fungal biofilter.

2. Materials and methods

2.1. Microorganisms

The fungal biofilter was inoculated with fungal biomass from a seed biofilter inoculated with *Paecilomyces variotii* and treating the same VOC mixture used in this study. Inoculation was conducted by mixing 50% of the packing material from the seed biofilter with 50% of sterilized perlite in a sterile chamber. The initial fungal biomass concentration in the biofilter was 4.1 g VSS L^{-1} bed. The bacterial biofilter was inoculated with aerobic activated sludge from the wastewater treatment plant attain a final concentration of 0.7 g VSS L^{-1} bed.

2.2. Chemicals and mineral salt medium

Propanal and hexanol with purity higher than 97% (reagent grade) were purchased from Sigma–Aldrich (Germany). Methyl Isobutyl Ketone (MIBK) and toluene were purchased from Reasol (México) with a 99.5% purity. The mineral salt medium (MSM) used for irrigation in both biofilters was described by Vergara-Fernández et al., 2012 with the only modification of 6 instead of 18 g l^{-1} $NaNO_3$ [17]. The pH was adjusted to 4.0 with HNO_3 for the irrigation of the fungal biofilter and to 7.0 with NaOH for the bacterial biofilter. The antibacterial agent Chloramphenicol (0.5 g L^{-1}) (Sigma–Aldrich, USA) was added to the MSM used for the irrigation of the fungal biofilter.

2.3. Experimental setup

Two glass columns of internal diameters 7.8 and 7.2 cm were used as fungal and bacterial biofilters, respectively (See supporting material Figure S1). Both columns were filled with sterile vermiculite (particle size 2.4–5.0 mm) to a final working volume of 2 L. Both packing media were then soaked with their corresponding MSM to obtain a 60% moisture and a wet packing density of 400 g L^{-1}.

The gaseous VOC mixture was obtained by addition of liquid propanal, MIBK and toluene using a syringe pump into a mixing chamber where the VOCs evaporated in a continuous air flow of 320 mL min^{-1}. An air stream, (0.290 L min^{-1}), saturated with hexanol vapor was combined with the main VOC-laden stream and the mixture further diluted with 3.4 L min^{-1} of water-saturated air to attain the target VOC concentrations (Propanal = 0.65 ± 0.16 g m^{-3}, MIBK = 0.96 ± 0.25 g m^{-3}, toluene = 0.99 ± 0.27 g m^{-3} and hexanol = 0.17 ± 0.04 g m^{-3}). Total organic C loading rate applied was 105.4 ± 23.8 g C m^{-3} reactor h^{-1} and 104.8 ± 20.5 for the fungal and bacterial biofilters, respectively. Finally, the water-saturated VOC stream was equally split and fed in parallel to the fungal and bacterial biofilters for 60 days. The operational strategy was the same for both biofilters: a startup period of 11 days with an empty bed residence time (EBRT) of 90 s to allow for microbial acclimation. The EBRT was then reduced to 60 s and maintained thereafter. On day 19, the air flow was reversed in both biofilters from a downwards to an upwards configuration to promote better biomass distribution. On day 48, propanal was removed from the VOC mixture to assess its influence on the biodegradation of the other VOCs. Biofilter irrigation (150 mL of pH adjusted MSM) was performed intermittently until day 32 and afterwards every five days. The pH of the leachate was measured and re-adjusted before recycling it when pH values fell below 5 in the bacterial biofilter leachate in order to avoid a pH-mediated inhibition and the proliferation of fungal communities. The pressure drop was weekly measured in both biofilters with a manual differential pressure meter.

At day 60, a series configuration was set by connecting the inlet of the fungal biofilter to the outlet of the bacterial biofilter. The entire water-saturated VOC stream (4.0 L min^{-1}) was fed to this combined system, which resulted in an overall EBRT of 1 min (30 s in each reactor). The VOC concentrations were adjusted to maintain a total carbon loading rate of 96.3 ± 4.9 g C m^{-3} reactor h^{-1} (Propanal = 0.47 ± 0.07 g m^{-3}, MIBK = 0.74 ± 0.07 g m^{-3}, toluene = 0.75 ± 0.07 g m^{-3}, hexanol = 0.13 ± 0.03 g m^{-3}). Results from the biofiltration experiments are expressed in terms of inlet loading rate, biofilter elimination capacity (EC) and percent removal efficiency (RE) as previously described [12].

2.4. VOC starvation experiment

An interruption of the VOC supply (while maintaining the air flow) to both biofilters was carried out on day 41 for 24 h to characterize the VOC desorption/adsorption dynamics and the recovery of the biodegradation performance following VOC supply. Both the total organic carbon and CO_2 concentrations were continuously monitored at the gas outlet of both systems during the experiment.

2.5. Analytical procedures

The VOC concentration was analyzed by GC-FID as described by García-Peña et al., 2008 [18] The total organic carbon concentration in the biofilter outlet during the VOC starvation experiment was measured with a PID Continuous Gas Monitor PI 201, HNU Systems Inc., previously calibrated with the VOCs mixture. CO_2 concentration was measured in an infra-red ZRH Fuji Electric analyzer (California Analytical Instruments) calibrated from 0 to 2000 ppm with N_2. When CO_2 concentrations exceeded that range an infra-red ZFP-9 analyzer (California Analytical Instrument) was employed.

The identification of the bio-degradation intermediates was carried out by solid phase micro extraction (SPME). A carboxen/polydimethylsiloxane (PDMS) fiber (Supelco, USA) was exposed for 3 min to the headspace of a 1.5 mL gas-tight vial containing 0.5 mL of the liquid sample. The liquid samples analyzed included the irrigation leachates and the outlet gas condensates (obtained by cooling the outlet stream to 4 °C) of both biofilters on days 47 and 67 (day 6 of in-series operation). The SPME fiber was then desorbed in a 6890 GC (Agilent Technologies) equipped with an Agilent 19091S-433 capillary column and a mass spectrometry detector MS 5975B VL MSD (Agilent Technologies). The injector temperature was maintained at 300 °C while the initial oven temperature steadily increased at 10 °C min^{-1} from 60 °C up to 140 °C. The total column flow was 23.1 mL min^{-1}, MS source temperature 230 °C and the quadrupole temperature 150 °C.

3. Results and discussion

3.1. Fungal biofilter

An intensive growth of the fungal biomass was observed from day 0 to day 11 concomitant with an increase in the total EC, which reached a maximum of 52 g C m_{bed}^{-3} h^{-1} on day 6. Complete biodegradation of propanal was observed from day 1 (Fig. 1 A), which can be attributed to the fact that the fungal inoculum was obtained from a biofilter treating the same VOC mixture and preferentially degrading propanal. The REs for MIBK increased during the first three days of operation up to a maximum of 73%, but

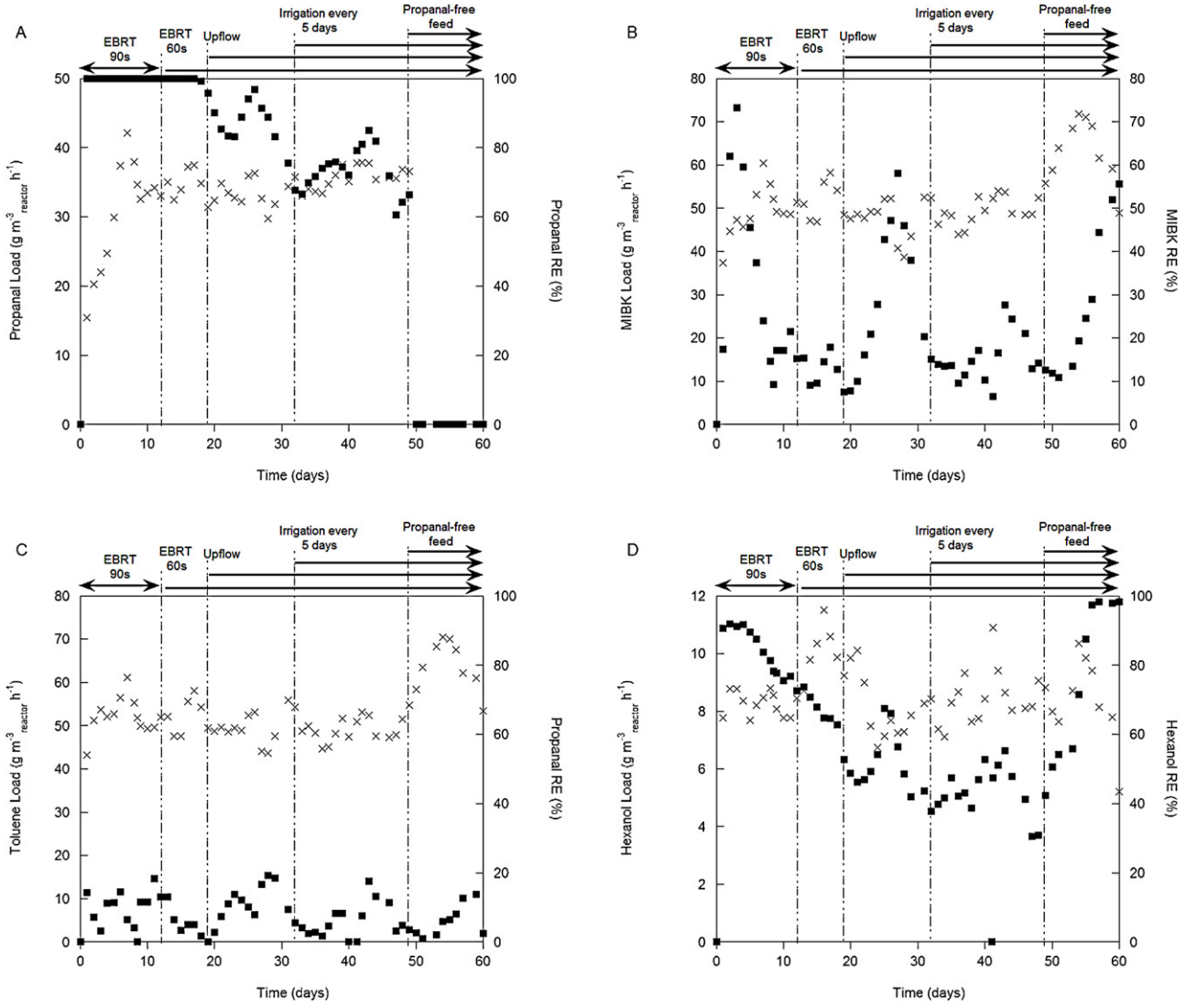

Fig. 1. Time course of the loading rate (×) and RE (■) of propanal (A), MIBK (B), toluene (C) and hexanol (D) in the fungal biofilter. Vertical dash-dotted lines indicate the changes in the operating conditions.

decreased afterwards to remain stable at 15.0 ± 5.3% (Fig. 1 B). Toluene biodegradation during the start-up period, as well as during the entire experiment, was poor and remained always below 20% (Fig. 1C). Hexanol was also efficiently degraded during the first 5 days of operation (RE of 91.0 ± 1.0%) but its removal started to decrease linearly from day 5 (Fig. 1D). The reduction of the EBRT from 90 to 60 s did not have a significant impact on the degradation of any of the target VOCs: propanal was fully depleted, MIBK degraded at ≈15%, toluene removal remained lower than 20% and the deterioration in hexanol biodegradation continued at similar rates.

The inversion of the gas flow direction on day 19 mediated an initial decrease in the RE of all treated VOCs, followed by a rapid recovery in the elimination of propanal, MIBK, toluene and hexanol. This change in the operational configuration of the biofilter promoted a more regular biomass distribution (by visual observation), with the subsequent increase in the total EC. Toluene EC also increased during this biomass growth period but remained below 20% (Fig. 1C), which confirmed the previously reported biodegradation preference pattern: oxygenated compounds > aromatic compounds [19]. VOC hydrophobicity might play an important role in the observed elimination pattern, since the preferred compounds, propanal and hexanol, also presented the lowest dimensionless Henry's Law constants (7.7×10^{-4} and 3.2×10^{-3} C_g/C_l, respectively), which might have resulted in higher mass transfer rates than those for MIBK and toluene ($H = 0.014$ and 0.27, respectively) [20]. However, the fluctuating elimination capacities of MIBK and hexanol recorded at constant VOC loading rate, suggest that microbial activity rather than mass transfer governed the biodegradation process in the fungal biofilter.

The periodical irrigation of the fungal biofilter every 5 days allowed for process stabilization, likely due to supplement of water and nutrients, and an enhanced washout of the accumulated metabolites. Under these operational conditions, the removal efficiencies achieved for propanal, MIBK, toluene and hexanol were 72.8 ± 6.6%, 15.0 ± 5.3%, 5.8 ± 4.7%, 43.4 ± 6.7%, respectively. On day 49, the polluted gas stream was deprived from propanal to assess the performance of the microbial community in the absence of the preferentially biodegraded VOC. Under this new operational scenario, hexanol RE rapidly increased up to an average of 96.0 ± 4.5% from day 56 (Fig. 1D), while MIBK biodegradation gradually improved from day 54 (five days after propanal supply was stopped) to finally achieve a RE of 56% on day 60. Despite that an increase in the toluene RE was also observed, the maximum RE achieved remained within the fluctuation range

Table 1
Average performance of the fungal, bacterial and two-stage biofilters during steady state periods (days 31–49 for fungal and bacterial biofilters and days 6–20 in two-stage biofilter).

Parameter	Fungal	Bacterial	Two stages
Loading rate ($g\,C\,m^{-3}$ reactor h^{-1})	110.2 ± 12.0	108.2 ± 8.1	98.0 ± 9.2
Average EC ($g\,C\,m^{-3}$ reactor h^{-1})	27.7 ± 8.9	40.2 ± 5.4	38.4 ± 8.0
C eliminated (%)	25.1 ± 10.2	37.1 ± 13.1	39.2 ± 8.9
C to CO_2 conversion (%)	45.9 ± 15.8	63.1 ± 11.5	86.9 ± 12.4
Pressure drop increase[a] (Pa m^{-1} bed)	91–912	91–372	1398–2003

[a] Pressure drop values account for the complete experimental period.

observed along the entire biofilter operation (Fig. 1D). Based on the results, a particular VOC biodegradation preference order can be established as follows: propanal > hexanol > MIBK > toluene. In this context, the occurrence of competitive inhibition among VOCs has been already described for fungi treating BTEX mixtures. For instance, García-Peña et al. (2008) described the preferential removal of toluene by *P. variotii* in the presence of benzene, ethyl-benzene and xylenes, which confirms that substrate competition is clearly VOC-specific [18]. On the other hand, the total C elimination capacities when propanal was removed remained similar to the previous steady state in the presence of the 4 VOCs ($23.8 \pm 12.2\,g\,C\,m^{-3}$ reactor h^{-1} *vs* $27.7 \pm 8.9\,g\,C\,m^{-3}$ reactor h^{-1}, respectively). The average carbon EC in the fungal biofilter ($27.7 \pm 8.9\,g\,C\,m^{-3}$ reactor h^{-1}) (Table 1) was in the range reported by García-Peña et al., 2008 (10–60 $g\,C\,m^{-3}$ reactor h^{-1}) at a loading rate of $250\,g\,C\,m^{-3}$ reactor h^{-1} (2.5 times higher than in the present study) in a *P. variotii* biofilter treating toluene or benzene.

Despite that high pressure drop, caused by the occupation of the free space by the mycelia, has been commonly pointed out as one of the main drawbacks of fungal biofiltration [16], the maximum pressure drop reached in the fungal biofilter after 60 days of operation was 912 Pa m^{-1} bed, which is an acceptable value for industrial biofiltration. Nevertheless, the final pressure drop in the fungal biofilter was approx. three times higher than the one recorded in the bacterial biofilter (Table 1).

3.2. Bacterial biofilter

Complete biodegradation of propanal was observed on day 11 in the bacterial biofilter for a 90 s EBRT after an initial period of rapid biodegradation followed by deterioration at day 5 and a final recovery of the biodegradation performance, which can be attributed to the acclimation of the inoculum to the pollutant mixture (Fig. 2A). The RE of MIBK and toluene remained low during this acclimation period and started to increase after the first week of operation (Fig. 2B, C). Finally, hexanol removal followed a similar trend to that of propanal during the acclimation period, to eventually become stable at RE ≈90% from day 11 (Fig. 2D). The decrease in the EBRT from 90 to 60 s did not have a significant impact on the elimination of propanal and hexanol, however MIBK and toluene achieved maximum removal efficiencies shortly after the EBRT was reduced (63.7% day 14, 100% day 13, respectively) followed by a sharp decrease in their abatement. This increase in MIBK and toluene at this reduced EBRT suggest that the bacterial biofilter was likely limited by microbial activity and the bacterial population totally acclimated, from the initial inoculation with activated sludge, during process operation at 60 s of EBRT. The decrease observed in MIBK and toluene RE from day 17 could not be attributed to nutrients or water content limitations since the irrigation with MSM performed on day 17 did not alleviate this deterioration.

Once the direction of the flow was inverted on day 19, an intensive biofilm growth was also observed all along the bed height in previously non-colonized zones, concomitant with an increase in the RE of MIBK, toluene and hexanol. This enhancement in the biodegradation performance was most significant in the case of MIBK, where REs increased from 10% to 70% by days 26 and 27. However, similarly to the fungal biofilter this temporary enhancement in the abatement performance was followed by decay in the degradation of MIBK, toluene and hexanol, which might be related to the accumulation of metabolites (Fig. 2). In this context, the characterization of the metabolites revealed the presence of 1-propanol and hexanal in both biofilters (see the Metabolites Identification section). In this context a cross inhibition effect might be hypothesized in the bacterial biofilter, where the average EC recorded during this stable period ($40.2 \pm 5.4\,g\,C\,m^{-3}$ reactor h^{-1}) was lower than the ECs commonly reported for conventional biofilters. This effect was more remarkable for toluene, where ECs higher than $100\,g\,m^{-3}$ reactor h^{-1} are commonly reported [21,22], while in this study the toluene EC never exceeded $48\,g\,m^{-3}$ reactor h^{-1}. When the feed stream was deprived from propanal (day 49), a fast increase in the RE of the remaining VOCs was observed, confirming the ability of the community to degrade them. A partial catabolic repression might be involved in the observed reduction in activity since the generalist microbial communities found in biofilters are often able to shift their metabolism in order to degrade all available pollutants under substrate-limited scenarios, while preferentially degrading some specific VOCs under non-limiting substrate conditions [23,24]. In our particular experiment, biofilter feeding with the four VOCs likely resulted in a non-limiting substrate scenario. However, when the system was deprived from propanal (the most easily transferable VOC based on its hydrophilic nature), the substrate availability in the biofilm was likely reduced despite the increase in the loading rate to restore similar carbon loadings, promoting the catabolism of the remaining VOCs. In addition, the fact that the total carbon EC remained similar in the presence and absence of propanal supports the hypothesis of a gradual shift in the metabolism to cover the bacterial carbon and energy needs.

Overall, the bacterial biofilter performed better than the fungal biofilter under stable operating conditions for all tested VOCs (Table 2). These findings can be attributed to the higher microbial diversity presumably established on bacterial biofilters inoculated with activated sludge despite the fact that they have been reported to show reduced mass transfer conditions when compared to fungal biofilters [9]. Recent studies have reported a high microbial diversity in biofilters treating gaseous VOCs mixtures [4,5], and the key role of biodiversity on the robustness and performance of biofiltration processes [25]. The VOC mineralization ratio also constitutes a relevant parameter for the comparison of both biofiltration systems. Thus, while the fungal biofilter converted only a $45.9 \pm 15.8\%$ of the C eliminated to CO_2, this ratio increased up to

Table 2
Average VOC-removal efficiency in the fungal, bacterial and two-stage biofilter during steady state periods (days 31–49 for fungal and bacterial biofilters and days 6–20 in two-stage biofilter).

Pollutant	Fungal	Bacterial	Two stages
Propanal	72.8 ± 6.6%	100.0 ± 0.0%	100.0 ± 0.0%
MIBK	15.0 ± 5.3%	25.4 ± 4.8%	30.0 ± 8.5%
Toluene	5.8 ± 4.7%	9.4 ± 4.1%	13.1 ± 6.4%
Hexanol	43.4 ± 6.7%	90.6 ± 4.1%	90.2 ± 3.1%

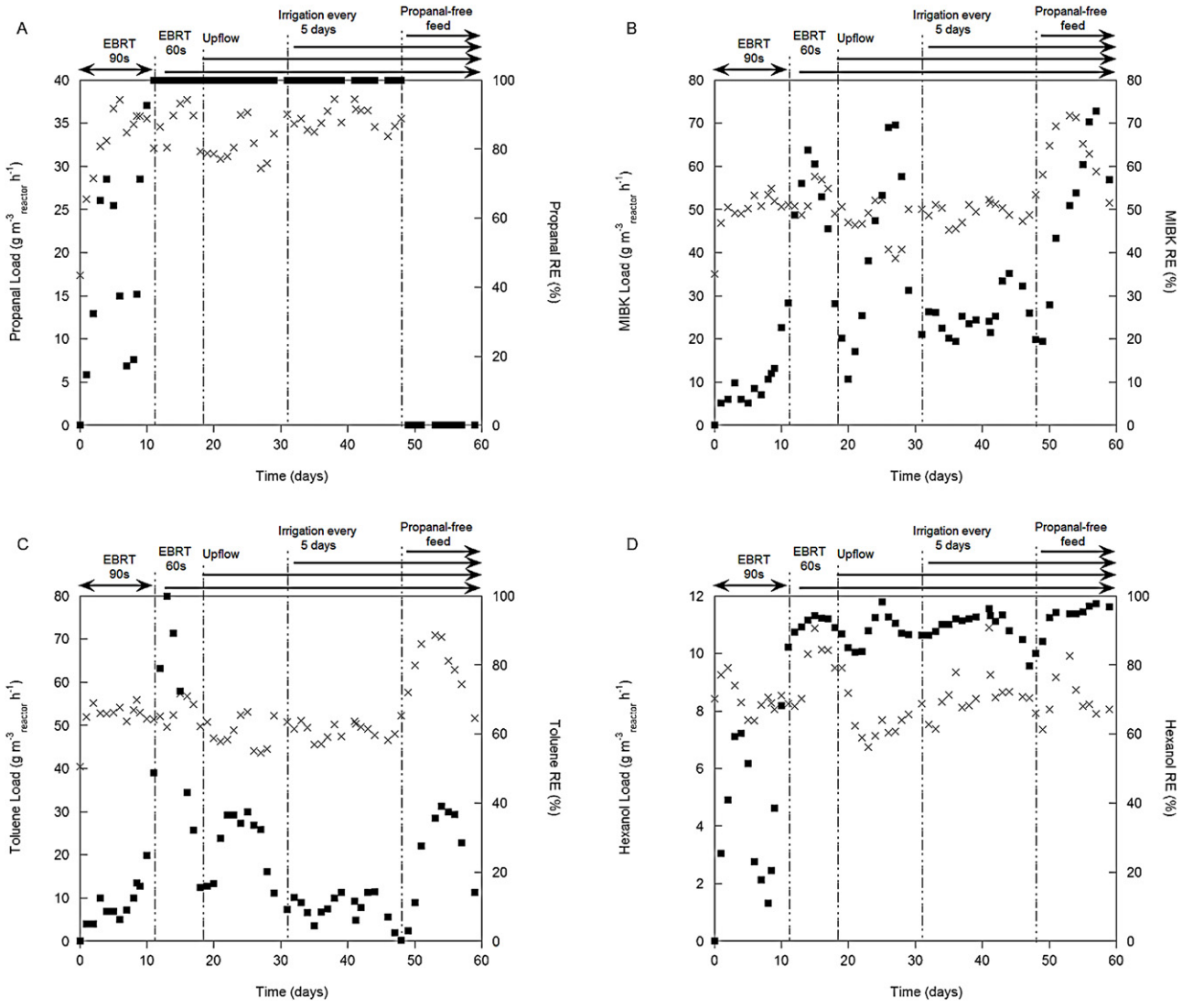

Fig. 2. Time course of the loading rate (×) and RE (■) of propanal (A), MIBK (B), toluene (C) and hexanol (D) in the bacterial biofilter. Vertical dash-dotted lines indicate the changes in the operating conditions.

63.1 ± 11.5% in the bacterial biofilter (Table 1). This suggests that the bacterial community was more efficient oxidizing carbon than the fungal community which showed higher biomass production. Moreover, the presence of mites in the bacterial biofilter but not in the fungal biofilter, which was confirmed by microscope observation of biofilm samples (See supporting material Figure S2), may have also contributed to the higher mineralization rates recorded in the bacterial biofilter and a lower increase in the pressure drop [26]. In terms of pressure drop, bacterial biofiltration exhibited final values 60% lower than those of fungal biofiltration, which is a significant figure from an economic viewpoint. In our particular case, the savings derived from the installation of a bacterial rather than a fungal biofilter after 20 years of operation would account for approx. 0.24 million€ (12,000 € $year^{-1}$).

3.3. Process response to VOC starvation

The concentration of organic C detected at the outlet of both biofilters did not decrease to zero immediately after the interruption of the VOC supply and gradually declined likely due to the desorption of the VOCs or bioreactor intermediates accumulated in the biofilter packing bed. Thus, the full desorption of the volatile organic C present in the fungal biofilter occurred in about 1.5 h (Fig. 3 A), while only 1 h was necessary in the bacterial biofilter to fully desorb all volatile organic carbon (Fig. 3 B). These findings confirmed the fact that more organic C was indeed retained in the fungal biofilter, which can be explained by the high affinity of fungi for hydrophobic VOCs and the aerial mycelia promoting the sorption of organics in the fungal biomass [10]. Surprisingly, the interruption in the VOC supply did not have a major impact in the CO_2 outlet concentrations for the 24 h in the absence of VOCs, which decreased from 853 to 606 ppm (compared to 447.2 ± 48.9 ppmv recorded in air) in the fungal biofilter (≈38%) (Fig. 4 A) and from 1506 to 994 ppm in the bacterial biofilter (≈52%) (Fig. 4 B). This sustained high CO_2 production in both biofilters in the absence of external C supply (based on the inert nature of the support) can be attributed to the metabolism of accumulated reserves and non-volatile metabolites. The presence of mites in the bacterial biofilter may have contributed to the sustained CO_2 production by promoting the bacterial biomass turnover.

When VOC supply was restored, the outlet concentration of organic C increased sharply up to a maximum of 1.45 g m^{-3} in both biofilters 9 min after feed resumption. Then, this concentration rapidly decreased in both biofilters probably due either to the fast kinetics of VOC adsorption in the packing material (commonly observed during process start-up in biofilters) or to an increased

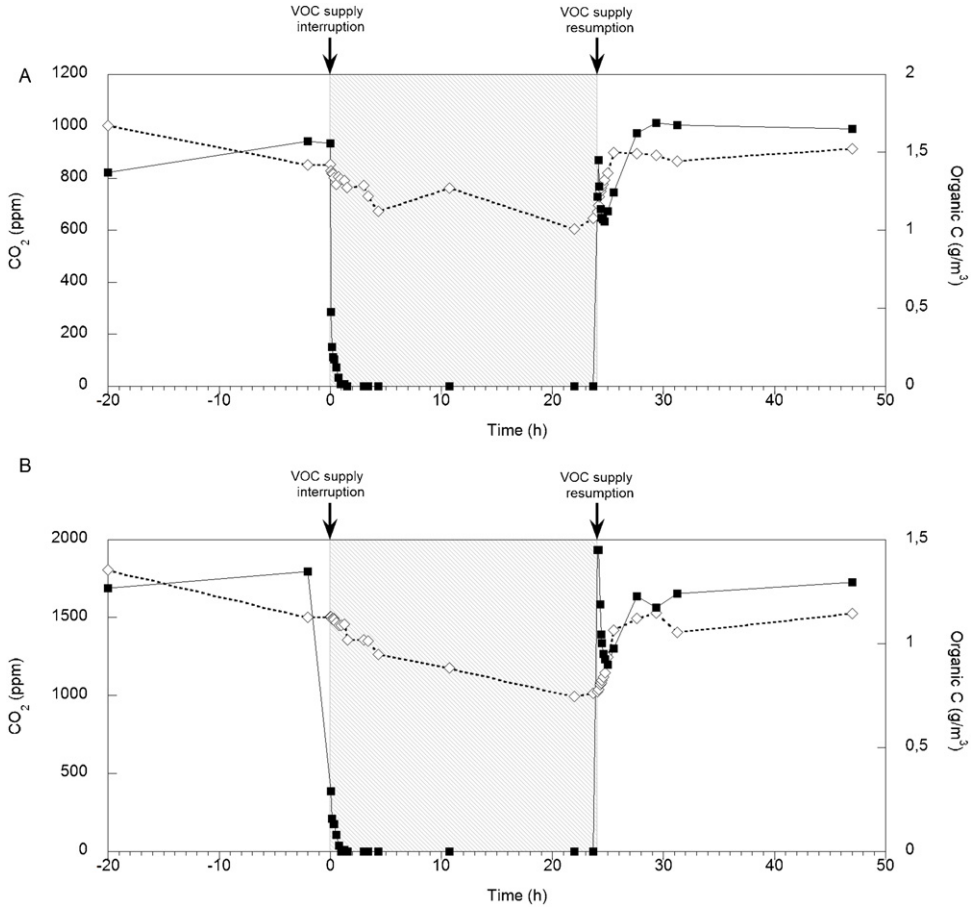

Fig. 3. Time course of the CO_2 concentration (◊) and organic C concentration (■) during the starvation experiment in the outlet of the fungal (A) and the bacterial biofilters (B).

pollutant uptake caused by the previous famine period. Both biofilters recovered steady REs and CO_2 production levels after 4 h. These results are in agreement with literature data where short recovery times were reported after starvation periods for both fungal and bacterial biofilters [7,13,27].

3.4. Metabolites identification

The identification of intermediate metabolites included the analysis of samples of the leachate and outlet gas condensate from both biofilters on day 47 and on day 6 of the combined bacterial–fungal operation. Two metabolites were identified by GC–MS with a match quality higher than 90%: Hexanal and 1-propanol. Despite that hexanal was detected in the outlet stream condensate of both biofilters, it was not present in the leachates, probably due to its higher volatility (Henry's law constant of $8.6 \times 10^{-3}\ C_g/C_l$) compared with that of hexanol (Henry's law constant $7.7 \times 10^{-4}\ C_g/C_l$), which was indeed detected both in the gas condensates and in the leachate of both biofilters [20]. The presence of hexanal was anticipated since hexanol biodegradation is mediated by the enzyme alcohol dehydrogenase (ADH), which oxidizes alcohols to aldehydes or ketones [15,28]. Surprinsingly 1-Propanol was detected in both the outlet condensates and leachates of both biofilters. This production of an alcohol from a more oxidized substrate such as propanal under aerobic conditions deserves further investigation. In this context, the production of biodegradation intermediates must be carefully assessed, even though they are in much smaller concentration that the inlet pollutants [15], as they might generate secondary pollution.

3.5. Fungal/bacterial coupled biofiltration

This configuration (bacterial → fungal reactors in series) was selected to avoid the carry-over of fungal spores and under the hypothesis that the most water soluble VOCs would be degraded in the first bacterial stage promoting the biodegradation of the hydrophobic VOCs by the fungal community. During the 16 days of stable operation of the two-stage bacterial-fungal biofilter the overall EC obtained ($38.4 \pm 8.4\ g\,C\,m^{-3}$ reactor h^{-1}) was slightly lower than the one obtained in bacterial biofiltration but significantly higher than the EC recorded for the fungal biofilter. The individual ECs of each biofilter under this configuration were half than those under single-stage operation due to the reduced EBRT in each individual biofilter (30 s instead of 60 s). Surprisingly, this reduction in the individual abatement performance of each stage was more significant in the bacterial biofilter (EC $20.1 \pm 5.9\ g\,C\,m^{-3}$ reactor h^{-1}, corresponding to a reduction in EC of 50.1%) than in the fungal biofilter (EC $18.9 \pm 7.9\ g\,C\,m^{-3}$ reactor h^{-1}, corresponding to a reduction in EC of 31.7%). The pressure drop in this two-stage biofilter doubled that of fungal biofiltration on day 60, which is consistent with the fact that gas flow was increased to maintain the same residence time (Table 1). On the other hand, a high and increasing VOC mineralization ratio (C produced as CO_2/C eliminated) was recorded, which finally became stable at ≈0.9. This sharp increase in the fungal mineralization ratio (from ≈46% during

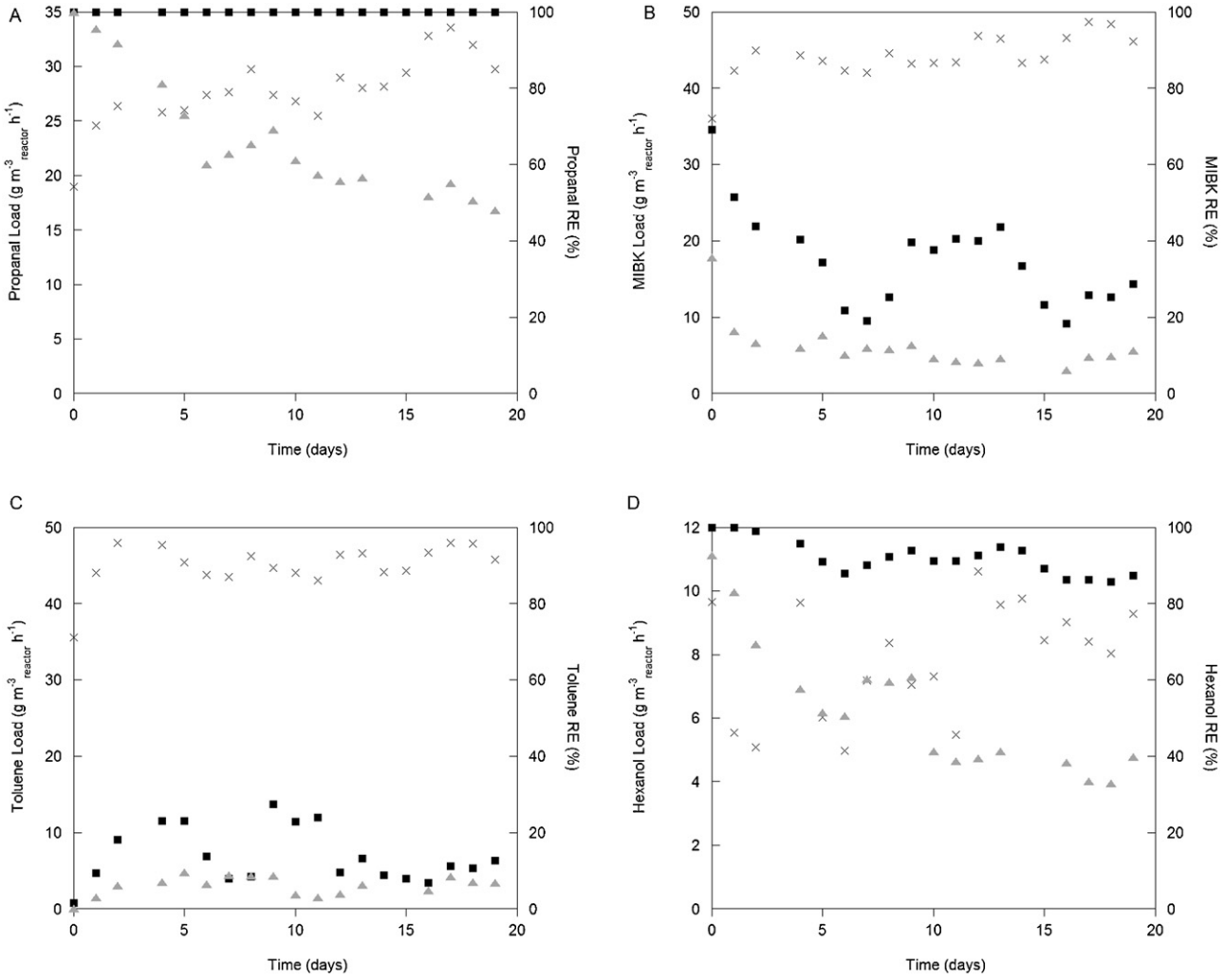

Fig. 4. Time course of the loading rate (×), bacterial stage RE (▲) and global RE (■) of propanal (A), MIBK (B), toluene (C) and hexanol (D) in the two-stage biofilter.

stable single operation to ≈97% when located after the bacterial stage) might be related to the mite infestation of the fungal biofilter. The partial mineralization ratios, (0.75 ± 0.40 and 0.97 ± 0.56 for the bacterial and fungal stages, respectively) supports the hypothesis of an increased CO_2 production from the mite-mediated biomass turnover.

The initial overall decreasing performance observed in the bacterial stage RE was attributed to the resumption of propanal supply to the system. Under steady state (days 5–19), propanal was removed by $57.7 \pm 6.0\%$ in the bacterial stage while the fungal biofilter operated as a polishing step to achieve a total RE of $100 \pm 0.0\%$ over the last 14 days of experimentation (Fig. 4 A). MIBK underwent low REs in the bacterial stage ($9.8 \pm 1.8\%$) and the fungal stage ($25.4 \pm 8.9\%$), with a total RE of $30.1 \pm 8.6\%$ (Fig. 4 B). The overall toluene RE remained low and fluctuating ($13.2 \pm 6.4\%$) (Fig. 4C) with an even biodegradation in both stages ($6.4 \pm 2.0\%$ in the bacterial stage and $7.8 \pm 5.6\%$ in the fungal stage). Both toluene and MIBK exhibited the fluctuating RE patterns already observed in the bacterial and fungal biofilters when operated separately, which again suggests the occurrence of a inhibitory metabolite accumulation. Hexanol was evenly eliminated in the bacterial stage ($44.7 \pm 9.9\%$) and in the fungal stage ($39.9 \pm 2.7\%$) with a total $90.1 \pm 3.1\%$ (Fig. 4 D). Therefore, the results here obtained ruled out the initial hypothesis of a complete removal of the hydrophilic VOC fraction in the bacterial biofilter followed by the removal of the most hydrophobic VOC in the fungal section.

3.6. Conclusions

This study constitutes, to the best of our knowledge, the first systematic evaluation of fungal and bacterial biofilters in terms of abatement capacity of a mixture of VOCs and pressure drop. Overall, bacterial biofiltration supported higher elimination capacities and mineralization ratios than its fungal counterpart. This observation can be explained partly by the fact that the bacterial biofilter developed a more diverse population, originated from the activated sludge, as compared to the fungal biofilter which was inoculated with an axenic culture, although the development of a secondary fungal population may not be ruled out. However, both biofilters showed an excellent robustness versus 24 h VOC starvation episodes. The steady state operation also showed a similar order in the biodegradation preference (propanal > hexanol > MIBK > toluene) with propanal partially inhibiting the biodegradation of the rest of compounds. When deprived from propanal, both biofilters were able consume the other VOCs in a larger extent as shown by the similar EC in the presence and absence of propanal. The two-stage bacterial/fungal biofilter provided high levels of VOCs mineralization. However, the results obtained did not validate the initial hypothesis of an enhanced VOC removal by combining a preferred removal of the hydrophilic VOC fraction in the bacterial biofilter with the removal of the most hydrophobic VOCs in the fungal section

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jhazmat.2013.01.064.

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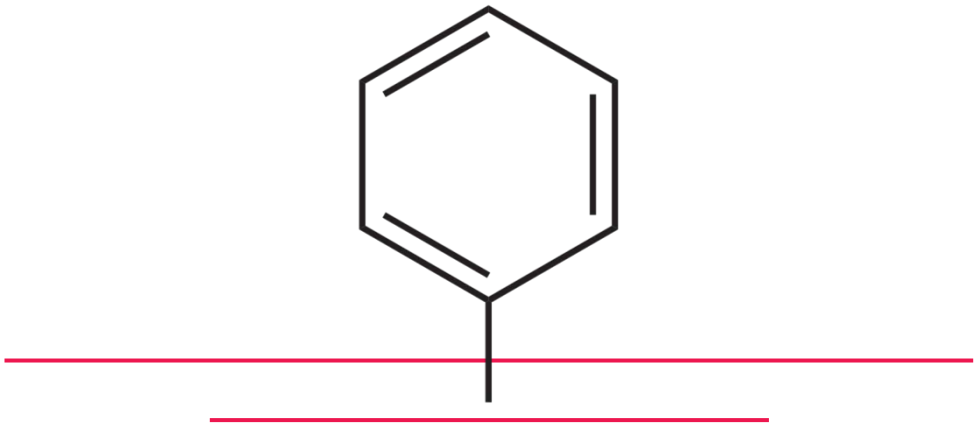
Chapter 8



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Step-feed biofiltration: A low cost alternative configuration for off-gas treatment

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ABSTRACT

Clogging due to biomass accumulation and the loss of structural stability of the packing media are common operational drawbacks of standard gas biofiltration inherent to the traditional biofilter design, which result in prohibitive pressure drop buildups and media channeling. In this work, an innovative step-feed biofilter configuration, with the air emission supplied in either two or three locations along the biofilter height, was tested and compared with a standard biofilter using toluene as a model pollutant and two packing materials: compost and perlite. When using compost, the step-feed biofilter supported similar elimination capacities (EC ≈ 80 g m^{-3} h^{-1}) and CO_2 production rates (200 g m^{-3} h^{-1}) to those achieved in the standard biofilter. However, while the pressure drop in the step-feed system remained below 300 Pa m_{bed}^{-1} for 61 days, the standard biofilter reached this value in only 14 days and 4000 Pa m_{bed}^{-1} by day 30, consuming 75% more compression energy throughout the entire operational period. Operation with perlite supported lower ECs compared to compost in both the step-feed and standard biofilters (≈30 g m^{-3} h^{-1}), probably due to the high indigenous microbial diversity present in this organic packing material. The step-feed biofilter exhibited 65% lower compression energy requirements than the standard biofilter during operation with perlite, while supporting similar ECs. In brief, step-feed biofiltration constitutes a promising operational strategy capable of drastically reducing the operating costs of biofiltration due to a reduced energy consumption and an increased packing material lifespan.

1. Introduction

Despite air pollution has traditionally received less attention than solid or water contamination, over the past decades there has been an increasing awareness to adequately treat off-gas emissions (Lebrero et al., 2011a). In this regard, biological techniques have emerged as efficient, economic and environmental-friendly waste gas treatment alternatives to their physical-chemical counterparts (Estrada et al., 2011). Of them, biofiltration is the oldest and simplest bioreactor configuration and still constitutes one of the most popular technologies applied to a wide variety of air pollutants and concentrations, ranging from malodors to industrial emissions (Kennes et al., 2009).

Unfortunately, biofiltration still suffers from major operational drawbacks inherent to its conventional design. Pressure buildup and bed channeling caused by biomass accumulation and filter media deterioration rank among the most important operational problems leading to a reduced packing material lifespan and an increased energy consumption (the main

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contributors to the operating costs in biofilters) (Prado et al., 2009). Uneven water distribution as a result of biomass accumulation and packing media deterioration is also another common operational issue in biofiltration significantly affecting the biological activity of biofilters. The impact of the above mentioned operational limitations is more severe in organic low-cost packing materials and during the treatment of high pollutant loading rates. Mite predation and operational strategies such as packing mixing and leachate recycling have been repeatedly tested in order to overcome these issues, although with a limited success (Roshani et al., 2012; Woertz et al., 2002). In addition, several studies have shown that pollutant biodegradation often takes place in the first 30% of bed height, which concentrates most of the biomass growth, the remaining 70% contributing to the polishing of the air emission at a significantly high energy cost (Lebrero et al., 2011b). Therefore, despite the important advances in biofiltration over the past three decades, the development of innovative design and operational strategies in biofilters is still needed to increase both their pollutant abatement performance and lifespan while decreasing the operating costs associated to energy and packing material requirements.

The present study evaluated the performance of an innovative step-feed biofilter configuration where the packed column is divided into three separate modules fed and irrigated independently. This configuration is expected to support a more homogeneous biomass distribution, lower overall pressure drops and a more efficient irrigation of the packing material. This operational mode, where a partially purified stream is mixed with a raw polluted stream before entering a new biofiltration module, has been successfully applied worldwide for an enhanced carbon and nutrient removal in wastewater treatment (Ge et al., 2012), but scarcely studied in the field of waste gas treatment (Mendoza et al., 2003). A conventional and a step-feed biofilter were comparatively evaluated in terms of elimination capacity (EC), CO_2 production, pressure drop and bed compaction using toluene as a model volatile organic compound (VOC) and two types of packing material: compost and perlite.

2. Materials and methods

2.1. Microorganisms

Biofilters were inoculated by mixing the packing material with fresh aerobic activated sludge from Valladolid Wastewater Treatment Plant (Valladolid, Spain), previously centrifuged at 10,000 rpm for 10 min and re-suspended in mineral salt medium (MSM). On day 61, a *Pseudomonas putida* F1 strain (DSMZ 6899) was inoculated in the perlite-based biofilters by irrigation of a bacterial culture in order to assess the potential occurrence of microbial activity limitation in the system.

2.2. Chemicals

Toluene was purchased from Fisher Scientific® (United Kingdom) with a purity higher than 99.5%. All other chemicals and reagents were purchased from Panreac® (Spain) with a purity higher than 99%. The MSM used for biofilter irrigation and activated sludge re-suspension was prepared according to Muñoz et al. (2008).

2.3. Experimental setup and operation

Two series of experiments were carried out in the same experimental setup: series 1 used compost as a packing material. The compost was characterized by a density (as received) of 0.387 kg L^{-1}, a wet density of 0.675 kg L^{-1}, a porosity of 66%, a pH of 7.7 and a water holding capacity (volume basis) of 1.26 L_{water} $L^{-1}_{compost}$ (TMECC, 2002). Series 2 used perlite as a packing material. The perlite was characterized by a density (as received) of 0.277 kg L^{-1}, a wet density of 0.317 kg L^{-1}, a porosity of 62%, a pH of 6.57 and a water holding capacity (volume basis) of 1.65 L_{water} $L^{-1}_{perlite}$. Both packing materials were experimentally characterized according to standard methods (TMECC, 2002). A jacketed PVC reactor (0.105 m inner diameter, 1.20 m height) was used as the control standard biofilter (Biofilter A), while three sequentially interconnected jacketed PVC reactors (0.10 m inner diameter, 0.40 m height) constituted the modules B1, B2 and B3 of the step-feed biofilter (Biofilter B). Both biofilters were packed with a final working volume of 8.6 L with the corresponding packing material in each experiment (compost or perlite). Both series 1 and 2 comprised the operation of biofilter A and B in parallel.

A synthetic toluene-polluted emission (2.50 ± 0.23 g m^{-3}) was obtained by mixing a $\approx$4 L min^{-1} toluene-saturated air stream (obtained by air-sparging into pure toluene) with a $\approx$13.2 L min^{-1} pre-humidified air stream (Fig. 1). The humidity of the inlet toluene-laden air stream was measured on-line by a thermohygrometer (Testo 605-H1, Testo AG, Germany) and ranged from 50 to 70%. The air flow fed to Biofilter A (standard biofilter) was adjusted to 8.6 L min^{-1} to provide an empty bed residence time (EBRT) of 1 min, which was maintained during the entire experimentation period. The Biofilter B (modular biofilter) was also operated at a global EBRT of 1 min under two different configurations: (i) a three-stage configuration where the 8.6 L min^{-1} toluene-laden air stream was split into three identical streams fed to the inlet of modules B1, B2 and B3, resulting in individual EBRTs of 60, 30 and 20 s, respectively; (ii) a two-stage configuration where the 8.6 L min^{-1} toluene-laden air stream was split into two identical streams fed only to the inlet of modules B1 and B2, resulting in individual EBRTs of 40, 20 and 20 s in modules B1, B2 and B3, respectively. The overall inlet loading rates were always maintained at 150.2 ± 14.1 g m^{-3} h^{-1}. Irrigation with MSM was performed at a rate of 30 mL $L^{-1}_{packing}$ every day except from day 19 to 54 in the compost experiment and from day 41 to 105 in the perlite experiment, when it was performed every two days (Estrada et al., 2011). An abiotic test was initially carried out in both biofilters (without packing material) for 48 h at an EBRT of 1 min to confirm the absence of toluene photolysis or adsorption in the systems and back mixing at the sampling ports in Biofilter B.

Gas samples of 100 μL were periodically drawn from the sampling ports located at the inlet and outlet of each biofilter and module by means of a gas tight syringe (Hamilton, USA) for toluene and CO_2 concentration analysis. Aliquots of biofilter leachate (when available) were periodically drawn for pH

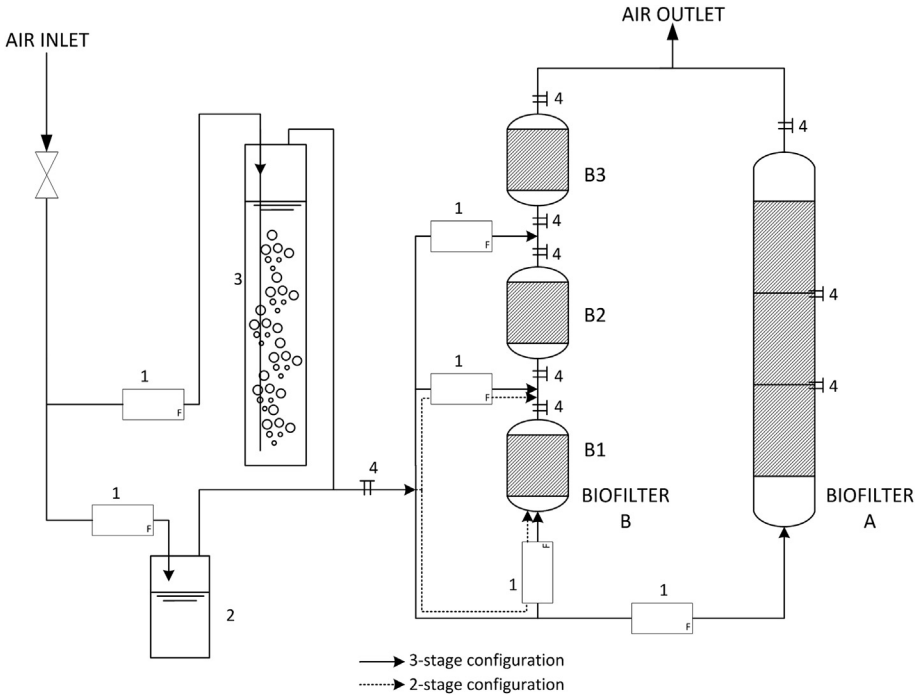

Fig. 1 – Schematic representation of the experimental set-up: 1. Gas flow controllers, 2. Toluene evaporation chamber, 3. Humidifying column, 4. Gas sampling ports.

and metabolites analysis. The pressure drop was measured by means of a U-tube manometer connected to the inlet and outlet of biofilter A and each module of biofilter B using water as the manometric fluid. Blank tests (empty reactor without packing material) were carried out in order to account for the pressure drop caused exclusively by the packing material and not by the piping or accessories. Bed compaction was estimated from the variations on the bed height directly measured on the biofilters. The water content was measured by dry weight at the end of the compost experiment as a key parameter in the development of the pressure drop across the bed. Compost samples were dried overnight at 100 °C and the water content was estimated by weight difference.

2.4. Analytical procedures

Toluene gas concentration was analysed in a Varian 3900 gas chromatograph equipped with a flame ionization detector and a SupelcoWax (15 m × 0.25 mm × 0.25 μm) capillary column. The injector, detector and oven temperatures were set at 200, 200 and 140 °C, respectively. N_2 was used as the carrier gas at 2 mL min^{-1}. CO_2 concentration was determined in a Varian CP-3800 gas chromatograph (Palo Alto, USA) coupled with a thermal conductivity detector and equipped with a CP-Molsieve 5A (15 m × 0.53 μm × 15 μm) and a CP-PoraBOND Q (25 m × 0.53 μm × 10 μm) columns. The oven, injector and detector temperatures were maintained at 45, 150 and 175 °C, respectively. Helium was used as the carrier gas at 13.7 mL min^{-1}.

Metabolites were qualitatively characterized by solid-phase microextraction (SPME) coupled to GC–MS as follows: 1.7-mL glass vials were filled with 1.5 mL of biofilter leachate and closed with Teflon/rubber caps. Then, an 85-μm Carboxen/PDMS SPME fiber (Supelco, Bellefonte, USA) was immersed into the leachate and maintained for 5 min. The SPME fiber was then injected and allowed to desorb for 1 min in an Agilent 6890N GC–MS equipped with a DB-WAX column (30 m × 0.250 mm × 0.25 μm) (J&W Scientific®, CA, USA). The injector temperature was set at 200 °C while the oven temperature was initially maintained at 40 °C for 4 min and then increased at 10 °C min^{-1} up to 200 °C. Source and MS quadrupole temperatures were set at 230 and 150 °C, respectively.

2.5. Process performance calculations

EC (g m^{-3} h^{-1}) was calculated as follows:

$$EC = \frac{(C_{in} - C_{out}) \times F}{V_{bed}} \quad (1)$$

where C_{in} and C_{out} represent the toluene concentrations at the inlet and outlet of the reactor (g m^{-3}), F is the gas volumetric flow rate (m^3 h^{-1}) and V_{bed} is the volume of the packed bed (m^3).

CO_2 production (g m^{-3} h^{-1}) was calculated by:

$$CO_2 \text{ production} = \frac{(CO_{2\,out} - CO_{2\,in}) \times F}{V_{bed}} \quad (2)$$

where $CO_{2\,in}$ and $CO_{2\,out}$ are the CO_2 concentrations at the inlet and outlet of the reactor in g m^{-3}, F is the gas volumetric flow rate in m^3 h^{-1} and V_{bed} is the volume of the packed bed in m^3.

The compression energy requirements were calculated by using the following expression to obtain the power requirement (P, kW) (Estrada et al., 2011):

$$P = \frac{F \times \Delta P}{0.7} \quad (3)$$

where F is the gas volumetric flow rate ($m^3\ s^{-1}$), ΔP is the pressure drop measured across the biofilter packing media (Pa m_{bed}^{-1}) and 0.7 stands for a standard blower efficiency of 70%. The energy requirements were obtained by integrating the area under the power curve over the time of the experiment.

The biofiltration efficiency parameter ε ($g_{toluene}\ N^{-1}\ h^{-1}$) was developed in order to carry out a fair performance comparison between the biofilters here evaluated and the available literature on toluene biofiltration. This parameter accounts for the mass of pollutant removed in a biofilter per unit of force applied and was defined as follows:

$$\varepsilon = \frac{EC}{\Delta P}[=]\frac{g \cdot m^{-3} \cdot h^{-1}}{Pa \cdot m_{bed}^{-1}}[=]\frac{g_{toluene}}{N \cdot h} \quad (4)$$

where EC is the toluene elimination capacity as described in equation (1) and ΔP is the pressure drop measured across the biofilter packing media (Pa m_{bed}^{-1}).

3. Results and discussion

3.1. *Compost-based biofiltration*

The performance of both biofilters was initially characterized by toluene adsorption onto the packing material within the first 3 days of operation, and the stabilization of ECs at $\approx$25 g m^{-3} h^{-1} by day 5 (Fig. 2A). On day 10, the ECs started to increase gradually in both bioreactors and reached steady ECs of $\approx 127 \pm 6$ g m^{-3} h^{-1} and 93 ± 7 g m^{-3} h^{-1} in biofilters A and B, respectively, from day 15 to 24. These values agree with previously reported toluene ECs $\approx$100 g m^{-3} h^{-1} recorded in biofilters packed with organic media (Gallastegui et al., 2011b, Kennes et al., 2009; Znad et al., 2007) (Table 1). During this start-up phase the pressure drop increased from 186 to 588 Pa m_{bed}^{-1} in biofilter A and from 79 (corresponding to 27, 82 and 109 Pa m_{bed}^{-1} in modules B1, B2 B3, respectively) to 225 (corresponding to 163, 108 and 436 Pa m_{bed}^{-1} in modules B1, B2 B3, respectively) in biofilter B (Fig. 2B). It is noteworthy that despite being able to remove 30% more toluene in this first period, biofilter B maintained 50% lower pressure drops than biofilter A. The same empirical findings can be observed for the rest of the experiment with compost, which constitutes an advantage of the step-feed configuration. On day 19, the irrigation was decreased to 30 mL $L_{packing}^{-1}$ every two days in an attempt to reduce the gradual pressure drop buildup in biofilter A. Despite the ECs in biofilter A and B remained constant during the first week of this new operational period, they gradually decreased to 68 ± 0.4 and 67 ± 3 g m^{-3} h^{-1} by day 34, respectively, which was likely caused by the decrease in the moisture content of the packing material as suggested by the absence of leachate and the visual observation of the compost media. However, the reduction of the irrigation frequency did not positively affect the pressure drop in biofilter A, which sharply increased from 1000 Pa m_{bed}^{-1} on day 28 to 4000 Pa m_{bed}^{-1}on day 32. This value was above the maximum recommended pressure drop of 1500 Pa m_{bed}^{-1} acceptable at industrial scale (Estrada et al., 2011). On the other hand, the pressure drop recorded in biofilter B throughout this operational period never exceeded 255 Pa m_{bed}^{-1}. During the operation with the 3-stage configuration (days 0–34), the average EC in biofilter A was 36.2% higher than in biofilter B. Both the slower start-up and the lower EC achieved during stable operation of the step-feed biofilter between days 15 and 30 (Fig. 2A) were attributed to the lower mass transfer capacity of biofilter B. This lower capacity was likely caused by the

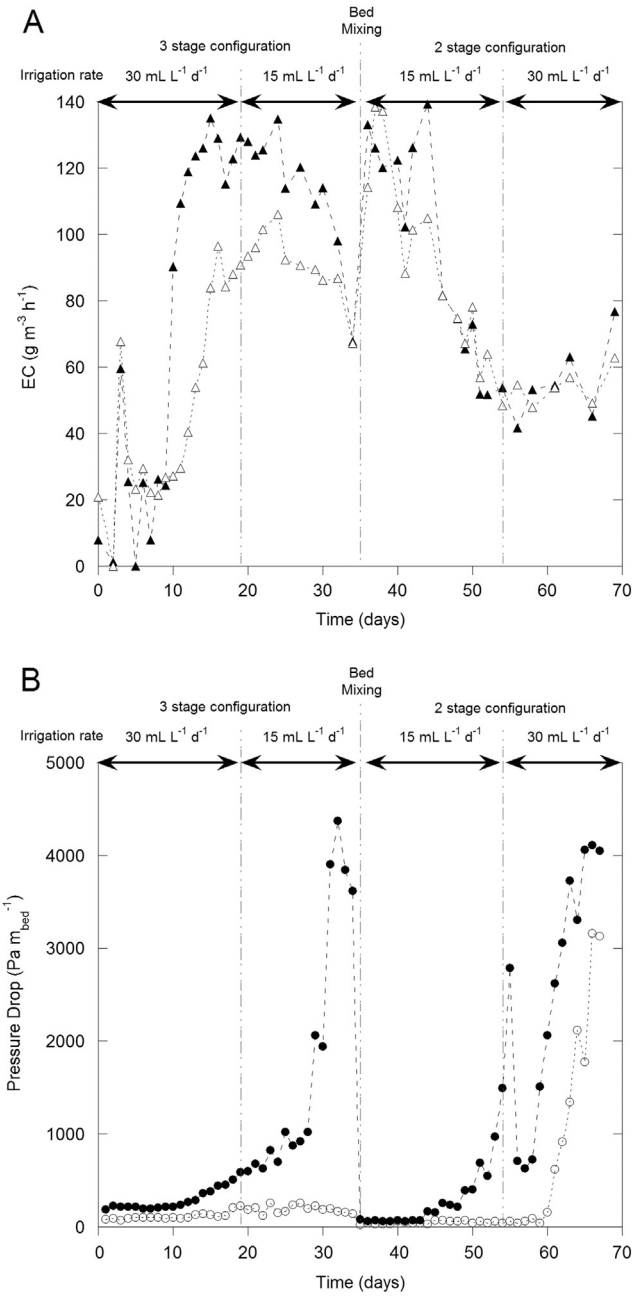

Fig. 2 – Time course of EC (A) in biofilter A (▲) and B (△), and pressure drop across the bed (B) in biofilters A (●) and B (○) during the 70 days of operation using compost as the packing material. Horizontal arrows indicate the operational periods and irrigation rates.

Table 1 – Compilation of toluene biofiltration data under the worst and best operating conditions reported.

Packing material	EC ($g\ m^{-3}\ h^{-1}$)	ΔP ($Pa\ m_{bed}^{-1}$)	ε ($g\ N^{-1}\ h^{-1}$)	Reference
Perlite (step-feed)	27	20	1.397	Present study
Perlite (standard)	9	1716	0.005	
Compost (step-feed)	138	30	4.707	Present study
Compost (standard)	45	3304	0.014	
Compost	95	98	0.969	(Maestre et al., 2007)
	95	4903	0.019	
Perlite *Fungal + mite predation	125	130	0.962	(van Groenestijn et al., 2001)
	80	130	0.615	
Vermiculite *Fungal	90	490	0.184	(García-Peña et al., 2001)
	90	3922	0.023	
Peat	93	98	0.948	(Álvarez-Hornos et al., 2008)
	93	1373	0.068	
Compost/Ceramic 50%	160	49	3.263	(Znad et al., 2007)
	160	196	0.816	
Coir Pith	97	39	2.467	(Krishnakumar et al., 2007)
Compost/Shells	82	588	0.139	(Vergara-Fernández et al., 2007)
	82	20,004	0.004	
ABONLIR (commercial soil)	138	392 (max.)	0.352	(Gallastegui et al., 2011b)

decreased gas turbulence in the packed bed due to the lower gas flow imposed in each module compared with biofilter A, leading to a lower mass transfer coefficient. Indeed, while the standard biofilter can be regarded as three sequential modules with individual EBRTs of 20 s, the step-feed biofilter operated with EBRTs of 60 s and 40 s in modules B1 and B2. Kim and Deshusses (2008) observed that a decrease in the gas velocity resulted in a significant decrease in the mass transfer in packed bed reactors such as biotrickling filters (Kim and Deshusses, 2008).

On day 34, the packing material in biofilter A and in each module of biofilter B was taken out from the reactors, mixed thoroughly and returned to each corresponding reactor. This procedure allowed for an instantaneous reduction in the pressure drop of biofilter A to initial levels concomitant with an increase in the EC up to $133 \pm 1\ g\ m^{-3}\ h^{-1}$ on day 36 (Fig. 1A). This rapid increase in the EC was probably due to a better distribution of the air flow and water content throughout the entire packing media after bed mixing. However, toluene biodegradation performance gradually decreased to $54 \pm 6\ g\ m^{-3}\ h^{-1}$ at day 54, concomitant with a rapid increase in the pressure drop along biofilter A up to 549 Pa m_{bed}^{-1}. From day 34 onwards, biofilter B was operated under a two-stage configuration and similar EC values to those obtained in biofilter A were achieved. Despite the increase in the EC up to $138 \pm 1\ g\ m^{-3}\ h^{-1}$ due to the above mentioned benefits derived from bed mixing, the EC of biofilter B also gradually decreased to $49 \pm 6\ g\ m^{-3}\ h^{-1}$ by day 54. The pressure drop under this 2-stage configuration remained below 300 Pa m_{bed}^{-1} (corresponding to individual pressure drops under 100 Pa m_{bed}^{-1} in each module after re-packing). The restoration of the initial irrigation strategy (30 mL $L_{packing}^{-1}\ day^{-1}$) at day 54 in order to increase the moisture content and therefore the EC, allowed for the stabilization of the EC in both bioreactors at $\approx 55\ g\ m^{-3}\ h^{-1}$. This intensification in packing media irrigation triggered a sharp pressure drop buildup in biofilter A up to 2785 Pa m_{bed}^{-1} by day 57 followed by a sudden decrease to 627 Pa m_{bed}^{-1}on day 59 (Fig. 2B). In this context, sudden pressure drop fluctuations in organic media-based biofilters have been associated to the dynamic nature of packing flooding episodes (Dorado et al., 2012; Ryu et al., 2010). In our particular study, water accumulation was repeatedly observed on the top of the bed of biofilter A following irrigation, which hindered water trickling through the bed. Finally, the packing bed broke on day 62 resulting in even higher pressure drops (4000 Pa m_{bed}^{-1} by day 69). On the other hand, the step-feed biofilter maintained low pressure drops (below 300 Pa m_{bed}^{-1}) despite the increase in irrigation frequency up to 30 mL $L_{bed}^{-1}\ day^{-1}$ on day 54. From this day onwards, the pressure drop rapidly increased up to 3128 Pa m_{bed}^{-1} by day 70 (corresponding to 27, 54 and 8607 Pa m_{bed}^{-1} in modules B1, B2 and B3, respectively) as a result of packing media flooding in module B3. The water content measured in modules B1, B2 and B3 was 0.59, 0.56 and 0.73 g $H_2O\ g_{compost}^{-1}$ (on wet basis), which agrees well with the high pressure drop developed in module B3 and the visual observations of bed flooding. The water content at the bottom, middle and top sections of the packed bed of biofilter A was 0.63, 0.69 and 0.87 g $H_2O\ g_{compost}^{-1}$ (on wet basis), which also confirmed that water accumulation in the upper part can result into the break down of the packed bed and high pressure drops.

During operation with the 2-stage configuration (days 35–70), the ECs in both biofilters remained similar. This increased toluene removal performance in the step-feed biofilter was attributed to an increased mass transfer capacity under the two-stage configuration, where modules B2 and B3 operated at an EBRT of 20 s, being comparable to biofilter A operation. However, both biofilters suffered a similar decrease in toluene biodegradation performance during this period, probably due to a partial microbial inhibition caused by the accumulation of inhibitory metabolites in the packing media. The pH values recorded in the leachate samples of both biofilters remained always above 6.0, which suggests that no severe acidification of the packing bed occurred during the entire experiment.

The economic benefits derived from the lower operational pressure drops along the 70 days of experimentation are inherent to the step-feed design, since the total air emission

only flows through 1/3 or 2/3 of the total bed height (in the 3-stage and 2-stage configurations, respectively), while in standard biofiltration the total air emission flows through the entire bed height. In fact, most of the pressure drop in biofilter B occurred in the modules B2 and B3 (those receiving the highest flow rates). By integrating the pressure drop values over the 70 days of operation, the step-feed configuration resulted in approximately 75% compression energy savings. In addition, the low pressure drops maintained in biofilter B allowed for a significant increase in the bed lifespan under this configuration. This fact entails significant savings in packing material and work costs, which account for 47% and 29% of the total operating costs in standard biofilters, respectively (Estrada et al., 2012).

At full scale, a three-module step-feed biofilter would entail increased construction and installation costs. Some additional investment costs would also be caused by the need of three parallel irrigation systems and gas flow distribution lines. However, these extra accessories are not complex from an engineering viewpoint since they are standard operations in most waste gas treatment facilities. Besides, recent studies have confirmed that the purchase of the packing material and liner and their installation are the main contributors to the investment costs in biofilters together with their engineering design (Prado et al., 2009). Therefore, since these costs would remain essentially unchanged in a step-feed configuration, the above mentioned benefits of the step-feed design would certainly outbalance slightly higher investment costs.

Surprisingly, the bed compaction presented similar values in both biofilters: 11.0% in biofilter A and 9.7% in biofilter B by day 34, and 17.0 and 16.6%, respectively, by the end of the experiment. This bed compaction data, together with the absence of a decrease in the pressure drop in biofilter A when the irrigation frequency was reduced, suggest that biomass growth, and not only media flooding or compost deterioration, were responsible of the recorded pressure drops.

3.2. *Perlite-based biofiltration*

When toluene biodegradation was carried out in perlite-based biofilters, the process was characterized by a low and fluctuating biodegradation performance within the first 10 days of operation regardless of the biofilter configuration used. Despite the increase in the overall toluene biodegradation performance during the last part of the operational period at an irrigation rate of 30 mL L^{-1} d^{-1}, process performance was much more unstable than in the case of compost biofiltration. Maximum toluene ECs of 53 ± 1 g m^{-3} h^{-1} and 54 ± 5 g m^{-3} h^{-1} were recorded by day 13 in biofilter A and by day 28 in biofilter B (Fig. 3A). During the operation under a three-stage configuration (days 0–70) no significant differences were observed between biofilter A (31 ± 12 g m^{-3} h^{-1}) and biofilter B (30 ± 11 g m^{-3} h^{-1}) regardless of the irrigation strategy. On day 19, a characterization of the metabolites present in the leachate of both bioreactors was carried out in order to assess the presence of potential inhibitory or toxic metabolites responsible for this low and unstable biodegradation, with o-cresol (97% quality match) and crotonic acid (95% quality match) identified as the major metabolites. The presence of metabolites such as o-cresol was not surprising since it is a common intermediate of toluene biodegradation found at high toluene loading rates and under oxygen limiting conditions (Yu et al., 2001). During the first 41 days of operation both biofilters underwent comparable pressure drop increases: from 68 to 392 Pa m_{bed}^{-1} and from 39 to 412 Pa m_{bed}^{-1} in biofilters A and B, respectively.

Despite the irrigation frequency was reduced on day 41 to 30 mL L_{bed}^{-1} every two days, no significant effect on the EC was observed, which remained low and highly fluctuating in both

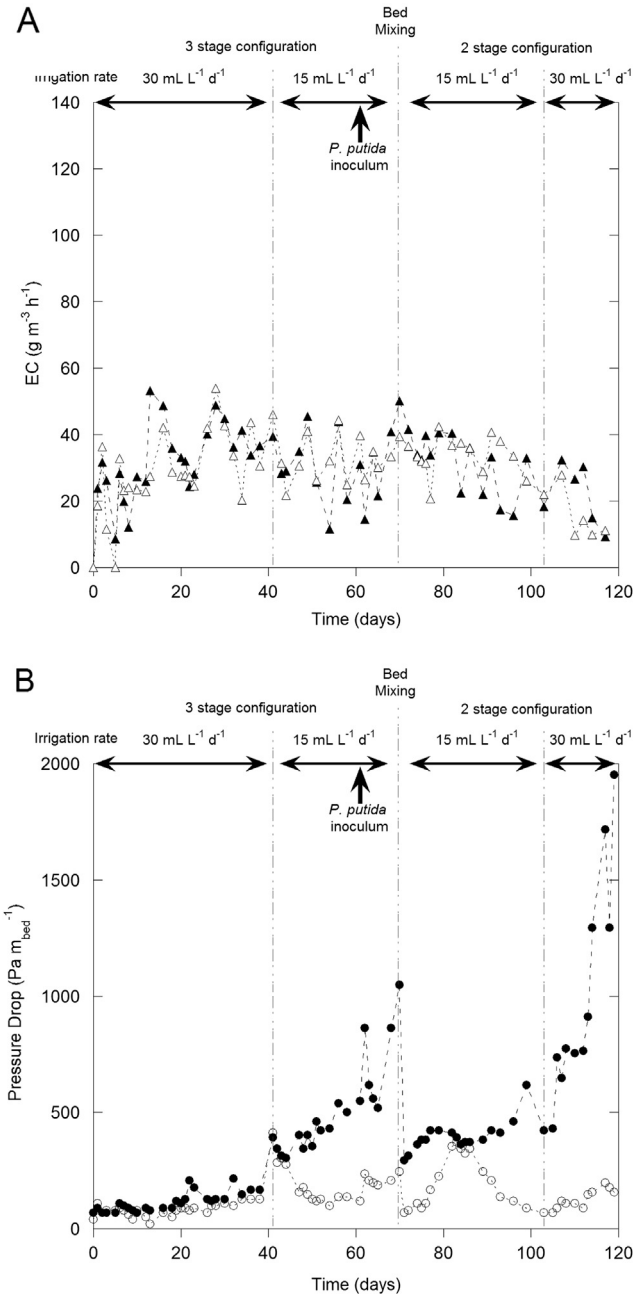

Fig. 3 – Time course of EC (A) in biofilter A (▲) and B (△), and pressure drop across the bed (B) in biofilters A (●) and B (○) during the 120 days of operation using perlite as the packing material. Horizontal arrows indicate the operational periods and irrigation rates.

biofilters. Unfortunately, this lower irrigation frequency did not mitigate the pressure drop buildup in biofilter A, which reached 1000 Pa m_{bed}^{-1} by day 70 (Fig. 3B). However, a gradual reduction in the pressure drop to 118 Pa m_{bed}^{-1} on day 51 was recorded in biofilter B, which suggests that while the pressure drop in the step-feed biofilter B was likely due to the flooding of the bed (especially in module B3, data not shown), the increase in pressure drop in biofilter A was caused by biomass accumulation. In order to shed light on the low and unstable ECs observed in both biofilters, a test to assess the occurrence of mass transfer limitations was carried out on day 54. The toluene gas concentration was increased from 2.50 ± 0.23 g m^{-3} to 3.96 ± 0.13 g m^{-3} (≈60% concentration increase) for 2.5 h while maintaining the EBRT at 1 min and the concentrations of CO_2 and toluene were continuously monitored. The EC significantly increased in both biofilters after 0.5 h at 3.96 ± 0.13 g toluene m^{-3}, to decrease shortly after to the initial levels (Fig. 4). This increase was higher in biofilter A, where the EC increased from 11 ± 3 g m^{-3} h^{-1} to 50 ± 11 g m^{-3} h^{-1}, while in biofilter B this increase accounted only for 34.3%. However, a concomitant increase in CO_2 production was not observed, which suggest that toluene was not completely oxidized in this period but transformed into metabolites which started to inhibit the system after 1 h of experiment.

Both biofilters were then inoculated with *P. putida* F1 on day 61 in an attempt to overcome any potential inhibition mediated by toluene degradation intermediates. This strain has been reported to have both a high toluene biodegradation capacity and resistance towards toluene and metabolites such as benzyl alcohol and o-cresol (Bordel et al., 2007). No significant increase in the EC and the CO_2 production rate were observed following *P. putida* F1 inoculation. Therefore, the overall results suggest mass transfer rather than biological activity limited toluene biodegradation during perlite-based biofiltration. However, since no microbial monitoring of the survival and activity of *P. Putida* F1 were conducted, it cannot be guaranteed that these bacteria remained active over the long-term biofilter operation. Therefore, the absence of a limitation in microbial activity cannot be completely ruled out based on the re-inoculation test.

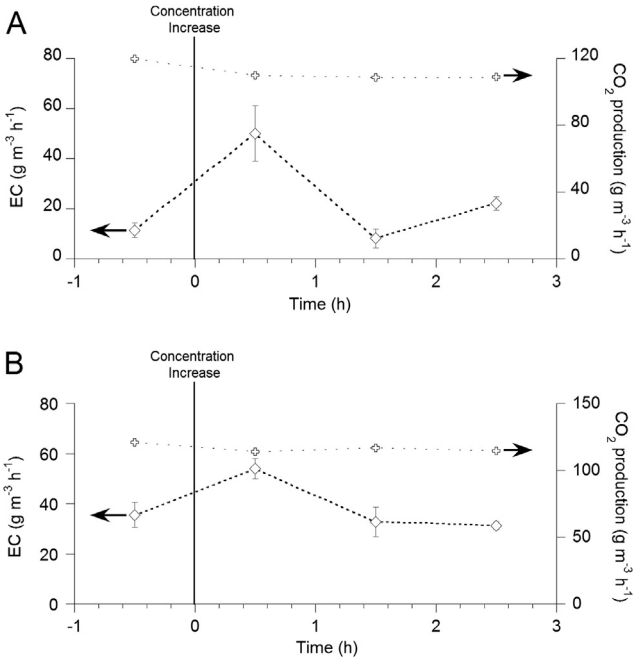

Fig. 4 – Time course of EC (◇) and CO_2 (⊕) production during the mass transfer experiment in biofilters A (A) and B (B).

On day 70, the packing material was taken out from the biofilters, mixed thoroughly and returned to each corresponding bioreactor, while biofilter B started the operation under a two-stage configuration. Unlike in the compost-based biofiltration, this action did not result in an increase in the EC of any of the biofilters. The restoration of an irrigation strategy of 30 mL L_{bed}^{-1} per day by day 103 did not mitigate the deterioration in toluene biodegradation in both bioreactors, which by the end of the experimentation supported ECs below 10 g m^{-3} h^{-1}. However, packing media mixing allowed for a reduction in the pressure drop of biofilter B from 245 to 68 Pa m_{bed}^{-1}, and from 1049 Pa m_{bed}^{-1} to 294 Pa m_{bed}^{-1} in biofilter A. Afterwards, the pressure drop in biofilter A gradually increased up to 1951 Pa m_{bed}^{-1} compared to 157 Pa m_{bed}^{-1} in biofilter B at the end of the experiment (Fig. 3B).

During the perlite-based biofiltration, no significant differences were observed between the performance of the step-feed and the standard biofilter in terms of EC. Despite the higher mass transfer potential of biofilter A due to the higher gas velocity in each section at similar concentration gradients, the performance of biofilter A was comparable to that recorded in biofilter B. In addition, the fact that no EC differences were recorded between the three-stage and two-stage configurations in biofilter B (the two-stage configuration holding a higher mass transfer potential than the three-stage one due to the higher gas velocity applied to the first two modules), together with the low ECs recorded at the end of the experimentation, suggested that both systems were gradually limited by microbial activity rather than mass transfer from day 70 onwards due to metabolites accumulation.

The compression energy savings estimated in biofilter B by integrating the pressure drop values along the experimentation period accounted for 65% compared to the compression energy requirements for gas circulation through biofilter A, which would result in total operating costs reductions higher than 9% (Estrada et al., 2012). No bed compaction was observed in the biofilters during the operation with the perlite packing.

3.3. CO_2 production

When using compost as a packing material, CO_2 production was linearly correlated to the EC regardless of the configuration tested. Hence, biofilter A produced approximately 2.7 g CO_2 $g_{toluene}^{-1}$ (r^2 = 0.950), while biofilter B produced approximately 2.9 g CO_2 $g_{toluene}^{-1}$ exhibiting a high linear correlation (r^2 = 0.936) (Fig. 5A and B). This high CO_2 production yield, compared to the theoretical stoichiometric production of 3.3 g CO_2 $g_{toluene}^{-1}$ for complete toluene oxidation (without biomass formation), was higher than the previously reported yield of 2.1 g CO_2 $g_{toluene}^{-1}$ (Bordel et al., 2007) in suspended growth bioreactors but agrees with the 2.8 g CO_2 $g_{toluene}^{-1}$ recently reported for toluene biofiltration in organic biofilters, highlighting

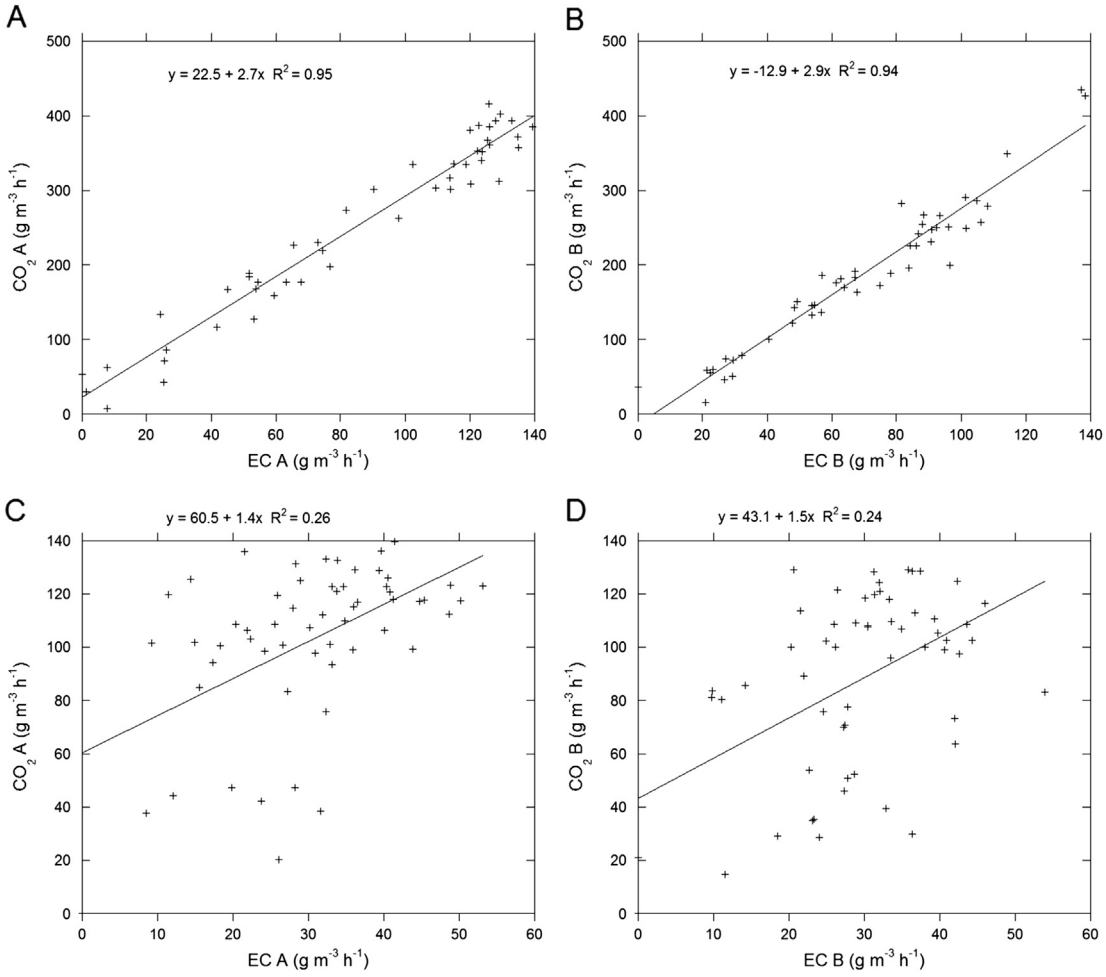

Fig. 5 – CO_2 production rate as a function of EC during the 70 days of operation with compost as a packing material in biofilters A (A) and B (B), and during the 120 days of operation with perlite as a packing material in biofilters A (C) and B (D). The fitting equation and correlation coefficient were obtained by linear regression of the experimental data.

the high mineralization capacity of both biofilters (Gallastegui et al., 2011b). No significant differences were observed between the initial and final period or between the three and two-stage configuration in biofilter B.

On the other hand, when perlite was employed as a packing material, CO_2 yields of 1.4 and 1.5 g CO_2 $g_{toluene}^{-1}$ were recorded in biofilters A and B, respectively. However, a poor linear correlation between CO_2 and EC was observed in both biofilter A ($r^2 = 0.262$, Fig. 5C) and B ($r^2 = 0.238$, Fig. 5D). This decoupling between EC and CO_2 can be attributed to the accumulation and further biodegradation of metabolites during periods of high EC, or, in general, to an unstable microbial metabolism. Moreover, no significant differences were observed after the inoculation with *P. putida* F1 or between the three and two-stage configuration in biofilter B.

3.4. *Elimination capacity vs. energy requirements*

Based on the results here reported and on previously published works on toluene biofiltration, the following rules of thumb can be established considering a realistic optimum biofilter operation at ECs around 100 g m^{-3} h^{-1} and pressure drops of 100–200 Pa m_{bed}^{-1}: ε values between 0.5 and 1 imply a good biofilter operation, while ε values higher than 1 represent an outstanding performance (a high abatement performance at a minimum energy consumption). On the other hand, ε values below 0.5 correspond to a deficient performance due to a limited EC or an excessive pressure drop across the bed. The concept of biofiltration efficiency can be applied to other pollutants or gas treatment processes, however, these empirical rules of thumb must be re-set for each new scenario (Table 1).

During the first 35 days of operation with compost as a packing material (Fig. 6A), biofilter A supported ε values below 0.5 $g_{toluene}$ N^{-1} h^{-1}, which increased up to 2 $g_{toluene}$ N^{-1} h^{-1} immediately after packing mixing to gradually decrease from day 48 onwards. On the other hand, the step-feed biofilter B exhibited higher ε than biofilter A during the first 35 days of operation ($\varepsilon \approx 0.5$ $g_{toluene}$ N^{-1} h^{-1}). Compost re-packing supported ε values over 1 $g_{toluene}$ N^{-1} h^{-1} for approximately 1 month, with the maximum ε achieved immediately after re-packing the 3 modules of biofilter B. Overall, toluene biofiltration with perlite supported lower ε values than those achieved with compost, mainly due to the limited EC and

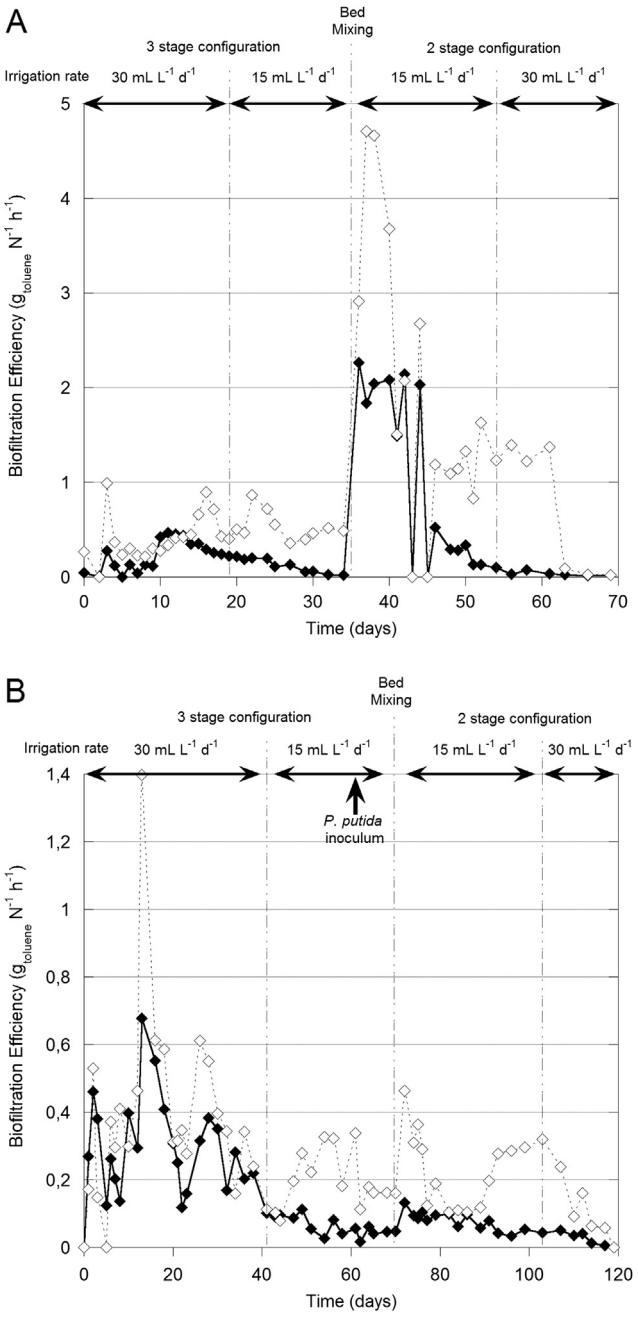

Fig. 6 – Time course of biofiltration efficiency (ε) in biofilters A (◆) and B (◇) during the compost (Fig. 5A) and the perlite-based biofiltration (Fig. 5B). Horizontal arrows dictate the operational periods and irrigation rates.

despite the lower pressure drops recorded (Fig. 5B). The step-feed configuration always exhibited higher ε than the standard perlite unit, but neither the inoculation with *P. putida* F1 nor packing material mixing caused a significant increase in the performance of perlite-based biofilters, which supported ε values below 0.5 $g_{toluene}\ N^{-1}\ h^{-1}$ during the most part of the experiment.

One of the most remarkable results of this study was the limited EC obtained in perlite-based biofilters both under the standard and step-feed configurations. These ECs were significantly lower compared to those here obtained in the compost-based biofilters or to those reported for typical organic packing materials in literature. However, a detailed literature review showed that inert packing materials commonly exhibit a low toluene abatement performance compared to organic packing materials, despite offering lower pressure drops and longer lifespans (Gallastegui et al., 2011a; Ortiz et al., 2003; Sakuma et al., 2006; Song and Kinney, 2005; Woertz et al., 2002). Other studies report high ECs when inert packing materials are mixed with organic materials (Znad et al., 2007) or used in fungal biofiltration (García Peña et al., 2001; Van Groenestijn et al., 2001). Recent publications have shown that a high diversity and functional redundancy are key aspects to achieve a stable and efficient long term operation in biofilters (Cabrol et al., 2012). Thus, despite the WWTP sludge is often considered a highly diverse inoculum, the indigenous microbial species (especially fungi) present in most composts have been shown crucial for the biodegradation of BTEX in biofilters (Prenafeta-Boldú et al., 2012). The high specific surface areas and the availability of micro and macro nutrients characteristic of compost probably also play a key role in the high ECs achieved. However, the main drawback of compost as a packing material is often its low lifespan, since biofilter A developed prohibitive pressure drops in only one month of operation. In this context, pressure drops of up to 2000 Pa m_{bed}^{-1} were recently achieved in only 30 days of operation in a biofilter packed with compost-covered clay pellets, which highlight the poor structural stability of organic packing materials (Dorado et al., 2012).

4. Conclusions

This study confirmed that step-feed biofiltration can provide similar toluene eliminations than standard biofilters while reducing energy requirements and increasing packed bed lifespans. The benefits provided by this configuration are more significant when implemented in biofilters packed with organic materials, which are more prone to clogging due to biomass accumulation and media deterioration.

Acknowledgements

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Methane abatement in a gas-recycling biotrickling filter: evaluating innovative operational strategies to overcome mass transfer limitations.

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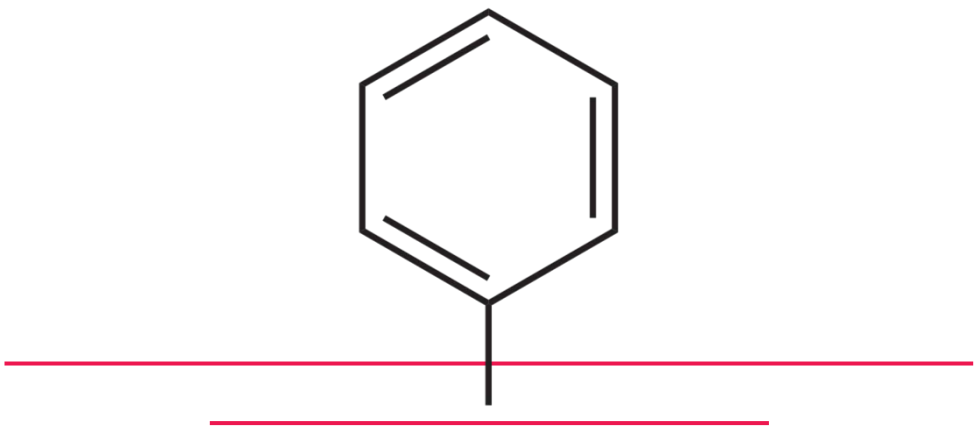
CEJ (Submitted for Publication)

Chapter 9



Methane abatement in a gas-recycling biotrickling filter: evaluating innovative operational strategies to overcome mass transfer limitations

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Abstract
The present study aimed at maximizing the performance of a standard biotrickling filter (BTF) devoted to the treatment of CH_4 at low concentrations by enhancing the mass transfer using optimum liquid recycling rates and an innovative gas recycling strategy. Internal gas recycling favored CH_4 abatement in the early stages of BTF operation and supported stable elimination capacities (ECs) above 30 g m^{-3} h^{-1} at an empty bed residence time of 4 min and a liquid recycling velocity of 5 m h^{-1}, which represented the highest ECs achieved in single phase BTFs to date. A comprehensive energy analysis confirmed that internal gas recycling could increase CH_4 abatement by 50% at only 10% higher operating costs. The BTF exhibited a high microbial diversity (Shannon-Wiener indices of 2.5-2.8) dominated by Type I methanothrophs, likely due to the presence of high Cu^{2+} concentrations. Mass transfer limitations from the aqueous phase to the microorganisms, attributed to biomass accumulation in the packing material, were identified under the long term operation.

Keywords: biotrickling filter, greenhouse gas, mass transfer, methane, polyurethane foam.

1. Introduction

Methane, with a global warming potential 20 times higher than that of CO_2, is nowadays the second most relevant greenhouse gas (GHG) emitted to the atmosphere. Atmospheric CH_4 concentrations in 2011 exceeded pre-industrial levels by 150% [1, 2], with anthropogenic emissions representing 50-65 % of the total CH_4emission inventory worldwide [1]. In this context, the increased public awareness of environmental problems and the urgent need to reduce anthropogenic GHG emissions worldwide are promoting an intensive research on the development of cost-effective and environmentally friendly CH_4 abatement technologies.

Methane emissions not suitable for energy recovery (methane content < 30%) have been traditionally treated using flaring or incineration as end-of-the-pipe technologies [3]. Unfortunately, while these oxidation technologies are only cost-effective for emissions containing CH_4 concentrations over 20%, more than 50% of the anthropogenic CH_4 is emitted at concentrations below 3% [4]. Dilute CH_4 emissions are typically found in old landfills fugitive emissions or gas recovery systems (0-20%), in ventilated coal mines (0.1 – 1 %) or in covered liquid manure storage tanks (0-3%) [5-9]. In this regard, biological technologies represent a promising end-of-the-pipe solution for the treatment of dilute off-gas emissions, biotrickling filtration being one of the most cost-effective configurations due to its robustness and low operating costs [10, 11].

However, pollutant mass transfer limitations often reduce the abatement potential and hinder the full-scale application of biotrickling filters (BTFs)

devoted to the treatment of highly hydrophobic compounds such as CH_4[12]. Most recent research studies have focused on CH_4 mass transfer enhancement by either applying complex bioreactor configurations such as horizontal biofilm, airlift or tailor flow reactors [13-15] or by adding non-aqueous phases and surfactants to conventional bioreactor configurations [4, 16]. However, both approaches have resulted in limited elimination capacities and entailed high operating costs [16]. Therefore, the development of simple and cost-effective bioreactor configurations and operational strategies devoted to CH_4 abatement will be crucial in the global fight against climate change.

The present study aimed at maximizing the abatement capacity of a standard, single-phase BTF treating dilute CH_4 emissions. First, the influence of the gas empty bed residence time (EBRT) and the linear liquid recycling velocity (U_L) on the abiotic k_{LACH4} and pressure drop in the BTF was characterized. Secondly, the influence of U_L, internal gas recycling and liquid media renewal rate on the CH_4 biodegradation performance of the BTF was evaluated. Internal gas recycling constitutes an innovative mass transfer enhancement approach based on the decoupling of the gas-liquid turbulence inside the reactor from the actual gas residence time. Finally, the dynamics of the microbial community structure responsible for CH_4 biodegradation were elucidated.

2. Materials and Methods

2.1 Chemicals

The mineral salt medium (MSM) used during the experimentation was a modified Brunner medium consisting of (gL^{-1}): $Na_2HPO_4{\cdot}12H_2O$, 6.15; KH_2PO_4, 1.52; $NaNO_3$, 0.61 (used instead of $(NH_4)_2SO_4$to prevent the inhibition of methanotrophs by ammonia [17]); $MgSO_4{\cdot}7H_2O$, 0.2; $CaCl_2{\cdot}2H_2O$, 0.05; EDTA, 0.005; $FeSO_4{\cdot}7H_2O$, 0.002; H_3BO_3, 0.0003; $CoCl_2{\cdot}6H_2O$, 0.0002; $ZnSO_4{\cdot}7H_2O$, 0.0001; $Na_2Mo_4{\cdot}2H_2O$, 0.00003; $MnCl_2{\cdot}4H_2O$, 0.00003; $NiCl_2{\cdot}6H_2O$, 0.00002; $CuCl_2{\cdot}2H_2O$, 0.00001. Cu^{2+} was supplemented to the MSM from a 10 g L^{-1} $CuSO_4$ stock solution to the target concentrations in order to avoid copper limitations. All chemicals were purchased from Panreac (Spain) with a purity higher than 99.0%. Methane (99.5% purity) and nitrogen (99.9% Purity) were supplied by Abello-Linde, S.A. (Spain), while silicone oil 200 cSt (99.9% purity) was purchased from Sigma Aldrich (USA).

2.2 Inoculum

The BTF was inoculated with methanotrophic cultures enriched from aerobic activated sludge from Valladolid wastewater treatment plant (Valladolid, Spain). Sludge samples were acclimated separately to CH_4 degradation for 37 days at Cu^{2+}concentrations of 5, 10, 25 and 50 μM in order to assess the influence of copper concentration on methane biodegradation. Methanotrophic cultures were enriched at 25 ºC in 1250 mL bottles containing 500 mL MSM and batchwise fed (8 amendments) with CH_4 at initial headspace concentrations of ≈ 14 g m^{-3}. Based on the negligible influence of Cu^{2+} concentration on the CH_4 biodegradation rate (data not shown), the BTF was inoculated with 300 mL of each culture and further operated at 10 μM Cu^{2+}.

2.3 Experimental set-up

A laboratory scale BTF consisting of a cylindrical jacketed PVC column (0.08 m inner diameter) was packed with polyurethane foam (PUF) to a working packed bed volume of 4 L. The packing material consisted of 1 cm^3 PUF cubes (Filtren TM 25280, Recticel Iberica S.L.) with a net density of 20–24 kg m^{-3} and a specific surface area of 1000 m^2 m^{-3}. MSM (1.2 ± 0.2 L) was continuously recycled into the BTF from an external 1.2 L jacketed holding tank stirred at 700 rpm (Agimatic-S, Selecta®, Spain) (Figure 1). All experiments were carried out at 20ºC.

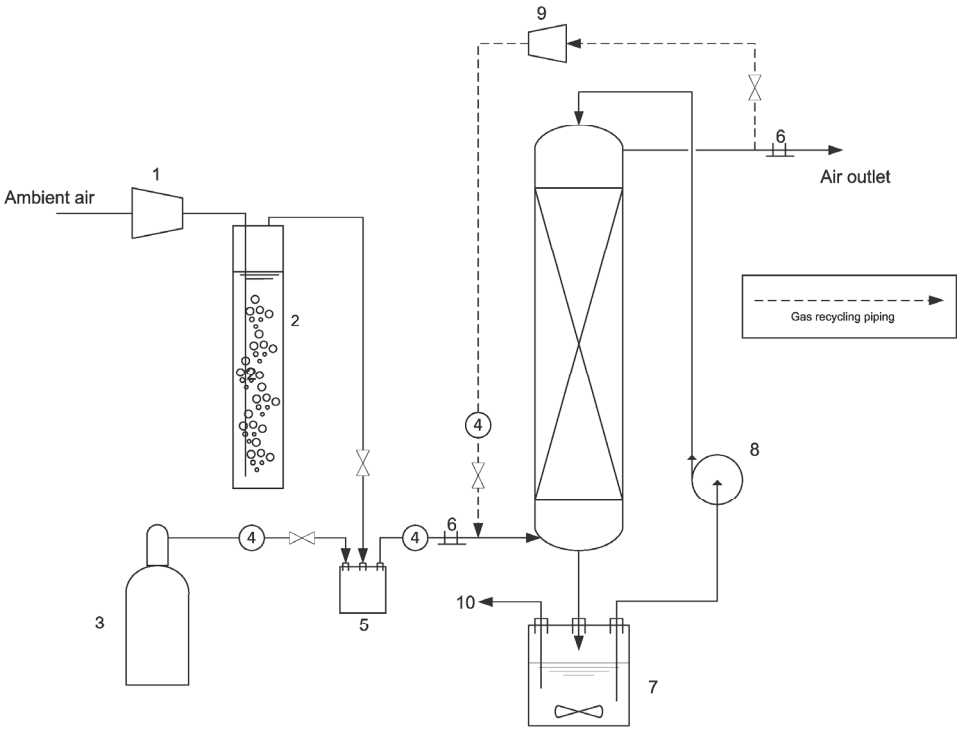

Figure 1. Schematic representation of the experimental set-up. 1. Ambient air compressor, 2. Humidifying column, 3.CH_4 reservoir, 4. Mass flow controllers, 5. Mixing chamber, 6. Gas sampling ports, 7. Stirred tank, 8.Liquid recycling pump, 9.Gas recycling compressor, 10.Liquid sampling port.

2.4 Influence of the EBRT and liquid recycling on k_LaCH_4 and pressure drop

The overall volumetric mass transfer coefficients for O_2 were determined at EBRTs of 12, 60, 120, and 240 s and liquid recycling velocities (U_L) of 0.6, 2, 3, 4, and 5 m h^{-1} using distilled water as the recycling liquid. N_2 was initially supplied to the BTF until the O_2 concentration in the liquid phase (recorded in the holding tank) reached ≈0 ppm. Then, air was supplied to the BTF while monitoring the increase in dissolved oxygen concentration. The experimental data were fitted to the model described by Lebrero et al. (2012) [18]. The overall k_La values for CH_4were estimated from $k_LaO_2$using the correlation reported by Yu et al. 2006 (Equation 1) [19]:

$$\frac{k_L a_{CH4}}{k_L a_{O2}} = \frac{\left(1/V_{m,CH4}\right)^{0.4}}{\left(1/V_{m,O2}\right)^{0.4}} \quad \text{Equation 1}$$

where the mass transfer coefficient of a target gas pollutant (k_LaCH_4) can be estimated from the coefficient of a reference gas (k_LaO_2 in the present study) previously determined in the same reactor under the same operating conditions by means of the molar volumes of the gaseous compounds ($V_{m,X}$).

The pressure drop across the packed bed was also recorded under all the EBRTs and U_L tested. Tests in the un-packed BTF were also carried out at all conditions assessed in order to account exclusively for the pressure drop caused by the packed bed.

2.5 Optimization of CH_4 biodegradation in the BTF

The synthetic methane-polluted emission fed to the BTF (15.3±0.5 g CH_4 m^{-3}, 2.2±0.1 %) was obtained by mixing a pure methane stream with a pre-humidified air stream in a mixing chamber. The emission flow-rate and CH_4 concentrations were regulated by means of mass flow controllers (Aalborg, USA), resulting in an EBRT of 4 min and an overall loading rate of 229±8 g m^{-3} h^{-1}. The internal gas recycling was carried out using an EVO 10 compressor (Electro A.D. S.L., Spain) by re-pumping 18 L min^{-1} from the

top to the bottom of the BTF and mixing this recycled air flow with the fresh methane-polluted emission (Figure 1). This innovative operational mode allowed the BTF to operate with a global EBRT of 4 min and the gas-liquid turbulence at an effective EBRT of 12.6 s.

The MSM renewal rate was set at 50 mL day^{-1} (dilution rate, D =0.045 d^{-1}) from days 0 to 47, 100 mL d^{-1} (D=0.09d^{-1}) from days 48 to 66, and 300 mL day^{-1} (D=0.27d^{-1}) from days 67 to 110 in order to avoid both nutrient limitation and the accumulation of toxic inhibitory metabolites in the recycling liquid. The liquid recycling rates tested in the BTF (200, 500, and 1500 mL min^{-1} corresponding to U_L of 2.3, 5, and 15 m h^{-1}) were controlled by means of a Dosapro series G™ A pump (Milton Roy Ltd., USA) and a 520-S pump (Watson Marlow, UK)at the highest flow rate.

Gas samples were periodically drawn from the sampling ports located at the inlet and outlet of the BTF to monitor the CH_4 and CO_2 concentrations. Liquid samples were periodically drawn from the stirred tank and filtered through 0.45 μm Millipore filters for the determination of pH, total organic carbon (TOC) and total nitrogen (TN) concentrations. Bed compaction was directly measured from the variations in the packed bed height in the BTF. The humidity of the inlet CH_4-laden air stream was measured on-line by a thermohygrometer (Testo 605-H1, Testo AG, Germany) and ranged from 40 to 60%. Water losses by evaporation in the system were balanced by addition of distilled water to keep a constant recycling liquid volume.

2.6 Analytical methods

The CO_2 and CH_4 gas concentrations were determined in a Bruker 430 gas chromatograph (Palo Alto, USA) coupled with a thermal conductivity detector and equipped with a CP-Molsieve 5A (15m × 0.53 μm × 15 μm) and a CP-PoraBOND Q (25m × 0.53 μm × 10 μm) columns. The oven, injector and detector temperatures were maintained at 45 °C, 150 °C and 175 °C, respectively. Helium was used as the carrier and make up gas at 6 mL $min^{-1}$and 24 mL min^{-1}, respectively. The pH was determined using a pH-meter Basic 20 (Crison, Spain), while the concentrations of TOC and TN were measured using a Shimadzu TOC-VCSH analyzer (Japan) equipped with a TNM-1 chemiluminescence module. Dissolved O_2 concentration in the holding tank was measured by means of an oxygen probe (Consort©, Belgium) connected to a multi-parameter analyzer C3020 (Consort©, Belgium) and a computer data logger as described elsewhere [20]. Pressure drop was measured by means of a U-Tube manometer connected to the inlet and outlet of the reactor using water as the manometric fluid.

2.7 Microbiological procedures

Biomass samples from the cultures acclimated at different Cu^{2+} concentrations (5, 10, 25 and 50 μM corresponding to samples A, B, C and D, respectively), from the mixed BTF inoculum (sample E) and from the BTF at days 38 (sample F) and 104 (sample G) were collected and stored at -20ºC in order to evaluate the richness and composition of the bacterial communities. The genomic DNA was extracted according to Lebrero et al. (2011) [23] . The PCR mixture (50 μL) was composed of 25 μL of BIOMIX ready-to-use 2× reaction mix (Bioline, Ecogen) containing reaction buffer, magnesium chloride, deoxynucleotide triphosphates (dNTPs), Taq polymerase and additives, 1 or 2 μL of the extracted DNA, PCR primers 968-F-GC and 1401-R (10μM) (Sigma- Aldrich, St. Louis, MO, USA) for bacterial 16S rRNA gene amplification, and Milli-Q water up to a final volume of 50 μL. The PCR thermo-cycling program used was previously described in Lebrero et al. (2011). The DGGE analysis of the amplicons was performed with a D-Code Universal Mutation Detection System (Bio Rad Laboratories) using 8% (w/v)

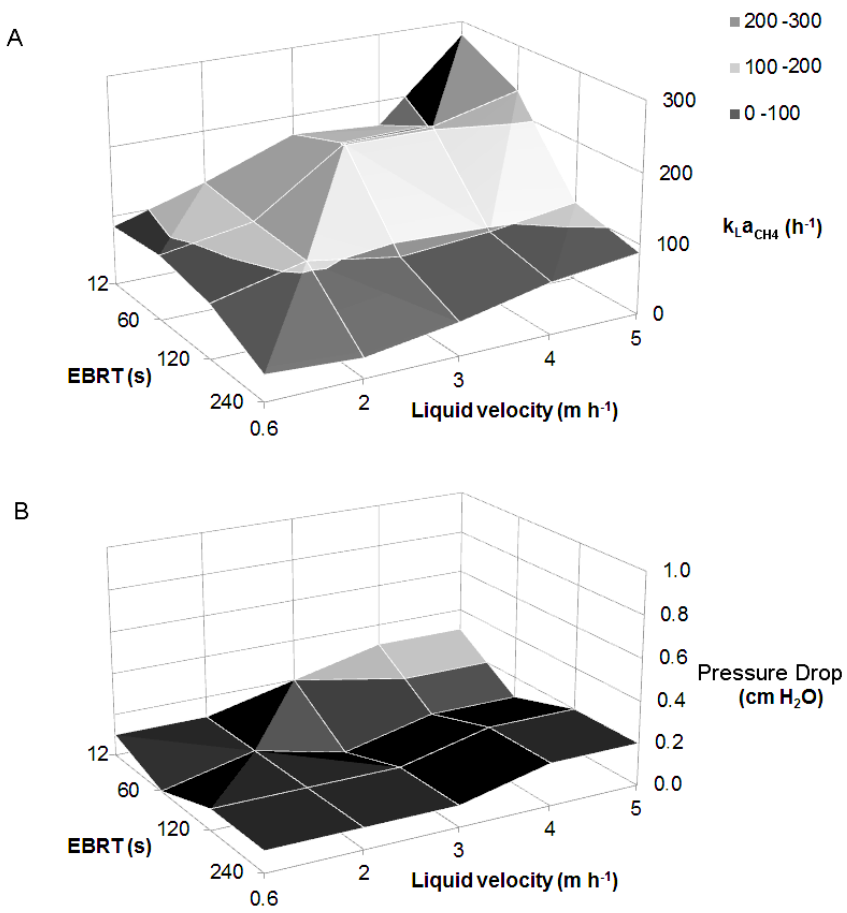

Figure 2. Influence of the gas EBRT and liquid recycling velocity (U_L) on k_La for CH_4 **(A)** and on the pressure drop in the packed bed **(B).**

polyacrylamide gel with a urea/formamide denaturing gradient from 45 to 65%. The DGGE running conditions were applied according to Roest et al. (2005) [21]. The gels were stained with GelRed Nucleic Acid Gel Stain (biotium) for 1 h and the obtained DGGE patterns processed using the GelCompar IITM software (Applied Maths BVBA, Sint-Martens-Latem, Belgium). After image normalization, bands were defined for each sample using the band search algorithm within the program. Similarity indices of the compared profiles were calculated from the densitometric curves of the scanned DGGE profiles by using the Pearson product–moment correlation coefficient [22]. The peak heights in the densitometric curves were also used to determine the Shannon–Wiener diversity index (H).

The most relevant bands were excised from the DGGE gel in order to identify the bacteria present in the samples above described. The procedure was previously described in Lebrero et al. (2011) [23]. The taxonomic position of the sequenced DGGE bands was obtained using the RDP classifier tool (50% confidence level) [24]. The closest matches to each band were obtained using the BLAST search tool at the NCBI (National Centre for Biotechnology Information) [25]. Sequences were deposited in GenBank Data Library under accession numbers KJ002507- KJ002532.

3. Results and Discussion

3.1 Influence of the EBRT and liquid recycling on k_LaCH_4 and pressure drop

The overall k_LaCH_4 values increased when increasing the liquid recycling velocity and decreasing the EBRT, with a maximum of 280±15 h^{-1} recorded at 5 m h^{-1} and 12 s (Figure 2A). CH_4 mass transfer exhibited a

low sensitivity towards variations in the gas EBRT at low liquid recycling velocities. For instance, the overall k_La_{CH4} increased from 37 to 85 h^{-1} (130%) when decreasing the EBRT from 240 to 12 s at a U_L of 0.6 m h^{-1}, while this increase accounted for 220% at a U_L of 5 m h^{-1}. These empirical findings were in agreement with the data reported by Kim and Deshusses (2008), where the volumetric mass transfer coefficient in the liquid film (k_La_w) for $CO_2$was not sensitive to variations in the gas flow-rate at the lowest U_L tested (0.1 m h^{-1}) [26]. This also suggested that the process was limited by mass transfer in the liquid side under low U_L, since only a moderate mass transfer improvement was observed when increasing the turbulence in the gas side. An increasing influence of the EBRT on the overall k_La_{CH4} was recorded at higher U_L (3, 4, and 5 m h^{-1}) likely due to a decrease in the mass transfer resistance in the liquid film (as a result of the higher liquid turbulence), concomitant with an enhanced transport in the gas side. Similarly, a reduced k_La_{CH4} sensitivity towards variations in U_L was recorded at high EBRTs. Therefore, these results suggest that attempts to overcome mass transfer limitations in BTFs by increasing the liquid recycling rate might be only cost-effective at low EBRTs. Internal gas-recycling can help reducing the mass transfer resistances while operating at high EBRTs, which can eventually boost CH_4 abatement in BTFs [13].

On the other hand, no significant influence of the EBRT and liquid recycling velocity on the pressure drop across the packed bed was recorded, with a maximum pressure drop variation from 0.1 to 0.3 Pa m^{-1}_{bed} under the conditions tested (Figure 2B). This finding was of key relevance for the implementation of internal gas-recycling strategies, since any additional energy requirement in this innovative operational mode would derive from the higher circulating flow rates rather than from an additional pressure drop mediated by the internal gas recycling.

3.2 Optimization of CH_4 biodegradation in the BTF

When the BTF was operated at an EBRT of 4 min and U_L = 2.3 m h^{-1}, the EC remained below 2 g m^3 $h^{-1}$with stable CO_2 production rates of ≈10 g m^{-3} h^{-1} (Figure 3). The high CO_2 production, above the expected levels according to the low CH_4 EC recorded, was attributed to the inoculum endogenous respiration. Then, U_L was increased to 5 m h^{-1} by day 13 in order to overcome possible mass transfer limitations in the system. This change in operational conditions corresponded to an overall abiotic k_La_{CH4} increase from 28 h^{-1} to 88 $h^{-1}$and resulted in fluctuating ECs (4.2 to 16.3 g m^{-3} h^{-1}) from day 14 to 31, which confirmed the occurrence of mass transfer limitation in the liquid side during the previous operational stage. CO_2 production rates gradually decreased in this period, which was attributed to the increasing contribution of anabolism (biomass growth) to CH_4 biodegradation compared to process start-up where endogenous respiration was the predominant process (Figure 3B). This fact was confirmed by visual observation of the significant biomass growth on the packing material and the low CH_4 mineralization observed in this period (24±12%).

Internal gas recirculation was implemented on day 31 at a rate of 18 L min^{-1}, resulting in a virtual EBRT of 12 s in the packing material while maintaining a global EBRT of 4 min and a CH_4 loading rate of 229±8 g m^{-3} h^{-1}. This strategy was expected to increase the mass transfer coefficient for CH_4 by a factor of 3, which agreed with the EC of up to 29 g m^{-3} h^{-1} recorded by day 32 (2.5 fold EC increase). This fact confirmed the potential of internal gas recirculation to enhance CH_4 mass transfer from the gas to the liquid phase. However, a sharp decrease in the EC to 0 g m^{-3} h^{-1} by day 35 was observed, which was attributed to nutrient limitation in the system. Thus, 500 mL of recycling cultivation broth were replaced by fresh MSM at day 38, which allowed to recover an EC of 27 g m^{-3} h^{-1} on day 39. The EC was then allowed to

gradually decrease again in order to confirm a potential nutrient limitation. The absence of CH_4 biodegradation recorded by day 42 coincided with negligible TN concentrations in the recycling liquid medium. Hence, NO_3^- concentrations in the BTF were daily restored from days 43 to 47 by adding 12 mL of a 100 g L^{-1} stock nitrate solution, which entailed TN concentrations of 72 ± 32 mg L^{-1} and steady EC of 21.7 g m^{-3} h^{-1} by day 48. Therefore, nitrogen was identified as the key limiting factor under internal gas recycling at 18 L min^{-1} and U_L of 5 m h^{-1}, and an increase in the MSM renewal to a D of 0.09 day^{-1} was implemented. This higher frequency in MSM exchange was able to maintain ECs of 18.5 ± 3.0 g m^{-3} h^{-1} for only 7 days. CH_4 biodegradation performance started to decrease again by day 55 likely due to the accumulation of inhibitory biodegradation metabolites. Hence, while a D of 0.09 day^{-1} was able to maintain TN concentrations at 149 ± 28 mg L^{-1}, TOC concentration in the recycling liquid increased from 77 to 161 mg L^{-1} from day 55 to day 66. The present empirical findings were in agreement with the deterioration in CH_4 oxidation activity observed by Mancebo et al. (2012) in an organic packing-based biofilter at high

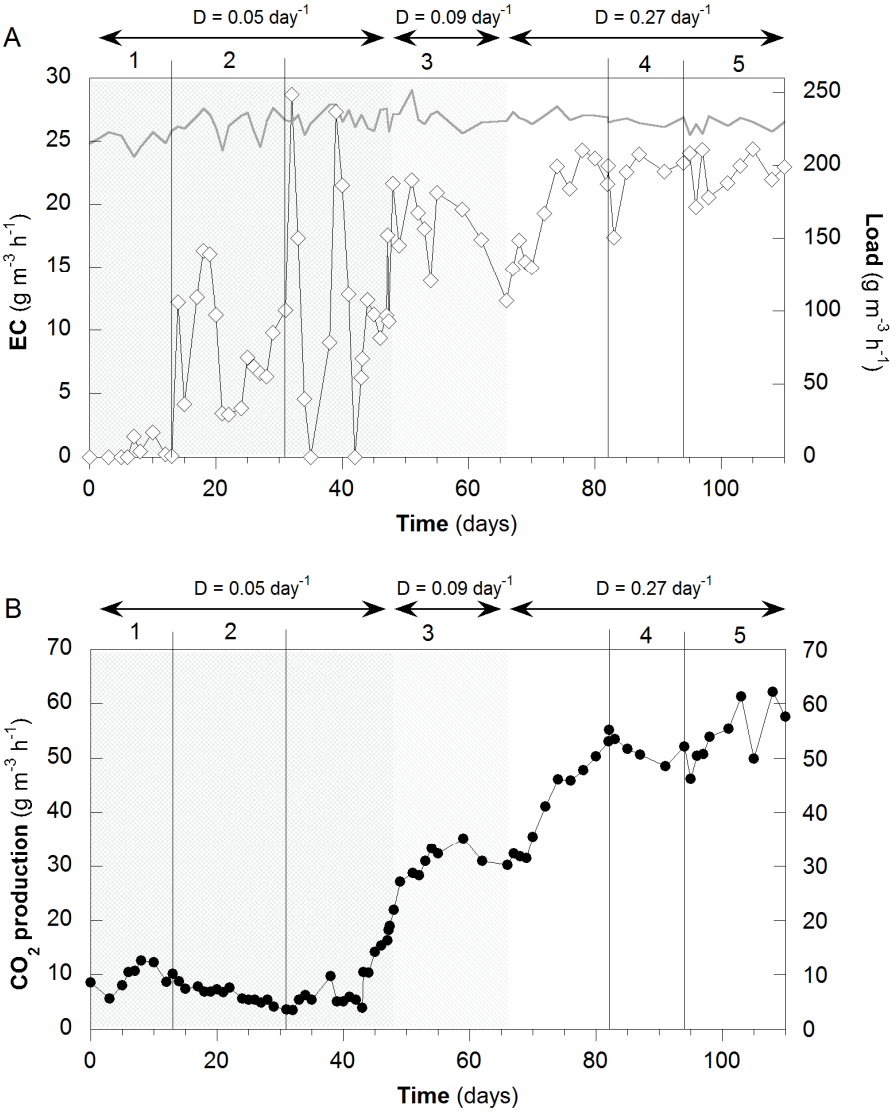

Figure 3. Time course of **(A)** Loading rate (grey line) and EC (white diamonds), and **(B)** CO_2 production rate (black circles) during CH_4 biodegradation in a BTF. Horizontal arrows indicate the MSM exchange rates, while vertical lines indicate the different operational stages: 1. U_L= 2.3 m h^{-1}, 2.U_L = 5 m h^{-1}, 3. Internal gas recycling at 18 L min^{-1}, 4. Gas recycling stopped, 5. U_L = 15 m h^{-1}.

dissolved organic carbon concentrations [27]. Therefore, a new MSM dilution rate of 0.27 $day^{-1}$was implemented from day 66 onward, which allowed to maintain TOC concentrations at ≈ 100mg L^{-1} and TN concentrations >100 mg L^{-1}. In this context, the EC gradually recovered to steady values of 22.2 ± 1.8 g m^{-3} h^{-1} from day 72 to 82 concomitant with a rise in CO_2 production up to 47.3 ± 4.1 g m^{-3} h^{-1} (89% CH_4 mineralization).

Internal gas recirculation was stopped at day 82 in order to confirm the potential of this operational strategy to enhance CH_4 mass transport in the BTF operating at the real EBRT of 4 min. Surprisingly, no deterioration in CH_4 biodegradation was observed following the interruption of the gas recirculation, with average ECs and CO_2 production rates of 22.2 ± 2.4 g m^{-3} h^{-1} and 51.9 ± 2.3 g m^{-3} h^{-1}, respectively, from day 82 to day 94. Based on the previous abiotic mass transfer characterization, k_LaCH_4 decreased from 280 to 88 h^{-1} when the EBRT increased from 12 s to 4 min. It can be hypothesized that biomass growth in the packed bed modified both the hydrodynamics and mass transfer processes in the BTF. Popat and Deshusses (2010) recently reported the complex and significant influence of biomass growth on mass transfer mechanisms in BTFs, where shifts in rate-governing steps were associated to a biomass-mediated modification of the interfacial area available for pollutant mass transfer [28]. Likewise, Arellano-García et al. (2013) demonstrated that the accumulation of biomass can modify the hydrodynamics of the recycling liquid from plug flow, affecting pollutant biodegradation in the BTF [29].

The influence of U_L on the EC in the absence of internal gas recirculation was further assessed by increasing the liquid recycling rate from 5 to 15 m h^{-1} on day 94. Despite an increase in mass transfer was expected from the extrapolation of the results presented in Figure 2A, the reactor maintained stable ECs of 22.5 ± 1.7 g m^{-3} h^{-1} from days 94 to 110 showing a slight increase in the CO_2 production rates with an average value of 54.2 ± 5.4 g m^{-3} h^{-1} (Figure 3). A mass transfer limitation test was carried out on day 97 in order to elucidate the rate-limiting step by increasing the inlet gas CH_4 concentration from 15 to 41 g m^{-3} for 3.5 h (2.8 fold increase) (Figure 4). The EC rapidly increased from 24.3 to 63.8 g m^{-3} h^{-1} (2.6 times increase) during this step CH_4 load increase, and concomitantly decreased to previous steady state values of 19 g m^{-3} h^{-1} when the inlet CH_4 concentration was decreased to 15 g m^{-3}. This test confirmed that CH_4 abatement in the BTF was mass transfer limited, ruling out a potential biological limitation [30]. In addition, the determination of CH_4 concentration in the

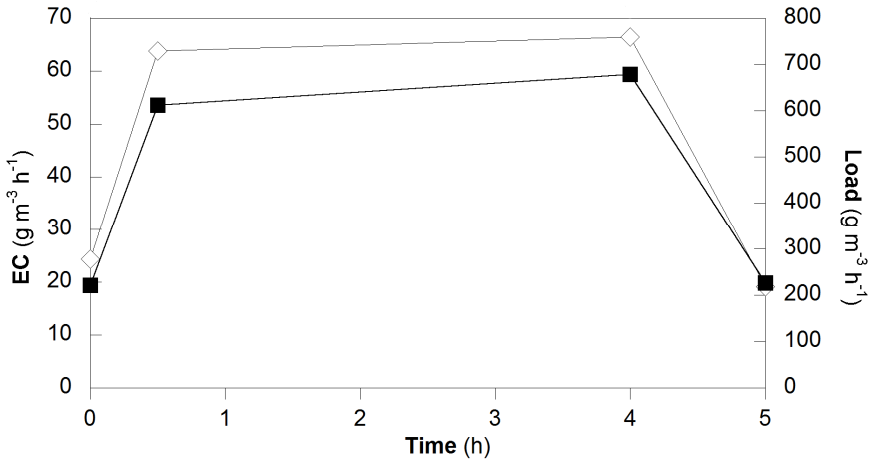

Figure 4. Time course of the EC (white diamonds) and loading rate (black squares) during the mass transfer limitation test.

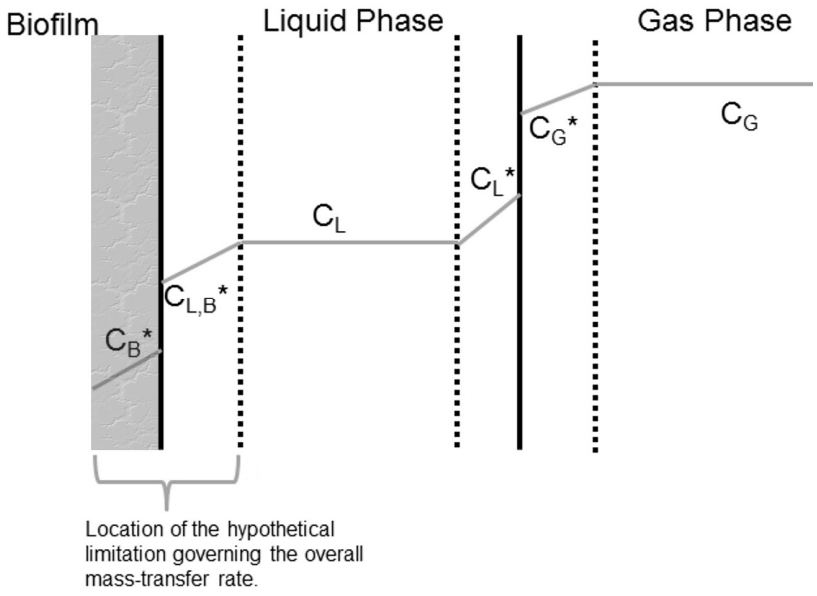

Figure 5. CH_4 concentration profile representing the mass transfer processes occurring in the BTF and the hypothetical mass transfer resistance governing the process. C_G = bulk concentration in the gas phase, C_G*= Gas phase concentration at the gas-liquid interphase,C_L*= Liquid phase concentration in equilibrium with the gas phase, C_L = bulk concentration in the liquid phase, $C_{L,B}$*= Liquid phase concentration at the liquid-biofilm interphase, C_B*= Biofilm concentration in equilibrium with the liquid phase.

liquid phase at the bottom of the column by day 100 revealed values of 0.22 ± 0.04 g m^{-3}, which were close to the theoretical equilibrium concentration of 0.40 g m^{-3} calculated by the Henry's law. This confirmed that CH_4 was effectively transferred to the liquid phase but suggested that the diffusive transport through the biofilm was the limiting mass transfer process. In view of the above-mentioned results, the occurrence of a mass transfer limitation between the liquid phase and the biofilm colonizing the packed bed might be hypothesized (Figure 5). This would explain the absence of increase in EC when increasing the gas velocity, and the high CH_4 concentrations recorded in the recycling liquid phase. Therefore, any operational modification to enhance the gas-liquid mass transfer would be unfruitful to increase CH_4 abatement. However, the increase in the U_L was also unsuccessful in enhancing the liquid-biofilm mass transfer, probably due to interfacial area decrease caused by biomass growth [28].

Finally, a significant packed bed compaction was recorded during BTF operation. A 22% bed compaction was recorded by day 87, increasing up to 34% by day 98 and to 35% by day 105. This high bed height decrease determined the effective EBRT and consequently the CH_4 load. Thus, the average ECs reported (≈22g m^{-3} h^{-1}) during the final stages of the experiment (days 94-110) should be corrected to account for the real packed bed volume, resulting in stable real ECs above 30 g m^{-3} h^{-1}[31]. To the best of our knowledge, these ECs were higher than any of the previously reported ECs in the scarce literature available to date for single phase BTFs treating CH_4. For instance, Avalos et al. (2012) found maximum ECs of ≈ 10 g m^{-3} h^{-1} in a stone-based BTF operated at 4.25 min of EBRT and CH_4 loads of 62 g m^{-3} h^{-1}, while Rocha-Rios et al. (2009) reached 22 g m^{-3} h^{-1} in a polyurethane foam-packed BTF operated at 4.8 min of EBRT and CH_4 loads of 140 g m^{-3} h^{-1}[32]. BTFs operated an EBRTs of 4-5 min support ECs similar to those obtained in full scale biofilters (BFs) operated at EBRTs sometimes exceeding 60 min (20-80 g m^{-3} h^{-1}) [3], while low EBRTs

Table 1. Comparative evaluation of energy consumption and GHG mitigation efficiency of internal gas recycling under two different scenarios. A reference (without gas recycling) and a gas recycle operation mode are considered for each scenario.

		Scenario 1		Scenario 2	
		Ref.	Recycle	Ref.	Recycle
Gas recycling ratio (Recycling flow/Feed flow)		0	18	0	1
Conditions	**V_L** ($m\ h^{-1}$)	5	5	2	2
	Virtual EBRT (s)	240	12	240	120
Energy consumed (energy units)		1	19	1	2
CH_4 removed* (CH_4 units)		1	3	0.25	0.75
CO_2 eq. removed (CO_2 units)		20	60	5	15
CO_2 eq. removed / energy consumed		20	3.2	5	7.5
Annual Operating Costs with gas recycling		1	3.6	1	1.1
Annual Operating Costs with EBRT increase**		1	2.5	1	2.5

*Comparative data based on the experimental data from the present study.

**Theoretically calculated by increasing the EBRT of the unit to achieve a similar CH_4 removal to that obtained under internal gas recycling.

(4.3 min) in BFs often result in lower ECs (e.g. ≈ 19 g $m^{-3}\ h^{-1}$) [33].

3.3 Energy considerations during operation with internal gas recycling

A cost-benefit analysis was conducted in order to evaluate the environmental sustainability of this operational strategy under two scenarios based on the overall k_La_{CH4} previously determined. Under reference scenario 1 (U_L = 5 m h^{-1}, EBRT = 240 s, no internal gas recycling) the BTF would require 1 energy unit and remove 1 CH_4 unit (or 20 CO_2 equivalent units) (Table 1). Thus, 20 CO_2 equivalents could be removed per unit of energy applied. The implementation of internal gas recycling to achieve a virtual EBRT of 12 s (by recycling 18 times the inlet flow rate) would increase the k_La_{CH4} by a factor of 3 (Figure 2A), removing 60 CO_2 equivalents. In this particular scenario, the energy consumption associated to gas pumping would increase by a factor of 18 (based on the fact that pressure drop remains constant, Figure 2B), and the efficiency would decrease from 20 to 3.2 units of CO_2 equivalents removed per unit of energy applied. This would result in an increase in the annual operating cost of 260 %, based on the fact that energy consumption in BTFs accounts for 22 % of the total operating costs (Estrada et al. 2012). On the other hand, an increase in the EBRT by a factor of 3 to achieve comparable ECs to those obtained under internal gas recycling would entail an increase in the operating cost of 150% (Estrada et al. 2011, 2012).

Nevertheless, internal gas recycling might be economically and energetically favorable under different operating conditions. The BTF would hypothetically remove 5 CO_2 equivalents under reference scenario 2 (U_L = 2 m h^{-1}, EBRT = 240 s and no internal gas recycling) and 15 CO_2 equivalents when internal gas recycling decreases the virtual EBRT to 120 s. Under this internal gas recycling, the BTF would double the energy consumption, resulting in removals of 7.5 units of CO_2 equivalent per unit of energy applied compared to 5 in reference scenario 2 (Table 1). In this case, the increase in the annual operating costs under internal gas recycling would account for only 10%, while an increase in the EBRT by a factor of 3 to achieve comparable ECs to those obtained under gas recycling would entail an increase in the operating cost of 150%

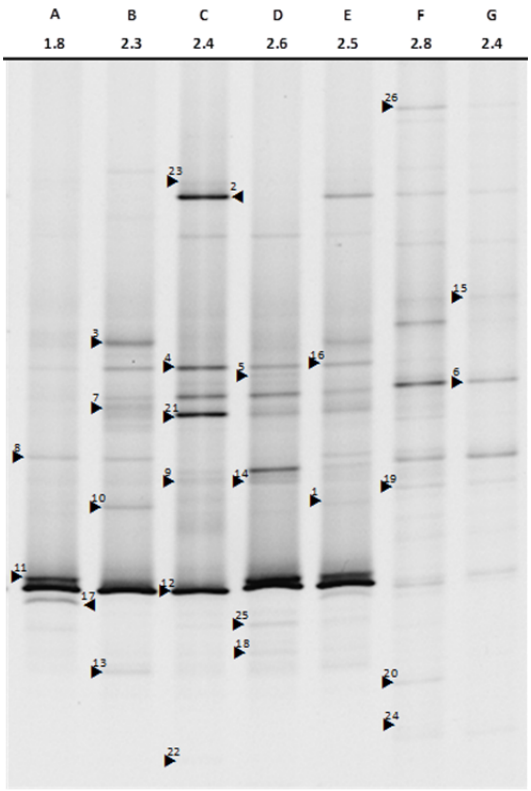

Figure 6. Bacterial DGGE profile of the four cultures acclimated at different Cu^{2+} (A, B, C and D corresponding to 5, 10, 25 and 50 ◎M, respectively), the mixed microbial inoculum (E), the population present in the BTF at day 38 (F) and 104 (G). The Shannon-Wiener diversity indices are indicated in the upper part of the gel. The sequenced bands are indicated by "▶" and the corresponding number of each band.

(Estrada et al. 2011, 2012). However, all these are hypothetical considerations and were not observed in the experimental operation of the BTF probably due to a biomass excessive growth which led to additional mass transfer limitations. Thus, finding optimal biomass content can be of key relevance for further optimization of the gas recycling strategy here proposed.

3.4 Bacterial population dynamics

The structure of the bacterial communities in the inocula and BTF was elucidated by sequencing 21 bands from the DGGE gel (Figure 6). The closest matches for each band, along with its similarity percentage and sources, are shown in Table 2 (Supplementary material). The phylum *Proteobacteria* was predominant in the cultures acclimated to different copper concentration and in the biotrickling filter regardless of the operational stage with 17 bands belonging to this phylum (DGGE bands1 to 7 and 13 to 22, Fig. 6). Most of these *Proteobacteria* were closely related to methane oxidizing bacteria (methanotrophs) and are often found at the anoxic/oxic interface of landfills, wastewater treatment plants, soils, rice paddies, peat bogs, wetlands and sediments[34, 35].

Aerobic methanotrophic bacteria obtain energy via CH_4 to CO_2 oxidation based on their ability to synthesize methane mono-oxygenases [36, 37]. Most methanotrophs belonged to the bacterial phylum Proteobacteria, in the classes Gammaproteobacteria (Type I) and Alphaproteobacteria (Type II) [38]. Type I methanotrophs, which include genera such as *Methylomonas, Methylobacter* and *Methylococcus*, [2] produce particulate methane monooxygenase (pMMO) and possess a more efficient CH_4-oxidizng metabolism than their type II counterparts [11, 35].

Both type I and II methanotrophs were identified in this work, type I being by far the most abundant type of methanotrophs. The closest relatives for DGGE bands 2-18 were type I methanotrophs [2], although the similarity was as low as 84 % for band 2 and 92 % for band 15. The preferential enrichment of type I methanotrophs was likely due to the high Cu^{2+} content (10 μM) in the mineral salt medium, which has been often correlated with the production of pMMO instead of the soluble form of the enzymes MMO [39]. Bands 19 and 20, which were relevant in the BTF but not in the initial inoculum belonged to the *Xanthomonagaceae* family and were related to other liquid or gas pollutant degraders. The DGGE band 21, specifically affiliated to the *Methylocystis* genus, was the only one belonging to type II methanotrophs. Bacteria belonging to the *Methylocystis*

genus have been also found in biofilters treating methane [40]. Finally, while the DGGE band 22 was 100% similar to a *Betaproteobacteria* isolated from a petroleum hydrocarbon-contaminatedwater[41], bands 23 to 26 were affiliated with the phylum *Chlamydae*, *Firmicutes, Gemmatimonadetes and Verrucomicribia,* but their similarity to the closest relatives ranged from 88 to 95%.

The Shannon-Wiener diversity indices for the methanotrophic cultures enriched at different Cu^{2+} concentrations revealed an increase in bacterial diversity at increasing Cu^{2+} concentrations in the cultivation medium (1.8, 2.3, 2.4 and 2.6 at Cu^{+2} concentrations of 5, 10, 25 and 50 μM, respectively). A high bacterial diversity (2.5-2.8) was maintained in the biotrickling filter from inoculation throughout the complete experimental period (Figure 6, samples E, F and G). However, a low similarity was observed between the inoculum and the community established in the reactor by day 38 (31.8% similarity) or by the end of the experiment (27.4% similarity). A significant evolution (66.4% similarity) of the communities governing CH_4 oxidation in the BTF was also observed from day 38 to 104. These results showed the progressive establishment of bacterial populations with high functional resilience and redundancy capable of maintaining high ECs despite the changes in their structure [42].

4. Conclusions

Internal gas recycling enhanced the performance of the BTF in the early stages of operation, which agreed with the data obtained in the abiotic mass transfer tests. Under certain operating conditions this strategy can theoretically result in a 50% improvement in the BTF energy use efficiency (CH_4 removal per unit of energy applied) at significantly lower operating costs than other strategies such as EBRT increase. However, the reactor faced additional mass transfer limitations not easily overcome by increasing the gas-liquid mass transfer as a result of biomass accumulation in the packed bed. CH_4 mass transfer from the liquid phase to the biofilm was identified as the limiting step during CH_4 abatement in our particular BTF. ECs higher than 30 g m^{-3} h^{-1} were achieved at an EBRT of 4 min and U_L of 5 m h^{-1}, which represent the highest ECs recorded in a single-phase BTF treating CH_4. A high diversity was found in the reactor through the experimental period showing high functional resiliency and redundancy and maintaining high EC despite changes in the bacterial community. Type I methanotrophs were dominant, proving that high Cu^{2+} concentrations in the recycling MSM was a successful strategy to promote their growth.

5. Acknowledgements

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Supplementary material

Table 2. RDP classification of the sequenced DGGE bands and corresponding matches (BLASTn) using the NCBI database with indication of the similarity percentages and sources or origin.

Taxonomic placement (50% confidence level)	Band	A	B	C	D	E	F	G	Closest relatives in Blast Name (accession number)	Similarity (%)	Source of origin
Phylum *Proteobacteria*											
	1								Uncultured bacterium (AF234724)	94	Nitrifying-Denitrifying Activated Sludge of an industrial wastewater treatment plant
Class *Gammaproteobacteria*											
	2			X		X	X	X	Bacterium enrichment culture HQ405606	84	Microbial community performing anaerobic oxidation of methane
	3	X	X	X	X	X			*Methylomonas* sp. (FR798969)	98	Methane oxidizing bacteria from wetland
	4	X	X	X	X	X	X		*Pseudoxanthomonas* sp. (FJ667504)	93	Activated sludge from an oil field wastewater-treating system
	5			X	X				*Methylomonas* sp. (FR798969)	97	Methane oxidizing bacteria from wetland
	6		X	X	X	X	X	X	*Methylomonas* sp. (FR798969)	93	Methane oxidizing bacteria from wetland
	7	X	X	X	X	X	X	X	*Methylomonas* sp. (FR798969)	98	Methane oxidizing bacteria from wetland
	13		X	X	X	X	X		*Methylococcaceae* bacterium (HF558990)	96	Methane-oxidizing communities from facultative ponds
	14				X				*Methylomonas* sp. (FR798969)	97	Methane oxidizing bacteria from wetland
Class *Gammaproteobacteria* Order *Methylococcales* Family *Methylococcaceae*											
	15						X	X	Uncultured *Methylobacter* sp. (GU472648)	92	Bacterial diversity from the oxic-anoxic interface of a meromictic lake
	16				X				*Methylomonas sp*. (FR798969)	97	Methane oxidizing bacteria from wetland
	17	X	X	X	X	X			*Methylomonas sp*. (FR798973)	99	Methane-oxidizing bacteria from a biofilter.
	18	X				X	X		*Methylomonas sp*. (FR798969)	95	Methane oxidizing bacteria from wetland

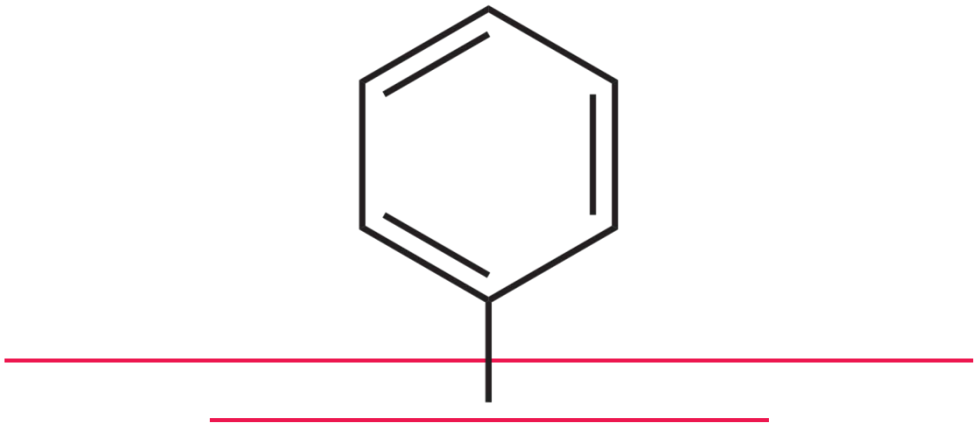
Table 2. (Continued)

Taxonomic placement (50% confidence level)	Band	A	B	C	D	E	F	G	Closest relatives in Blast Name (accession number)	Similarity (%)	Source of origin
Class Gammaproteobacteria Order *Xanthomonadales* Familiy *Xanthomonadaceae*											
	19						X	X	Uncultured bacterium (KC308294)	97	Hydrocarbon-degrading bacterial communities
Class Gammaproteobacteria Order Xanthomonadales Familiy Xanthomonadaceae Genus *Rhodanobacter*											
	20						X		Uncultured bacterium (FM213061)	99	Biotrickling filter removing H_2S from water treatment sludge
Class *Alphaproteobacteria* Order *Rhizobiales* Family *Methylocuystaceae* Genus *Methylocystis*											
	21		X	X	X	X	X	X	*Methylocystis* sp. (AJ458503)	99	Type II methane-oxidizing bacteria.
Class *Betaproteobacteria* Order *Rhodocyclales* Familiy *Rhodocyclaceae* Genus *Methyloversatilis*											
	22			X					*Methyloversatilis universalis* (KC577607)	99	Methylotrophic bacteria from filtration waters of landfills
Phylum *Chlamydiae* Class *Chlamydiae* Order *Chlamydiales* Familiy *Parachlamydiaceae* Genus *Neochlamydia*											
	23	X	X	X		X			*Neochlamydia hartmannellae* (NR025037)	93	Culture collection
Phylum *Firmicutes* Class *Bacilli* Order *Bacillales* Family *Paenibacillaceae*											
	24						X		Uncultured *Paenibacillus* sp. (JN038219)	91	Petroleum-contaminated soil
Phylum *Gemmatimonadetes* Class *Gemmatimonadetes* Order *Gemmatimonadales* Familiy *Gemmatimonadaceae* Genus *Gemmatimonas*											
	25			X	X	X	X		Uncultured bacterium (AF268993)	88	Activated-sludge wastewater treatment systems
Phylum *Verrucomicrobia* Class *Verrucomicrobiae* Order *Verrucomicrobiales* Familiy *Verrucomicrobiaceae* Genus *Prosthecobacter*											
	26						X	X	Uncultured bacterium (JQ713535)	95	Activated sludge

Biocatalytic coatings for air pollution control: a proof of concept study on VOC biodegradation.

José M. Estrada, Óscar I. Bernal, Michael C. Flickinger, Raúl Muñoz, Marc Deshusses (Unpublished Manuscript)

Chapter 10



Biocatalytic Coatings for Air Pollution Control: a proof of concept study on enhancing the rate of VOC Biodegradation

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Abstract

Despite biofilm-based biotechnologies exhibit a large potential as end-of-the pipe solutions for off-gas treatment, the high water content of biofilms often entails pollutant mass transfer limitations, which ultimately limit their widespread application. The present study constitutes a proof of concept on the applicability of bioactive latex coatings to air pollution control in order to maximize the biodegradation of volatile organic compounds (VOCs) using toluene as the model pollutant. The results showed that the methodology here proposed successfully entrapped *Pseudomonas putida* F1 cells preserving toluene degradation activity. Bioactive latex coatings showed specific elimination capacities 10 times higher than those supported by agarose-based biofilms, overcoming the strong mass transfer limitations encountered in conventional biofilms. Drying and starvation were identified as key factors inducing a gradual deterioration of the biodegradation capacity in these innovative coatings. This study constitutes the first application of bioactive latex coatings to VOC abatement, which represents a promising engineering approach towards biological air pollution control.

Keywords: air pollution, latex coating immobilization, *Pseudomonas putida*, toluene, VOC.

Introduction

Biotechnologies have emerged in the past three decades as cost-effective off-gas treatment techniques for air pollution control (Kennes et al. 2009), entailing important environmental and economic advantages over their physical/chemical counterparts (Estrada et al. 2012, Gabriel and Deshusses 2004). Biotechnologies have been applied to a wide range of gas emissions containing odours, volatile organic compounds (VOCs), H_2S or CH_4, biofilters and biotrickling filters being the most common bioreactor configurations (Iranpour et al. 2005, Lebrero et al. 2011, Nikiema et al. 2007).

Nowadays, most research efforts in the field are focused on improving the abatement performance of these biological techniques and on enlarging their applicability to a wider range of pollutants and concentrations (Kennes et al. 2009). In this regard, despite recent advances in the

field of microbial ecology have shed light in the previously undisclosed complexity of biological gas treatment processes, the number of microbial approaches to achieve these goals has been rather limited to date (Cabrol et al. 2010, Ralebitso-Senior et al. 2012). Most of the strategies recently applied to overcome mass transfer limitations, such as membrane bioreactors and two phase partitioning bioreactors, entail either increased operating costs or a high operational complexity (Kraakman et al. 2011, Lebrero et al. , Muñoz et al. 2012). In this context, the development of advanced biotechnologies based on cell immobilization into hydrophobic solid supports constitutes a suitable strategy to improve the off-gas treatment performance at reduced operating cost. Bioactive polymeric coatings can be engineered as nanoporous matrixes containing active cells and can adhere to a surface as active covers or paints (Flickinger et al. 2007). This innovative biocatalytic approach, despite having been successfully applied at laboratory scale for H_2 production and CO_2 photosynthetic assimilation, has never been applied to air pollution control (Fidaleo and Flickinger 2011, Gosse et al. 2012). The use of bioactive polymeric coatings in off-gas treatment biotechnologies would likely enhance direct pollutant uptake from the gas phase, thus avoiding mass transfer limitations associated to the water layer surrounding biofilms in most bioreactor configurations.

This work constitutes a proof of concept study on the applicability of bioactive latex coatings to air pollution control. The feasibility of producing active coatings for the biodegradation of toluene as a model VOC was assessed. The areal elimination capacity (EC), the specific EC and mineralization yields of these innovative bioactive latex coatings were determined and compared to water-based agarose biofilms and liquid cultures. Finally, the effects of drying and storage under starvation conditions on coating activity were also assessed. This study constitutes, to the best of our knowledge, the first application of bioactive latex coatings to gas phase VOC abatement.

Materials and Methods

Chemicals and Mineral Salt Medium

The mineral salt medium (MSM) employed for the cultivation of *Pseudomonas putida* F1 (PpF1) and the preparation of the agarose-based gels was composed of (g L^{-1}): KNO_3, 1; KH_2PO_4, 1; K_2HPO_4, 1; NaCl, 1; $MgSO_4$, 0.2; $CaCl_2$; 0.02 and 1 mL L^{-1} of a trace elements solution containing H_3BO_3, 0.06; $MnCl_2{\cdot}4H_2O$, 0.1; $CoCl_2{\cdot}2H_2O$, 0.12; $ZnCl_2$, 0.07; $NiCl_2{\cdot}6H_2O$, 0.025; $CuCl_2{\cdot}2H_2O$, 0.015; $NaMoO_4{\cdot}2H_2O$ 0.025, (pH = 6.7±0.1). Toluene was purchased from BDH (USA) with a purity of 99.5%. Molecular biology grade agarose at was purchased from Fischer Biotech (USA).

Strain selection and cultivation

PpF1 (Trevisan) Migula (ATCC® 700007™) was selected in the present study due to its ability to degrade toluene. Stock cultures were incubated under sterile conditions and orbital shaking at 30ºC in 1 L air-tight glass bottles containing 250 mL of liquid MSM. Toluene was daily supplied as carbon and energy source at initial headspace concentrations of ≈19 g m^{-3}. Bottles were aerated in a sterile cabinet for at least 1 h prior to toluene addition in order to avoid O_2 limitations. Biomass concentration in the cultures was monitored by absorbance measurements (OD_{620}) and correlated to dry weight (DW) concentrations using a specific calibration curve.

Toluene biodegradation in suspended culture tests

Preliminary toluene biodegradation tests were carried out in suspended PpF1 cultures in order to estimate the specific EC of the model bacterial strain. Sterile glass bottles of 1L were filled with 250 mL of MSM, inoculated with PpF1 at 41, 45, 62 and 97 mg DW L^{-1}, closed with teflon septum caps and provided with toluene at

headspace concentrations of 2.8, 3.0 and 5.9 and 1.1 g m^{-3}, respectively. The biodegradation tests were incubated at room temperature under orbital shaking for 4 h. Gas samples were periodically drawn from the bottle's headspace to assess the time course of toluene and CO_2 concentrations until complete toluene depletion. Abiotic tests were carried out under similar conditions to rule out any potential abiotic toluene losses.

Agarose-based biofilm preparation

Aliquots of PpF1 liquid cultures were centrifuged in 50 mL falcon tubes at 7155 × g for 15 min in a Super T21 centrifuge (SORVALL®, USA). The supernatant was then discarded and each biomass pellet re-suspended in 0.5 mL of fresh MSM. This concentrated cell suspension was re-centrifuged in Eppendorf vials at 9029 × g (miniSpin plus centrifuge, EPPENDORF, Germany) for 5 min and the corresponding biomass pellet was re-suspended again in 0.5 mL of fresh MSM. An agarose-MSM solution at 0.8% was pre-heated to boiling and allowed to cool at room temperature to 60ºC. Then, 0.75 mL of the agarose solution were mixed with the 0.5 mL concentrated cell suspension. This 1.25 ml agarose-PpF1 cells suspension (estimated final temperature of 44ºC) was then poured and distributed over square plastic plates. The resulting artificial biofilm was allowed to cool down at room temperature and introduced in the experimental chamber for testing.

Bioactive latex coating preparation

PpF1 cells were harvested from fresh stock cultures on day 2 or 3, corresponding to the exponential growth phase of the cultures previously described, and concentrated to a 0.5 mL cell suspension. An aliquot of 0.25 mL of this concentrated cell suspension was mixed with 0.25 mL of liquid SF012 latex (Rhoplex™ SF-012, organic solvent-free acrylate copolymer latex paint binder, 43.5 % solids, maximum viscosity 300 cP, minimum film formation temperature 0 ºC, pH 7–8, prepared without biocides; Rohm and Haas Co., Philadelphia, PA) as described by Gosse et al. (2012), resulting in final biomass concentrations of 13.5±0.2 mg DW mL^{-1}. Coatings were subsequently prepared by homogenously spreading 50 µl of the cell/latex slurry over a scribed 14-mm diameter circle (1.54 cm^2) centered on one end of a dry, sterile 3MM chromatography paper strip (2 cm × 14 cm). Freshly prepared coated strips were then wetted by capillarity with MSM by submerging the uncoated end of the paper strip into vertical Balch tubes containing 10 ml of liquid MSM (Gosse et al. 2012). Tubes containing the paper strips were sealed and stored at room temperature before or in between experiments.

Experimental set-up

The experimental chamber was built using a 5 L TEDLAR bag cut open on one side for the preparation and manipulation of the samples inside it. The chamber was equipped with a fan (NIDEC BETA M35105, flow rate 3.68 m^3 min^{-1}) in order to provide turbulence and mixing in the chamber headspace. Agarose biofilm plates or coated paper strips were located inside the chamber perpendicularly to the fan and in parallel to the air flow (Figure 1A). The open side was re-sealed before the beginning of the experiment and the chamber was filled with 4 L of air using a valve located at the top of the chamber. Liquid toluene was injected through the septum to obtain an initial toluene gas concentration of 1.13±0.15 g m^{-3}. Toluene and CO_2 concentrations were periodically monitored by sampling out the gas phase for 4 to 7 hours (depending on the degradation rates observed). Control tests were carried out in the absence of biological activity to confirm the absence of abiotic toluene losses by photolysis, adsorption or toluene leak.

Influence of cell concentration on the toluene biodegradation performance of agarose-based biofilms

Seven agarose-based biofilms were prepared as previously described in 16 cm^2 plates (resulting in a biofilm thickness of 0.8 mm) and tested in the experimental chamber (Figure 1B). The biofilms contained biomass concentrations of 13, 14, 18, 21, 24, 46 and 56 mg DW mL^{-1}.

Influence of biofilm area on the global toluene EC in agarose-based biofilms

Toluene biodegradation was assessed in the experimental chamber using larger flat plastic plates supporting biofilm areas of 16, 27, 36 and 44cm^2 with a PpF1 concentration of 14.0±0.4 mg DW mL^{-1} (Figure 1B). PpF1 cells were harvested from fresh toluene degrading cultures on day 2 or 3 (corresponding to the exponential growth phase to avoid any potential biomass aging effect). The free software ImageJ (NIH-USA) was employed to accurately determine both the biofilm area and thickness.

Toluene biodegradation in dry bioactive latex coatings

Tests were carried out in the experimental chamber using a batch of 4 coated strips (containing 13.5±0.2 mg DW mL^{-1}) attached to a plastic support providing a total coated area of 6.16 cm^2 (Figure 1C). This batch of 4 coated strips was daily tested over 4 consecutive days with overnight wet storage in a Balch tube at room temperature.

Toluene biodegradation in wet bioactive latex coatings

Tests were carried out in the experimental chamber using a batch of 4 coated strips (containing 13.5±0.2 mg DW mL^{-1}) attached to a plastic support providing a total coated area of 6.16 cm^2. The uncoated end of the strips was permanently submerged in liquid MSM during the experiment, which maintained the paper constantly wet by capillarity (Figure 2D). This batch of 4

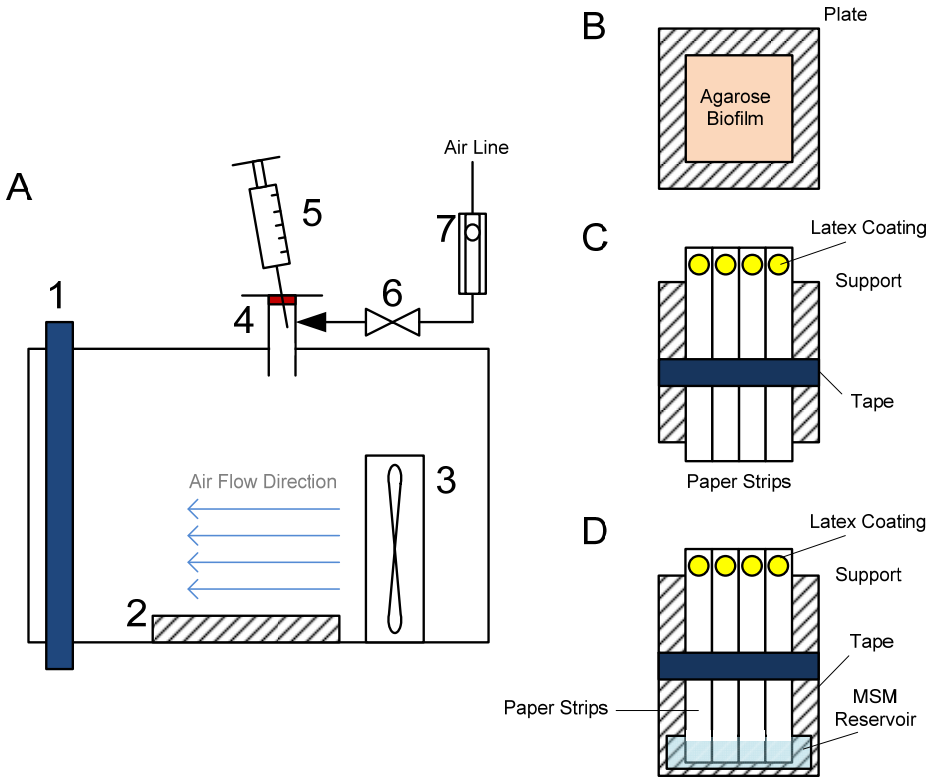

Figure 1. A. Schematic representation of the experimental set-up. 1. Air-tight chamber closure, 2. sample case, 3. fan, 4. septum, 5. syringe for liquid toluene addition, 6. Air inlet valve, 7. Rotameter. **B.** Top view of the agarose biofilm plate. **C.** Top view of a set of paper strips attached to the plastic support in dry tests. **D.** Top view of a set of paper strips attached to the plastic support in wet tests.

coated strips was consecutively tested for 4 days as previously described in the dry tests.

Bioactive latex coating starvation tests

Five batches of 4 coated strips were prepared and their toluene biodegradation performance was initially evaluated under wet conditions in order to provide an reference EC. Then, the strips were stored at room temperature in the Balch tubes without any carbon source supply for 0, 1, 2, 3 and 5, and their toluene biodegradation performance compared to the reference EC in order to assess the loss of activity of the coating.

Scanning electron microscopy (SEM)

Scanning electron microscopy images from the bioactive latex coatings before and after the biodegradation assays were taken from samples dried overnight at 100ºC in a FEI XL30 SEM-FEG microscope (FEI, Oregon, USA) using accelerating voltages of 5-10 kV.

Analytical procedures

Toluene and CO_2 gas concentrations were analyzed in an SRI 8610C Gas Chromatograph (California, USA) equipped with an FID and TCD detectors. A capillary DB-624 column (Agilent, California, USA) and a HayeSep® D packed column (Restek, Pensilvania, USA) were used for toluene and CO_2 determination, respectively. The temperatures of the oven, injector and FID detector were maintained at 120ºC, 120 °C and 160ºC. The oven, injector and TCD detector temperatures were maintained at 50ºC, 50ºC and 150ºC, respectively.

A correlation between biomass concentration and OD_{620} was carried out by filtering culture samples of different OD_{620} though a pre-dried and pre-weighted 0.2 μm filter (Pall Corporation, USA). The filters containing the biomass were then dried overnight at 100ºC. Sample analyses were carried out in duplicate. Culture absorbance was measured in a Smart Spec™ 3000 spectrophotometer (BIO-RAD, California, USA) at a wavelength of 620 nm.

Results and Discussion

Toluene biodegradation in agarose-based biofilm experiments

Toluene removal and CO_2 production in the test chamber confirmed that the biofilm preparation methodology proposed was successful at preserving the activity of PpF1 cells in an agarose matrix at different cell concentrations. The biomass concentrations tested in the agarose biofilms (13.2 - 45.8 mg DW mL^{-1}) were in the lowest range of the values reported for a *P. putida* biofilm developed in a biofilter treating phenol (30 – 100 mg TSS mL^{-1}) (Converti et al. 1997) or for other heterotrophic biofilms (9-110 mg TSS mL^{-1}) (Zhang and Bishop 1994). Constant average toluene areal ECs of 0.031 ± 0.005 mg cm^{-2} h^{-1} and average carbon mineralizations of 84±11% were recorded regardless of the biomass concentration in the biofilm (Data not shown). The areal EC here obtained was three times higher than the 0.01 mg cm^{-2} h^{-1} reported by Moller et al. (1996) for the biofilm of a toluene-degrading biofilter (Møller et al. 1996). This high areal EC would result in an overall biofilter abatement performance superior to those typically reported in standard biofilters. For instance, ECs of 3×10^6 g m^3 h^{-1} would be achieved in a biofilter based on the experimental areal ECs obtained in the present study and assuming a typical compost specific surface area of 5.12 m^2 g^{-1} (Maestre et al. 2007), which is 4 orders of magnitude higher than the typical ECs reported for toluene in organic packed biofilters (≈1×10^2 g m^3 h^{-1}) (Estrada et al. 2013b). This high toluene abatement performance can be explained by the high turbulence caused by the fan inside the experimental chamber, resulting in a theoretical gas velocity over the biofilm of 11.8 m s^{-1}. In this context, the operation of a compost biofilter (bed porosity of 0.43) at an EBRT of 1 min and a bed height of 1 m

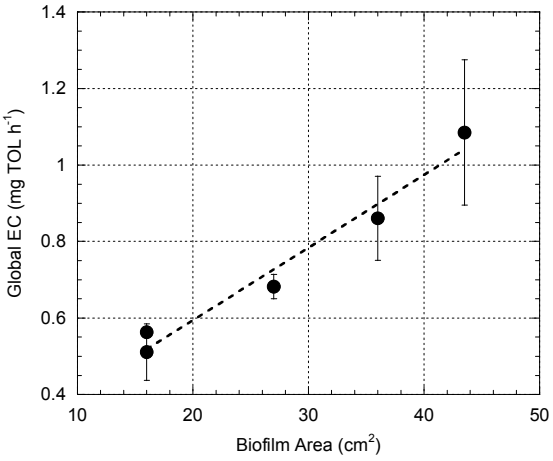

Figure 2. Global EC in the system as a function of the biofilm area for biofilms with a biomass DW of 14.0±0.4 mg mL^{-1}.

results in typical gas velocities of ≈ 0.04 m s^{-1}.

The fact that the areal EC remained constant regardless of the biofilm cell concentration clearly indicated that toluene biodegradation in the agarose-based biofilm was mass transfer limited. This result is in agreement with regular observations in biofilters, where the accumulation of biomass over time did not result in enhanced pollutant ECs (Cox et al. 1997, Song and Kinney 2005). Toluene mass transfer limitation was further confirmed in the toluene biodegradation assays conducted at 4 different biofilm areas. The areal ECs remained constant (0.028 ± 0.005 mg cm^{-2}), while the overall ECs in the system increased linearly at increasing biofilm areas (Figure 2). A hypothetical oxygen mass transfer limitation was ruled out since the maximum theoretical oxygen transfer capacity in the system doubled the maximum oxygen needs based on the correlation between mass transfer and the molecular volume of the compound to be transferred (Estrada et al. 2013a).

The similarity of real biofilms (high water content in an exopolymer matrix) with agarose based biofilms suggests that mass transfer limitations are likely to occur in full scale biofilters based on their low gas linear velocities (≈3 orders of magnitude lower than in our experimental set-up). The results here obtained showed that the agarose gel methodology is suitable for the preparation of model biofilms with controlled area, thickness and biomass content.

Toluene biodegradation in non-wetted bioactive latex coatings

The methodology used for the preparation of biological latex coatings successfully entrapped the PpF1 cells. Surface imaging allowed the observation of the rod-shaped void footprints created by shrank cells during latex drying (Figure 3A). In addition, the coating filled the void spaces between the cellulose fibers in the support paper (Figure 3B).

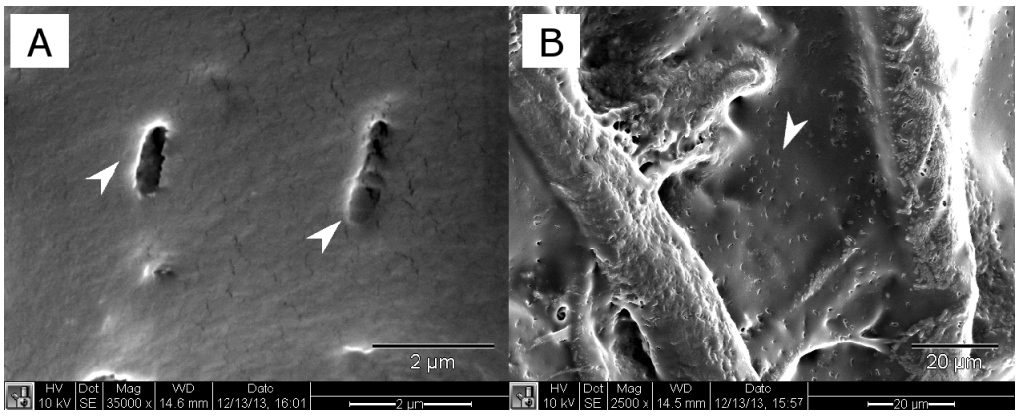

Figure 3. SEM imaging of the biological latex coating. White arrows indicate the footprint created by two *P. putida* cells (A) and the multiple cell footprints in the coating covering the space between two paper fibers (B).

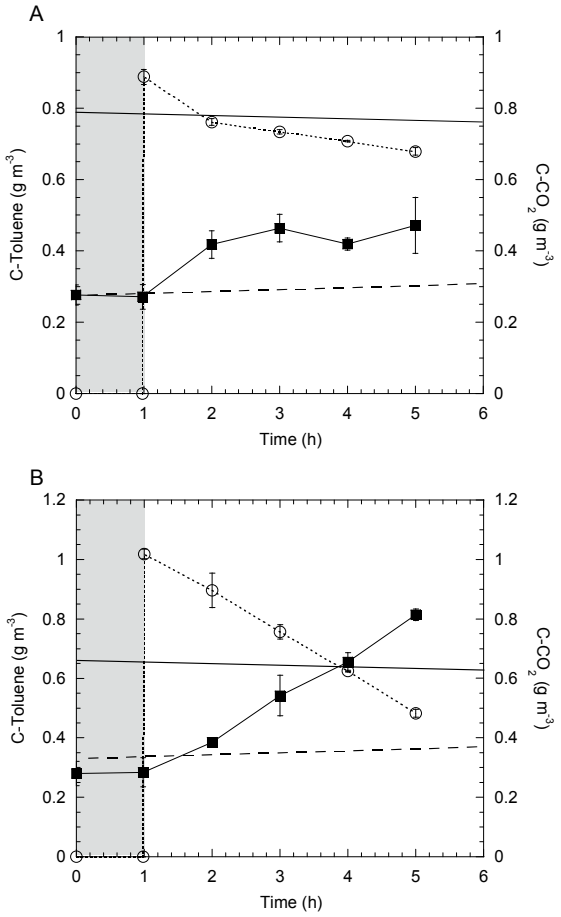

Figure 4. Time course of C-toluene (○) and C-carbon dioxide (■) concentration in the chamber headspace during the bioactive latex-based toluene biodegradation experiments with non-wetted (A) and wetted (B) strips containing a cell concentration of 13.5±0.2 mg DW mL^{-1}. Continuous and dashed lines represent toluene and CO_2 C concentrations, respectively, during blank tests. Shadowed area represents the lag phase before toluene was introduced in the experimental chamber.

The removal of toluene and the increase in CO_2 concentration in the headspace of the experimental chamber in 4 experiments conducted with non-wetted paper strips confirmed the presence of biological activity in the coatings (Figure 4A). Control tests were carried out with latex coatings in the absence of PpF1, which ruled out any potential toluene adsorption or chemical reaction effects mediated by the latex or the paper strips. Hence, this work constitutes the first latex-based immobilization of *P. putida* F1 retaining toluene degradation activity. CO_2 production due to an active endogenous respiration was negligible based on the absence of CO_2 concentration increase during the 1 h lag phase observed immediately before toluene injection (Figure 4). The average areal ECs using the latex-based coatings (0.095±0.031 mg cm^{-2} h^{-1}) doubled the areal ECs recorded in the agarose-based biofilms at similar cell concentrations (13.5±0.2 mg DW mL^{-1} in the latex coating vs. 14.0±0.4 mg DW mL^{-1} in the agarose gel). The average carbon mineralization efficiencies achieved were 66±26%, which were lower than those recorded in the agarose-based biofilm. The toluene biodegradation rate gradually declined along the experimentation time likely due to the progressive loss of humidity in the strip (Figure 4A and 5A).

Toluene biodegradation in wetted bioactive latex coatings

The continuous humidification of the latex-based coatings supported an increase in the areal ECs up to 0.230±0.074 mg cm^{-2} h^{-1} and constant degradation rates throughout the entire monitoring period, thus confirming the negative effects of paper strip drying on toluene biodegradation (Figure 4B, 5B). The average toluene mineralization also increased up to 88±10%. At this point, it is important to remark that the latex coatings here prepared were intended to be preserved and tested under sufficient water activity since no osmoprotectant molecules were added to the latex formulation (Gosse et al. 2012). Recent studies devoted to investigate the effect of drying on bioactive latex coatings activity revealed that the addition of glycerol and/or sucrose preserves biological activity during drying periods (Flickinger et al. 2009, Gosse and Flickinger 2011).

Sequential toluene biodegradation in bioactive latex coatings

An increase in biological activity was observed in the sequential biodegradation of toluene, this effect being higher in the experiments carried out with wetted strips.

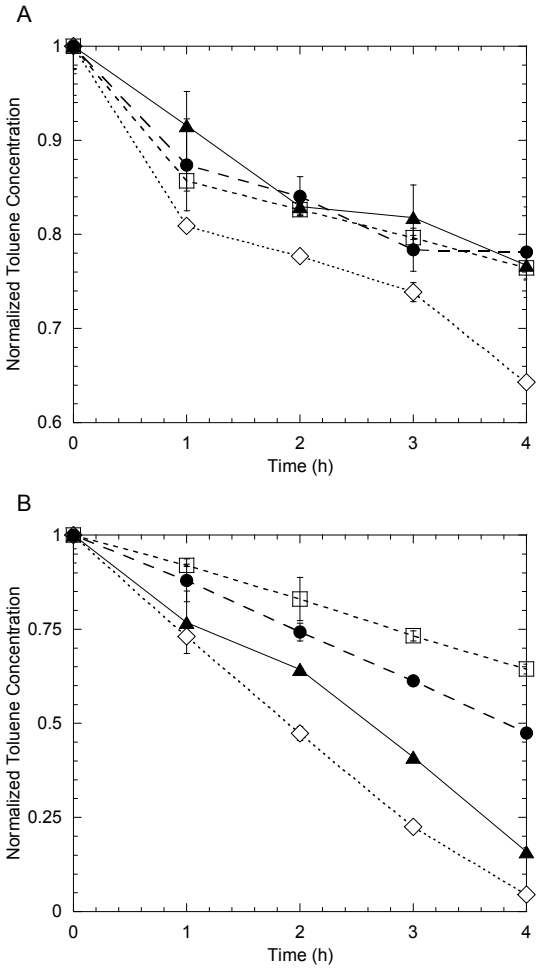

Figure 5. Time course of the normalized toluene concentration in the chamber headspace during the biological latex coating experiments with non-wetted (A) and wetted (B) strips during consecutive toluene biodegradation experiments carried out on day 1 (□), day 2 (●), day 3 (▲) and day 4 (◇). Coatings were prepared with a cell concentration of 13.5±0.2 mg DW mL^{-1}.

The areal ECs increased by 44% and 130% from day 1 to day 4 compared to the reference test (day 0) under non-wetted and wetted conditions, respectively (Figure 5A and B). Bacterial growth either on the coating or on the supporting paper was hypothesized as the main mechanism underlying this increase in toluene degradation. The latex circular coatings attached to the paper strips were cut, attached to new sterile strips and re-tested under wetted conditions. A decrease in the areal ECs from 0.31±0.04 to 0.17±0.02 mg cm^{-2} h^{-1} was observed, which confirmed that biomass was able to grow in the nearby uncoated area of the paper strips, significantly increasing the active area for toluene removal. Cell growth in the coating was also observed by comparing the initial SEM images of coatings with those after 4 toluene biodegradation experiments (data not shown). Cell overgrowth is a non-desired phenomenon both in laboratory studies and full scale applications, where the area and activity available for biocatalysis is ideally expected to be controlled. In this context, the use of non-growth/nitrogen limited culture media has been successfully applied as a strategy to limit biomass growth in bioactive latex coatings (Fidaleo et al. 2006, Flickinger et al. 2007).

On the other hand, toluene biodegradation in suspended PpF1 cultures yielded specific EC ranging from 240 to 635 mg g $_{DW\ biomass}^{-1}$ h^{-1}. While the lowest EC was recorded at 45 mg DW L^{-1} and 2.8 g m^{-3} of initial toluene concentration, the highest EC was recorded at 62 mg DW L^{-1} and 5.9 g m^{-3}. The agarose-based biofilms supported specific ECs of 34 ± 11 mg g $_{DW\ biomass}^{-1}$ h^{-1}, one order of magnitude lower than those obtained in liquid cultures and in the bioactive latex coatings under wetted conditions (321 ± 96 mg g $_{DW\ biomass}^{-1}$ h^{-1})(Figure 6).

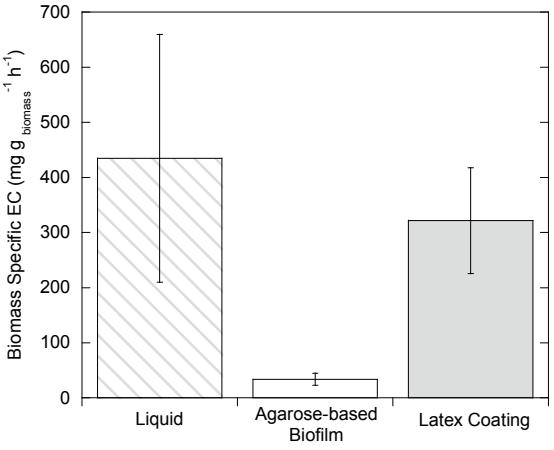

Figure 6. Specific EC for the different tests carried out in the present work in 4 liquid suspended growth tests, 6 water-based biofilm tests (biomass DW of 14.0±0.4 mg mL^{-1}) and 6 latex coating wet tests (biomass DW of 14.0±0.4 mg mL^{-1}).

The strong mass transfer limitations identified in the agarose-based biofilms were overcome in the latex coatings. The biomass embedded in latex was able to biodegrade toluene at rates which are 10 times higher than those recorded in agarose-based biofilms under similar operating conditions. The high areal ECs achieved were attributed to the enhanced direct pollutant uptake by the cells entrapped in the nanoporous latex structure, whose nutrients and water requirements were fully satisfied by capillarity. These results obtained for toluene (considered as a moderately hydrophobic compound, dimensionless Henry's law constant, H = 0.29) suggest a promising abatement performance of bioactive latex coatings when applied to the treatment of highly hydrophobic compounds such as hexane (H = 74) or CH_4 (H = 34) (Sander 1999). Hence, the application of biological coatings in air pollution control represents a new engineering approach to enhance the performance of biological off-gas treatment processes, often limited by pollutant mass transfer from the air emission to the microorganisms (Kraakman et al. 2011). The potential enhancement of oxygen mass transfer should also be explored in processes such as biogas sweetening, where oxygen solubility is limiting (Rodriguez et al. 2013). The contribution of toluene diffusion along the wet paper strips to the total toluene removal should also be assessed in future research, but it is expected to be minimal based on the low solubility and diffusion rates of toluene in water.

Effect of toluene starvation on the biological activity of bioactive latex coatings

Starvation of coated cells for 1 day did not entail any effect on the areal EC during toluene biodegradation. However, cell starvation for two and three days resulted in a loss of activity of 18% and 51%, respectively. Likewise, a 68% of activity loss was recorded after five days of starvation (Figure 7).

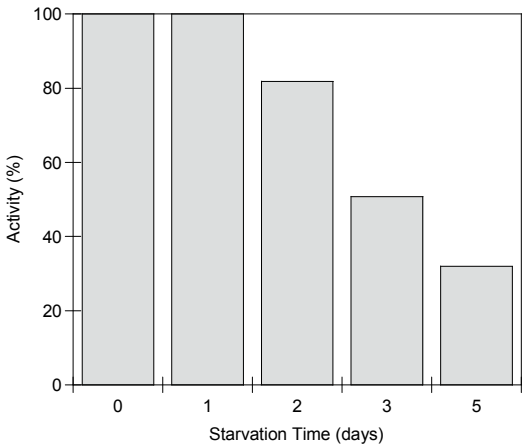

Figure 7. Influence of the starvation time on the toluene biodegradation activity of bioactive latex coatings.

The results obtained were in agreement with previously reported maintenance of toluene degrading activity after short term starvation periods (8 hours) and the complete loss of activity after 300 h of starvation in *P. putida* suspended cultures (Jenkins and Heald 1996, Muñoz et al. 2008). Likewise, a complete loss of activity has been reported in toluene-treating biotrickling filters after 5 days of starvation, even when maintaining air flow and irrigation (Cox and Deshusses 2002). Enzymes responsible for toluene degradation get inactivated under low substrate availability but incubation at lower temperatures (4ºC) or cryopreservation at -20ºC can attenuate this effect and preserve activity, respectively, in the particular case of cell coating with latex (Jenkins and Heald 1996). The effect of carbon source starvation on cell activity in bioactive latex coatings has been scarcely addressed in the previous research since coating activity is often immediately evaluated after preparation (Gosse et al. 2012). However, cell inactivation is a major concern in full scale bioreactor applications or in laboratory research in order to have biocatalytic materials available *off the shelf* (Flickinger et al. 2007). In this context, Swope and Flickinger (1996) reported a high cell viability (>90%) and enzyme inducibility for immobilized *E. coli* cells even after 17 days of starvation under non-wetted conditions using a non-growth

mineral medium and including glycerol in the polymer formulation (Swope and Flickinger 1996). The performance of low-cost preservation techniques such as low temperature, cryopreservation, non-growth medium and osmoprotectant molecules addition needs to be assessed in order to enhance the robustness of bioactive latex coatings in air pollution control applications. A high robustness towards starvation periods is crucial since biological treatment units have to cope regularly with fluctuations in pollutant concentration or maintenance facility shutdowns (Lebrero et al. 2010).

Conclusions

PpF1 cells were successfully immobilized in bioactive latex coatings and further tested for its ability to degrade toluene. This innovative cell immobilization methodology supported high toluene mineralization ratios and specific ECs 10 times higher than those obtained in agarose-based biofilms, overcoming the strong mass transfer limitations found. Operational issues such as coating drying and robustness towards starvation emerge as the main challenges to biological activity in latex coatings. This study constitutes the first application of bioactive latex coatings to air pollution control, which represents a promising alternative to overcome the mass transfer limitations commonly found in biofilters or biotrickling filters for air pollution control.

Acknowledgements

Dr. Alexis Wells Carpenter (Weisner Research Group, Department of Civil and Environmental Engineering, Duke University) is gratefully acknowledged for her help with SEM imaging. This research was supported by the Spanish Ministry of Economy and Competitiveness (BES-2010-030994 contract and CTQ2012-34949 project).

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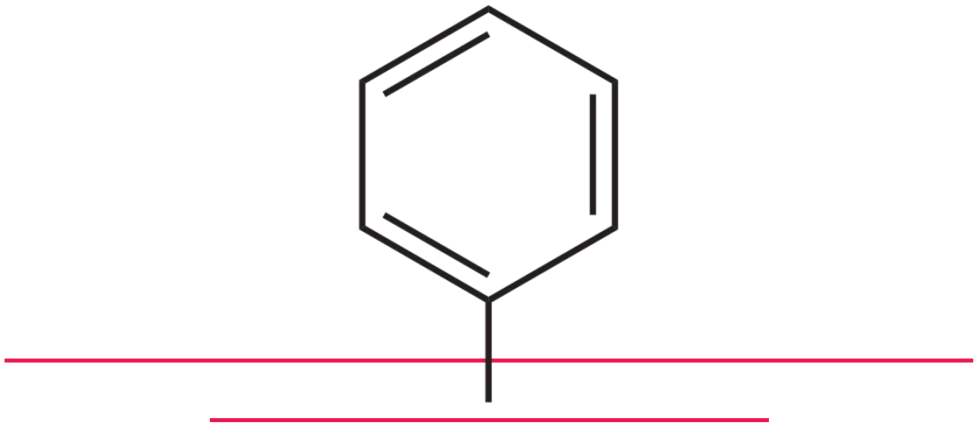
Conclusions and future work.

Chapter 11



The results obtained in the present thesis confirmed the superior performance of biotechnologies for air pollution control when compared to their physical/chemical counterparts. The application of the IChemE Sustainability Metrics was a successful tool for providing intercomparative data from several air treatment technologies in terms of environmental, economic and social performance under an odour treatment scenario. A high energy consumption, generation of hazardous wastes and high reagents/materials consumption were identified as the main drawbacks of physical/chemical technologies. Biotrickling filters, activated sludge diffusion and hybrid technologies emerged as the most promising technologies for odour abatement in wastewater treatment plants. Interestingly, the technologies with the highest investment costs (biotrickling filters and biofilters) presented the lowest operating costs and vice versa (as observed for incineration and activated carbon adsorption), while process economics were strongly influenced by design and operating factors (**Chapter 3**).

An in-depth economic analysis revealed that biological technologies exhibited the lowest operating costs while being less sensitive to variations in design parameters or commodity prices than physical/chemical technologies. Biotrickling filtration was identified as the most cost-effective alternative for odour treatment in the long term (20 years) and its costs were highly influenced by water prices. In this context, the use of partially treated effluents in WWTPs could reduce the total costs of a biotrickling filter by more than a 50%. A geographical analysis showed the relevance of local markets on the process economics. Finally, a full-scale experience-based robustness evaluation carried out in collaboration with CH2M Hill proved that activated carbon and the hybrid technologies were the most robust technologies, while biotechnologies exhibited a robustness equivalent to that of chemical scrubbers (**Chapter 4**).

Despite the sustainability and sensitivity analyses were performed in a particular odour treatment scenario, most of the results obtained could be extrapolated to other gas treatment scenarios. For instance, energy, material or water requirements are closely related to the gas flow to be treated and the pollutant concentration, and therefore the comparative performance conclusions here drawn will not differ

substantially in other case study scenarios. However, a specific analysis for each scenario is of key relevance and highly recommended for an adequate technology selection (**Chapters 3 and 4**).

Since biotechnologies represented the most sustainable alternative for air pollution control, different strategies were investigated in order to overcome their main limitations and promote their applicability. From a biological perspective, **Chapter 6** demonstrated that traditional inocula enrichment techniques for off-gas treatment based on toxicity tolerance is inefficient when pollutants are present at trace level concentration, since inocula exhibit a poor biodiversity and a low abatement performance. On the other hand, an enrichment strategy based on exposure at trace level pollutant concentrations resulted in more diverse communities, with limited toxicity tolerance but highly efficient for the treatment of low pollutant concentrations. These results provided new insights in the development of inoculum preparation protocols in order to optimize the start-up period and the stability of bioreactors.

Chapter 7 constituted the first systematic comparative evaluation of fungal and bacterial biofilters, with bacterial biofilter showing higher elimination capacities and mineralization ratios than its fungal counterpart for a VOC mixture. These results were attributed to the diversity of the inoculum used in the bacterial biofilter, given the key role of an adequate initial community. Inhibitory effects mediated by the presence of propanal on the biodegradation of the rest of compounds were observed in both biofilters. This work also evaluated the performance of an innovative sequential two-stage biofilter based on the advantages of fungal biofiltration for the biodegradation of highly hydrophobic pollutants.

Biofilters are nowadays one of the most applied air pollution control techniques due to the experience gathered in their design and operation and their cost-effective operation. However, clogging due to an excessive biomass accumulation or a loss of stability in packing media structure often limits their long-term operation. In **Chapter 8**, a novel step-feed biofiltration design was able to reduce the energy requirements and increase the packed bed lifespan compared to standard biofilters while supporting similar toluene removal efficiencies. This is of key relevance when organic packing

materials are used, which are more prone to clogging due to biomass accumulation and media deterioration.

In order to overcome the mass transfer limitations often found in biotrickling filters, the potential of a gas recycling strategy to increase the turbulence in the reactor while decoupling the real gas residence time from the mass transfer coefficient was explored in **Chapter 9**. Internal gas recycling enhanced the performance of the reactor in the early stages of operation, which agreed with the data obtained in independent abiotic mass transfer tests. Under certain operating conditions, this strategy can theoretically result in a 50% improvement in the biotrickling filter energy use efficiency. However, the bioreactor faced additional mass transfer limitations as a result of biomass accumulation in the packed bed, which were not overcome by increasing the gas-liquid mass transfer. Mass transfer from the liquid phase to the biofilm was identified as the limiting step.

Chapter 10 presented a successful proof of concept study assessing the immobilization of *Pseudomonas putida* F1 cells in bioactive latex coatings and the performance of this strategy for toluene removal. This innovative methodology supported high toluene mineralization ratios and specific elimination capacities 10 times higher than those obtained in water-based biofilms, overcoming the strong mass transfer limitations found in conventional biofilms. This study constituted the first application of bioactive latex coatings to air pollution control, which represents a promising alternative to overcome the mass transfer limitations commonly found in biofilters or biotrickling filters.

Despite the advances presented in this thesis towards the widespread use of biological technologies for air pollution control, their improvement and optimization constitute niches for future research. The continuous evaluation and scale-up of of design and operational strategies such as step-feeding and internal gas recycling or bioactive coatings will be of paramount importance. The popularization of new molecular biology techniques such as pyrosequencing will be of capital significance for a better understanding of the microbial ecology and its role in air pollution treatment processes. Mass transfer limitations in biotrickling filters and packing material lifespan

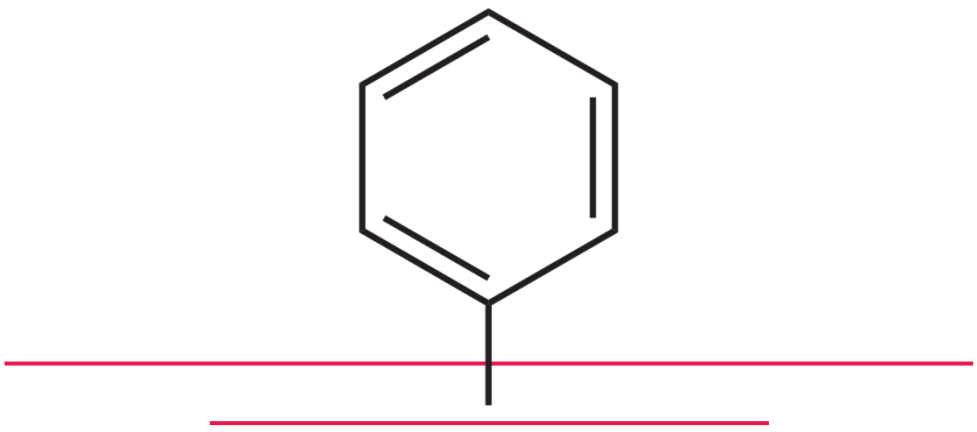
are still issues subject to research despite the promising results here presented. Based on the results here obtained, further lines of research in the field should focus on:

- A continuous sustainability evaluation of new or upgraded air pollution treatment technologies. An effort should be made in collaboration with private companies and public institutions in order to collect up-to-date data from full scale facilities to identify the real technology needs in the field. For instance, innovative strategies such as activated sludge recycling or centrate denitrification have recently emerged to prevent odour release in wastewater treatment plants.
- The role of non-active microbial species present in biological reactors (those who do not directly participate in pollutant biodegradation) is a topic of capital interest to be investigated in collaboration with microbiologists and ecology experts, which can bring new insights on their influence on the performance and robustness of biotechnologies for air pollution control.
- The application of sequential biological processes based on microbial speciation for the treatment of complex mixtures should be further explored based on the results obtained in **Chapter 6**.
- Step-feed biofiltration should be evaluated at full scale in order to confirm its real potential for energy and material savings based on the promising results obtained at laboratory scale. An estimation of its full economic implications must be developed taking into account the expected increased investment costs.
- New microbiological or technological approaches should be explored in order to improve mass transfer in biotrickling filters treating highly hydrophobic pollutants. In view of the present results, the influence of biomass accumulation on pollutant mass transfer in packed bed reactors must be systematically evaluated in order to fully understand its consequences and develop methods to reduce this negative impact.
- The application of bioactive coatings for air pollution control opens a totally new field that will require intense research prior to its application. The results here obtained support the potential of this approach as a technology platform for air pollution control. Operational issues such as biomass overgrowth and the effect of

coating drying on pollutant biodegradation performance were identified as the main technology limitations to be further investigated.

About the author

Chapter 12



Bio

José Manuel Estrada Pérez (Madrid, 1986) started his Chemical Engineering studies in 2004 at the University of Valladolid. Between 2009 and 2010 he spent 6 months at the École Nationale Supérieure de Paris - ParisTech (France) within the frame of the Erasmus Program successfully developing his Final Year Project on algal biomass valorization. José Manuel graduated in March 2010 and joined in April 2010 the VOCs and Odours Treatment Group head by Professor Raúl Muñoz in the Environmental Technology Research Group (Department of Chemical Engineering and Environmental Technology - University of Valladolid), being awarded with a FPI Grant by the Spanish Ministry of Science and Innovation.

His PhD studies initially focused on the sustainability and economic analysis of odour treatment technologies, within the research line recently started at that time by Dr. Raquel Lebrero in the group and actively collaborating with MSc. Bart Kraakman, principal technologist at the engineering firm CH2M Hill. José Manuel developed his experimental work in the field of biotechniques for air pollution control including odours, VOCs and methane. The candidate carried out within his PhD studies two 5-month research stays at Universidad Autónoma Metropolitana (2011, Mexico City, México) under the supervision of Professor Sergio Revah, and at Duke University (2013, Durham, NC, USA) under the supervision of Professor Marc Deshusses.

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International book chapters

Estrada J.M, Lebrero R, Quijano G, Kraakman B, Muñoz R (2014) Odour abatement technologies in WWTPs: energetic and economic efficiency. In: Sewage Treatment Plants: Economic Evaluation of Innovative Technologies for Energy Efficiency. IWA Publishing. Edited by: Tsagarakis K, Stamatelatou K. ISBN: 9781780405018.

Estrada J.M, Lebrero R, Quijano G, Kraakman N.J.R, Muñoz R (2013) Strategies for Odour control. In: Odour Impact Assessment Handbook. Edited by: Belgiorno V, Naddeo V, Zarra T. John Wiley & Sons, Ltd, UK. 85-125. ISBN; 978-1-119-96928-0.

National book chapters

Estrada J.M, Muñoz R, Elias A (2014) Tratamiento de gases y olores por biofiltración. In: Biotecnología y MedioAmbiente. 2nd Edition. Edited by: Marín I, Sanz J.L , Amils R, Editorial Ephemera.162-177. ISBN: 978-84-616-7027-7

Estrada J.M, Lebrero R, Quijano G, Muñoz R (2013) Tecnologías de eliminación de olores en EDARs. In: Tecnologías Avanzadas para el tratamiento de Aguas residuales. 2nd edition. Edited by: Mosquera A. 277-312. Lápices 4. ISBN-13: 978-84-692-5028-0.

Lebrero R, **Estrada J.M** (2010) Fuentes de Olores. In: Caracterización y Gestión de Olores en Estaciones Depuradoras de Aguas Residuales. Edited by: Muñoz R, Lebrero, R, Estrada J.M. 1-12. ISBN: 978-84-693-4273-2.

Lebrero R, **Estrada J.M**, Escorial G (2010) Legislación y evaluación de impacto. In: Caracterización y Gestión de Olores en Estaciones Depuradoras de Aguas Residuales. Edited by: Muñoz R, Lebrero, R, Estrada J.M. 1-12. ISBN: 978-84-693-4273-2.

Martin M.J, Anfruns A, Lebrero R, **Estrada J.M**, Canals C, Vega E (2010) Procesos de adsorción. In: Caracterización y Gestión de Olores en Estaciones Depuradoras de Aguas Residuales. Edited by: Muñoz R, Lebrero, R, Estrada J.M. 1-12. ISBN: 978-84-693-4273-2.

Contributions to conferences

5th International Conference on Biotechniques for Air Pollution Control and Bioenergy 10-13 Sept 2013, Nimes, France:

Estrada J.M, Quijano G, Lebrero R, Muñoz R. Step-feeding: an operational strategy towards a low-cost biofiltration. (Oral Presentation).

NOVEDAR Young Water Researchers Workshop: Innovative technologies for the XXI Century WWTP and future perspectives. 9-10 May 2013, Santander, Spain:

Estrada J.M, Lebrero R, Quijano G, Muñoz R. Odour abatement in Wastewater Treatment Plants: Selection Criteria Based on Sustainability, Robustness and Economic Analysis. (Oral Presentation).

5th IWA Conference on Odors and Air emissions jointly Held with the 10th Conference on Biofiltration for Air pollution Control. 4-7 March 2013, San Francisco, California, USA:

Kraakman B, Estrada J.M, Lebrero R, Josef Cesca, Muñoz R. Evaluating odour control technologies using reliability and sustainability criteria. (Oral Presentation).

Estrada J.M, Hernandez S, Muñoz R, Revah S. Treatment of VOC mixtures in fungal and bacterial biofilters: a comparative abatement evaluation. (Oral Presentation).

Odours in the Environment 2012. 27-28th Nov 2012, Madrid, Spain:

Lebrero R, Kraakman B, Estrada J.M, Muñoz R. Análisis de robustez de tecnologías para el tratamiento de olores. (Oral Presentation).

Estrada J.M, Kraakman B, Quijano G, Lebrero R, Muñoz R. Selección de alternativas para el tratamiento de olores: sostenibilidad y sensibilidad económica. (Oral Presentation).

Clean Water Through Bio-and Nano-Technology. May 7-9th, 2012, Lund, Sweden:

Estrada J.M, Rodriguez E, Quijano G, Muñoz R. Tailoring inocula acclimation for the biological treatment of odour and VOCs. (Poster).

WEF Odors and Air Pollutants. April 15-18, 2012. Kentucky, USA:

Kraakman B, Estrada J.M, Lebrero R, Cesca R, Muñoz R. Sustainability and Robustness Assessment of Odor Control Technology at Water Treatment Plants. (Oral Presentation).

Biotechniques for Air pollution control IV. October 12-14, 2011. A coruña, Spain:

Lebrero R, Estrada J.M, Muñoz R. A comparative study of one and two-liquid phase biotrickling filters for odour removal in WWTP. (Oral Presentation)

Lebrero R, Estrada J.M, Muñoz R, Quijano G. Toluene mass transfer characterization in a biotrickling filter. (Poster).

IWA Water & Industry 2011. Valladolid, Spain, 2011:

Lebrero R, Estrada J. M, Muñoz R Odour abatement in biotrickling filters: effect of EBRT on methyl mercaptan and VOCs removal. (Oral Presentation).

Estrada J.M, Kraakman N.J.R, Lebrero R, Muñoz R.A sustainability analysis of odour abatement technologies. (Oral Presentation).

Other merits

Stays abroad

Dept. of Process and Technology, Universidad Autónoma Metropolitana (Mexico City, México): August 2011- December 2011.

Professor Sergio Revah. Project: comparative evaluation of fungal and bacterial biofiltration.

Dept. of Civil and Environmental Engineering, Deshusses Lab., Duke University (Durham, NC, USA): August 2013 – December 2013.

Professor Marc Deshusses. Project: development of innovative bioactive coatings for air pollution control (in collaboration with North Carolina State University).

Committees and Reviewer Experience

Member of the organizing committee of the International Water Association Specialist Conference: Water and Industry conference 2011(Valladolid, Spain).

Member of the scientific committee of the International Water Association Specialist Conference: Water and Industry conference 2011 (Valladolid, Spain).

Member of the organizing committee of the NOVEDAR technical seminar: Characterization and Management of Odours in wastewater treatment plants 2010 (Valladolid, Spain).

Reviewer for Chemical Engineering Journal since 2013, Chemosphere since 2014.

Supervision

Master Thesis and Research Project: Anna Irrek (Oct 2012-Jan 2013) "Methane abatement optimization in a Biotrickling filter" Valladolid University (Spain).

Teaching

Environmental and Process Technology (2012-2013). Assistant Lecturer. Industrial Engineering Degrees. 1st Course. University of Valladolid (Spain) Cord Subject. 2 ECTS credits

Environmental and Process Technology (2013-2014). Assistant Lecturer. Industrial Engineering Degrees. 1st Course. University of Valladolid (Spain) Cord Subject. 2.4 ECTS credits