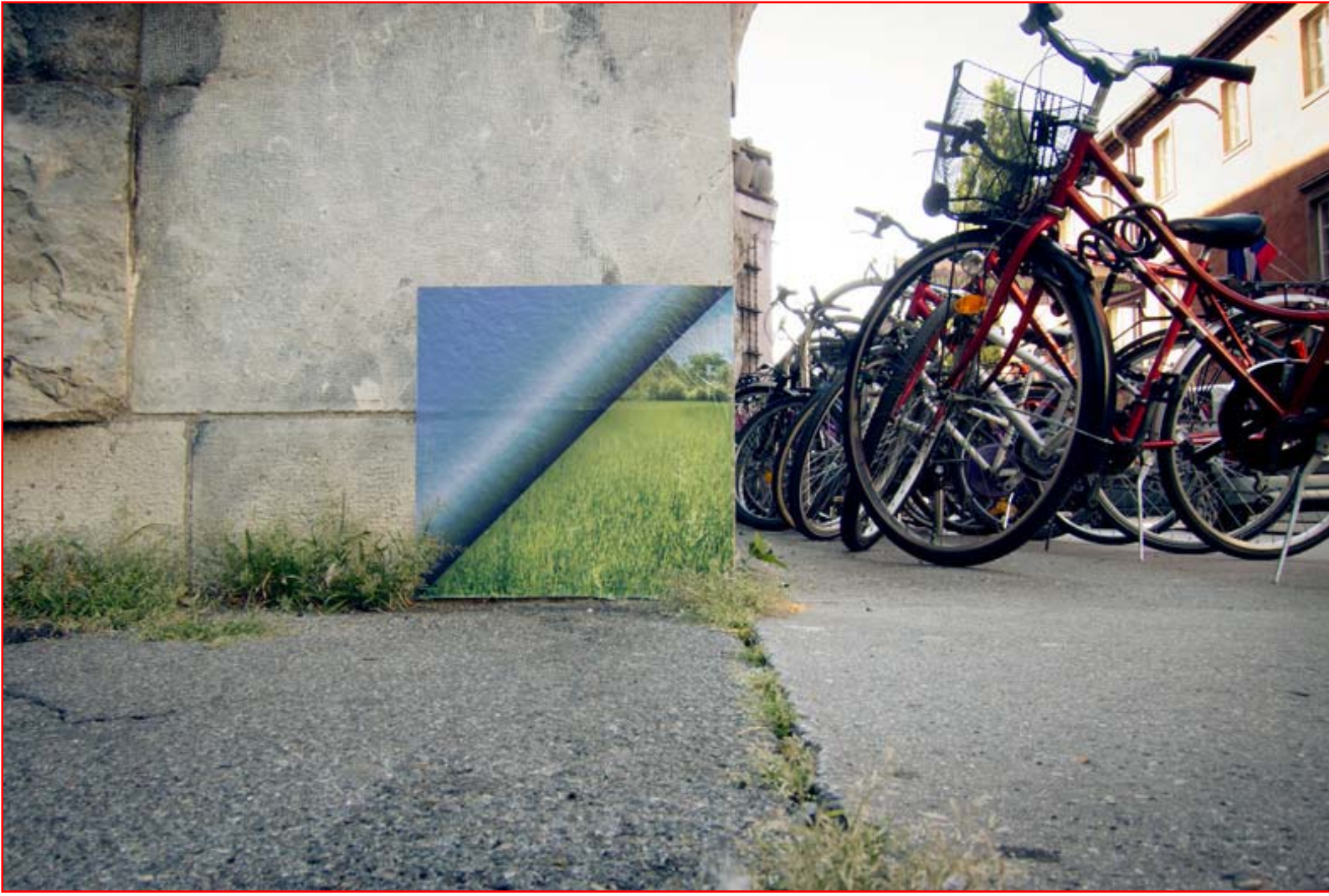

[APPLICATION OF A NEW PELLETISED ORGANIC BED FOR TEX BIODEGRADATION IN CONVENTIONAL BIOFILTERS]

Ingeniaritza Goi Eskola Teknikoa
Escuela Técnica Superior de Ingeniería
Bilbao

eman ta zabal zazu
UPV EHU

BIOFILTRACIÓN

GORKA GALLASTEGUI

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SUMMARY

Today, biological degradation technologies for treating contaminated gas emissions are a reliable and competitive alternative to conventional physical and technical processes. They have emerged in response to the global research requirement for developing efficient, eco-friendly and viable technological alternatives.

Across the range of bioreactors, conventional biofilters are seemingly simple systems as far as installation and operation are concerned. Nevertheless, the lack of a standardised methodology for control and decision-making is currently hindering their commercial implementation.

Initial studies conducted by the Biofiltration research group at the Engineering Faculty in Bilbao (University of the Basque Country, UPV/EHU) focused on searching for a new packing material to treat industrial gas flow contaminated with hydrogen sulphide (H_2S). This material should have certain physical and chemical properties to guarantee the optimum performance of conventional biofilters and, additionally, improve upon the features of other materials used to date in these bioreactors.

The selected material was an agricultural waste supplied by the Spanish company SLIR (Specialised Engineering in Recycling Agricultural Residues) and its commercial name is ABONLIR^{TM}. This material is a compost obtained by mixing pig manure and sawdust, and the pellets are manufactured by mechanical compression without the addition of any chemicals. Biofilters packed with this material for treating H_2S rendered a quick start-up and good performance over time, with the main properties of the organic waste remaining after use. These results led to further research focused on studying the material's response for treating other contaminants, such as TEX (toluene, ethylbenzene and *para*-xylene), which are emitted in a wide variety of industrial processes.

The main objective of this doctoral thesis is to establish the technical feasibility of biofiltration when ABONLIR^{TM} organic waste is used as a packing material for treating gas emissions contaminated with toluene, ethylbenzene and *para*-xylene.

Initially, the unsuccessful start-up of the laboratory biofilters for treating TEX was due to the absence of indigenous specific biomass in this organic packing material, thereby necessarily requiring the addition of an active inoculum. A standardised methodology or protocol for obtaining an active inoculum from a wastewater treatment sludge was established, and certain strategies for successfully acclimating biomass were proposed, such as the selection of the feeding mode (continuous vs. discontinuous) or the benefits/drawbacks of adding a readily degradable carbon source.

Considering the aim was to achieve high removal efficiency after the start-up period, the most relevant operating parameters on the removal efficiency of the three pollutants were analysed, and a recommended range or strategy for optimum performance was established. Thus, a periodic irrigation strategy every 25 days at a 5/1 ($kg_{Material}/L_{Nutrients}$) ratio was recommended, as a considerable increase in the overall removal efficiency of the bioreactors

was recorded when this irrigation frequency was applied. As far as the moisture content in the packing material is concerned, the optimum values were found to be in the 15 – 30 % range, which are considerably lower than those values found in the literature for organic support materials.

The next step involved bacterial and fungal community characterisation in biofilters treating toluene, ethylbenzene and *para*-xylene under xerophilic conditions and inoculated with the same toluene-degrading enrichment culture. Two main conclusions were reached: toluene and ethylbenzene biofilters recorded relatively low biodiversity and a similar microbial composition profile. By contrast, the biofilter exposed to *para*-xylene had a more complex microbial community, which was also more similar to the indigenous microbial population identified in the ABONLIRTM packing material.

The highest elimination capacities achieved for the three contaminants after stable performance for more than 400 days were 138, 170, and 128 g m^{-3} h^{-1} for toluene, ethylbenzene and *para*-xylene, respectively. The average removal efficiency achieved in each case was high enough for these biofilters to reliably treat industrial emissions (0–5 g m^{-3} contaminant range).

The simultaneous feeding of toluene and *para*-xylene showed that a remarkable inhibition effect took place in *para*-xylene biodegradation due to the presence of toluene. By contrast, an enhancing effect of toluene biodegradation was recorded due to the presence of *para*-xylene.

The robustness of the packing material was supported by the negligible pressure-drop value recorded during operating periods of more than 550 days (under the experimental conditions tested). Based on this result, the minimum replacement frequency recommended for the packing material was once per year.

The expertise gained with the ABONLIR™ organic material provided the grounds for further research on other possible waste materials generated in very large amounts, such as the Electric Arc Furnace (EAF) slag produced in the steel industry. Mixing organic material with EAF slag would bring additional benefits, as the system's robustness and contaminant adsorption capacity would be improved, and no replacement would be required or its life span could be extended.

In short, the adequacy of using the ABONLIR™ packing material for TEX biofiltration purposes has been proven under specific experimental conditions. This novel use in biofiltration brings added value to this organic material whose only commercial exploitation at present is as agricultural fertilizer. Future research on mixtures of the organic material and other residues would therefore render new applications for these wastes, favouring their management and promotion and the improvement of biofiltration as a clean and reliable technology.

Gaur egun, kutsaturiko aire korronteak garbitzeko, degradazio biologikoan oinarrituriko prozesuak alternatiba teknologiko fidagarriak eta lehiakorrak dira tratamendu fisiko-kimikoekin alderatuz. Ikuspuntu ekonomikoa, ingurumenaren babesa eta tratamenduaren eraginkortasuna kontuan hartuta, etorkizunerako aukera baliotsuak dira biotratamenduak .

Bioteknologiaren arloan, bioiragazki konbentzionalak bereziki aipagarriak dira, instalakuntzari eta erabilerari dagokienez, eskuragarriak eta sinpleak baitira. Dena den, euren behin betiko ezartze-komertziala bermatzeko, zenbait kontrolerako metodologia eta erabakiak hartzeko protokolo garatu behar dira oraindik ere.

Euskal Herriko Unibertsitatearen (UPV/EHU-ren) Bilboko Ingenieritza Goi Eskola Teknikoko "Bioiragazketa" taldean, hidrogeno sulfuroa hautatu zen lehendabizi, biodegradazioa aztertzeko eta bioiragazkailu konbentzionalak diseinatzeko. Beraz, material-euskarri egokia eta berriztatzailea aurkitzea izan zen lehenengo helburuetariko bat. Bioiragazkien funtzionamendu bikaina zihurtatzeko ezaugarri fisiko-kimiko egokiak eta bibliografian erabilitako beste ohiko euskarriekin aldean prestazio hobeak zituen material berriaren bila hasi zen lanean taldea.

Bilaketaren ondorioz, ABONLIR^{TM} izena duen txerri-zimaurrez eta zerrautsez osaturiko nekazal betegarri peletizatua hautatu zen. Euskarri organiko hau erabiliz, H_2S-a degradatzeko bioiragazkailuak oso azkar hasi ziren abian, oso eraginkorrak izan ziren, eta materialaren oinarrizko ezaugarriak funtzionamendu-denboran zehar ia-ia ez ziren aldatu. Ondorioz, prozesu industrial ugaritan igortzen diren beste konposatu batzuk degradatzeko aukerak aztertzeko ikerketa-lerro berria ireki zen. Zehatz-mehatz, ohiko diren TEX taldeko konposatuak (toluenoa, etilbentzenoa eta xilenoak) hautatu ziren.

Tolueno, etilbentzeno eta *para*-xilenoz (TEX-z) kutsaturiko gas korronteen tratamenduraren bideragarritasun teknikoa ABONLIR^{TM} euskarri organikoz beteriko bioiragazkailuetan aztertzea da doktoretza-tesi honen helburu nagusia.

Euskarri organikoaren jatorrizko biomasaren urritasuna dela eta, ez zen lortu TEX konposatuak degradatzeko bioerreaktoreak abiaraztea eta, ondorioz, araztegiko basa-laginak erabilita, aklimatazio eraginkorra eta abiarazketa azkarra lortzeko estrategia edo protokolo esperimentala diseinatzea ezinbestekoa izan zen. Proposatutako protokolo hau araztegiko basa osoa erabiltzean datza eta zenbait erabaki praktiko hartzeko balio du; hala nola, noiz erabili behar den aklimatazio jarraia edo/eta ez-jarraia (etena) eta zein kasutan komeni den erabiltzea oso erraz asimilatzen den karbono-iturria.

Epe-luzeko eraginkortasun handia ziurtatzeko, funtzionamendurako aldagai esanguratsuenen eragina aztertu zen. Lehendabizi, ureztatze maiztasuna aztertu zen eta, 25 egunean behin egitea proposatu zen, 5/1 (w/v) ($kg_{Materiala}/L_{Elikagaia}$) erratioa erabilita.

Euskarri organikoan bertan atxikitariko ur edukiak duen garrantzia dela eta, ABONLIR^{TM} materialarekin lan egiteko hezetasun tarte egokiena zehaztu zen. Tarte egokiena %15 – 30 zela ondorioztatu zen, balio horietatik at eraginkortasun maila nabarmen jaisten zelarik.

Era berean, material organi honekin beteriko eta baldintza xerofilikoetara ohituriko hiru bioiragazkietan (tolueno, etilbentzeno eta para-xilenoaren tratamendurako bioiragazkietan

hurrenez hurren) nagusi zen komunitate mikrobianoaren (bakterien eta onddoen) karakterizazioa burutu zen. Bertan, toluenoaren eta etilbentzenoaren bioiragazkiek antzeko profil mikrobiarra eta biodibertsitate baxu samarra zituzten. Beste alde batetik, *para*-xilenoaren kasurako profil mikrobiarra eta ABONLIR™ materialeko bertako populazioa antzekoak ziren eta ikuspuntu filogenetikoari dagokionez, aurreko bi bioiragazkiekin alderan komunitate mikrobianoa askoz konplexua izan zen.

Funtzionamendu egonkorrean 400 egunetik gora eduki ostean, ezabapenerako gaitasun maximoak 130, 170 eta 128 g m^{-3} h^{-1} izan ziren tolueno, etilbentzeno eta *para*-xilenoaren kasurako hurrenez hurren. Lorturiko batezbesteko ezabapen-eraginkortasunek TEX konposatuen arazketa ziurtatzen dute industria mailan (0 – 5 g m^{-3}).

Zenbait kutsatzaile aldi berean elikatzen zirenean, euren arteko efektu sinergikoak eta antagonikoak aztertu ziren. Horrela, toluenoaren presentziak *para*-xilenoaren degradazioa inhibitu zuela ikusi zen. Aitzitik, *para*-xilenoaren presentziak toluenoaren arazketa sustatu zuen. Toluenoaren eraztun aromatikoa erasotzea errazagoa izateak azaldu lezake jokabide hau.

Materialaren organikoaren gogortasuna frogatua izan zen 550 eguneko lanaldi jarraituen ostean karga galerak arbuiagarriak izan baitziren. Ondorioz, ikerturiko lan baldintzetan jardun ezkero, aski izango da material betegarriaren ordezkapena urtean behin egitea prozesuaren arrakasta ziurtatzeko.

Betegarri organikoarekin buruturiko lan-esperientziez baliatuz, beste zenbait hondakin-materialekin ere entseguak egiten hasi zen. Zehazki, altzairugintzaren ekoizpenean sortutako Arko Elektrikodun Labeko sarra beltzak hautatu ziren: izan ere, material ez-organiko honek ABONLIR™ euskarriarekin nahasterakoan, ezaugarri hobeak izango dituen betegarri berria lortu daiteke. Hobekuntzak hurrengoak izan daitezke: sistemaren gotortasun-maila handiagotzea, materialaren ordezkapenik gabeko iraupena luzatzea edota adsortzio ahalmen handiagoa lortzea.

Labur, doktoretza-tesi honetan ABONLIR™ materialaren bideragarritasuna egiaztatu da TEX konposatuekin kutsaturiko gas korronteen tratamenduan erabilitako bioiragazkietan betegarri gisa erabiltzeko. Erabilera berri honek, orain arte nekazaritzan ongarri bezala baino erabili ez den material honi balio erantsia ematen dio. Era berean, material organiko hau beste hondakin batzuekin nahastuz gero helburu bikoitza bermatzen da: kutsagarri gaseosoen bioiragazketa garbia eta eraginkorra izatea alde batetik, eta hondakin hauen berrerabilpena, balorazioa eta kudeaketa erraztea bestetik.

Las tecnologías de degradación biológica son, hoy en día, una alternativa real y muy competitiva frente a los tratamientos físico-químicos convencionales para corrientes gaseosas contaminadas. Dan respuesta a la necesidad de búsqueda de técnicas eficaces pero respetuosas con el medio ambiente sin olvidar que deben ser económicamente viables.

Dentro de estas tecnologías, los biofiltros convencionales destacan por su aparente simplicidad tanto en el manejo como en su instalación, pero todavía quedan por desarrollar protocolos de control y toma de decisiones que normalicen su implantación comercial.

Los primeros estudios realizados por el grupo de Biofiltración de la Escuela Superior de Ingeniería de Bilbao de la Universidad del País Vasco UPV/EHU, se centraron en la búsqueda de un material soporte para el tratamiento de corrientes contaminadas con sulfuro de hidrógeno (H_2S). El material debía presentar unas características físico-químicas que garantizasen el buen funcionamiento de los biofiltros convencionales y que además, superasen en prestaciones a otros materiales empleados en la bibliografía hasta el momento.

Como resultado, el material seleccionado fue un residuo agrícola peletizado formado por serrín de madera y purines de cerdo, que se comercializa bajo el nombre de ABONLIR^{TM}. Este soporte permitió el rápido arranque y eficaz funcionamiento con H_2S sin perder sus prestaciones básicas, lo que abrió una nueva línea de investigación para analizar sus posibilidades de aplicación para otros contaminantes, como son los denominados TEX (tolueno, etilbenceno y xilenos) emitidos por un amplio espectro de procesos industriales.

El principal objetivo de esta tesis doctoral fue investigar la viabilidad técnica de la biofiltración utilizando el material soporte orgánico ABONLIR^{TM} para el tratamiento de corrientes gaseosas sintéticas contaminadas con tolueno, etilbenceno y para-xileno.

El infructuoso arranque de los biofiltros para degradar los TEX debido a la ausencia de biomasa indígena específica en el soporte, obligó al diseño de una estrategia de arraque eficaz y rápida a partir de la aclimatación de lodo de una EDAR. Este protocolo propuesto se basa en la utilización del lodo completo e incluye recomendaciones prácticas de aplicación como el régimen de aclimatación más adecuado (continuo versus discontinuo) y la limitación en el uso de una fuente de carbono de asimilación rápida.

Para asegurar una elevada eficacia de eliminación tras el arranque, se estudió la influencia de los parámetros de funcionamiento más relevantes. Así, se estableció que la frecuencia de riego recomendada era de una vez cada 25 días con un ratio 5/1 ($kg_{RELLENO}/L_{NUTRIENTES}$). Dada la importancia que tiene el contenido de agua retenido por el propio soporte orgánico, a continuación se estableció el rango de humedad adecuado para el funcionamiento con el material seleccionado. Se concluyó que el intervalo óptimo era de 15 – 30 % ya que para valores inferiores la eficacia de eliminación disminuía notablemente.

Asimismo, se realizó la caracterización de la comunidad microbiana (bacteriana y fúngica) en condiciones xerofílicas de tres biofiltros preparados con el soporte orgánico para el tratamiento de estos compuestos. Se concluyó que los biofiltros de tolueno y etilbenceno presentaban una relativa baja biodiversidad y un perfil microbiano similar. En el caso del

biofiltro de para-xileno, éste mostró una comunidad microbiana mucho más compleja desde el punto de vista filogenético, siendo su perfil microbiano más parecido a la población microbiana indígena presente en el relleno original.

Las capacidades de eliminación máximas obtenidas tras períodos de operación estable superiores a 400 días fueron 130, 170 y 128 g m^{-3} h^{-1} para el tolueno, etilbenceno y *para*-xileno respectivamente. Las eficacias de eliminación promedio obtenidas aseguran la degradación de estos compuestos en el intervalo habitual en emisiones industriales (0 – 5 g m^{-3}) durante largos periodos de operación.

La alimentación simultanea de varios contaminantes permitió establecer los efectos sinérgicos o antagónicos entre ellos. Se concluyó que la degradación del *para*-xileno sufría un claro efecto inhibidor por la presencia del tolueno. Sin embargo, se observó el efecto contrario con respecto a la degradación de tolueno, que se veía incrementada por la presencia de para-xileno. Este comportamiento puede estar relacionado con la facilidad de ataque del anillo aromático del primero.

La robustez del relleno orgánico se vio confirmada por las despreciables pérdidas de carga medidas tras periodos de operación en continuo de 550 días. Este hecho permitió establecer que, en las condiciones de operación ensayadas, bastaría con reemplazar el material soporte una vez cada año o año y medio como mínimo.

Tomando como base la experiencia adquirida con el material ABONLIR™, se comenzó a investigar otros posibles materiales residuales (concretamente escorias de Horno de Arco Eléctrico para la producción de acero) que aportarán al material orgánico propiedades complementarias, como por ejemplo aumentar la robustez del sistema, prolongar aun más el tiempo de uso sin reemplazo o incrementar la capacidad de adsorción.

En resumen, en esta tesis doctoral quedó demostrada la validez del material ABONLIR™ como material de relleno en biofiltros convencionales para el tratamiento de los denominados TEX en las condiciones experimentales ensayadas. Este uso novedoso en biofiltración aporta un valor añadido al material cuya única salida comercial actualmente es la de abono agrícola. Así mismo, la posibilidad de emplear mezclas de este soporte orgánico con otros residuos abriría una variedad de nuevas aplicaciones con un doble objetivo: la biodegradación eficaz y limpia de contaminantes gaseosos y la reutilización de residuos favoreciendo su valorización y gestión.

SCOPE AND OBJECTIVE

The main objective of this work was to investigate the technical feasibility of biofiltration when using an innovative organic agricultural packing material for the treatment of three of the main aromatic hydrocarbons (toluene, ethylbenzene and *para*-xylene) discharged in industrial air emissions.

This goal was divided into the following partial objectives:

- Establish a standardised methodology for obtaining an active inoculum from sewage sludge in order to shorten the start-up period in conventional biofilters.

- Validate prior acclimation strategies experimentally by starting up three conventional biofilters packed with ABONLIR™ for the treatment of toluene, ethylbenzene and *para*-xylene.

- Define a water irrigation protocol for short-term and long-term operation when using the organic packing material under several organic loading scenarios. In this case, *para*-xylene was selected as representative contaminant.

- Determine the performance of the organic packing material in long-term operation by monitoring the control of several key parameters. In this case, toluene and ethylbenzene were selected as representative contaminants.

- Study the macrokinetics of three lab-scale biofilters treating toluene, *para*-xylene and mixtures of both compounds. A study was made of the relationship between the microbial growth rate and biochemical reaction rate and the interactions between both chemical compounds.

- Analyse the microbial communities of bacteria and fungi in samples collected from three laboratory-scale biofilters packed with ABONLIR™ and used for the treatment of gas streams containing toluene, ethylbenzene and *para*-xylene, respectively. Characterization was carried out by culture-independent molecular methods.

- Apply the expertise from the ABONLIR™ experience to novel materials for biofiltration purposes.

1. UP-TO-DATE TECHNOLOGICAL DEVELOPMENT

1.1. Fundamentals of air contaminated by Volatile Organic Compounds (VOCs)

1.1.1. Volatile Organic Compounds: TEX

Air pollution is one of the main environmental problems in many countries. Based on a dataset of 48 countries spanning the period 1990 to 2006, it was concluded that individuals do not grow accustomed to air pollution and that air pollution significantly reduces current life satisfaction (Menz, 2011).

Among the most frequently present categories of air pollutants, Volatile Organic Compounds (VOCs) have been recognized as one of the most important groups of air toxins that play key roles in atmospheric chemistry.

There is no clear or unanimous definition of a VOC: The US EPA defines VOCs as substances with vapour pressure greater than 0.1 mm Hg; the Australian National Pollutant Inventory affirms it is any chemical based on carbon chains or rings with a vapour pressure greater than 2 mm Hg at 25 °C, and the EU considers it to be any chemical with a vapour pressure greater than 0.074 mm Hg at 20 °C. Chemicals such as CO, CO_2, CH_4, and sometimes aldehydes, are often excluded.

VOC compounds are commonly found in the atmosphere at terrestrial level in different urban and industrial atmospheres. The hundreds of existing atmospheric VOCs are produced both by anthropogenic activities (such as motor vehicle exhaust, motor vehicle fuel evaporative losses, different industrial processes, petroleum storage and distribution and refineries, surface coating and solvent use, domestic wood heaters, biomass burning, environmental tobacco smoke, use of solvent, glues and cleaners in arts and crafts, landfills and agricultural activities) and by natural biogenic processes (such as emissions from trees and vegetation, forest fires produced by natural causes, or anaerobic marshy bog processes, among others).

It is well known that they are ubiquitous, even being detected in vegetation in the proximities of Mount Everest, in marine waters all over the planet, as well as in sediments and air in Antarctica (Domingo and Nadal, 2009).

The main environmental problems raised by VOC emissions are as follows: increase in the global greenhouse effect, reduction in stratospheric ozone and photochemical formation of ozone at terrestrial level. Photochemical smog is caused by the photochemical reaction of NO_X ($NO + NO_2$) and VOCs in the presence of sunlight.

VOCs may also pose a potential threat to human health. This health hazard is a consequence of the great mobility and capacity of VOCs to be inhaled by people working or living in places with high concentrations. In the literature, several VOC exposure-monitoring studies have reported that indoor VOC concentrations are generally higher than outdoor ones (Wang et al., 2009). This finding is crucial since many people in developed societies spend most of their time indoors. For instance, Jones (1999) stated that US residents on average spent 88 % of their day inside buildings, and 7 % in a vehicle.

Although short-term exposure to particular concentrations of some VOCs present in the air is not considered acutely harmful to human health, long-term exposure may result in mutagenic and carcinogenic effects. Median personal exposures to several VOCs have been associated

with excess lifetime cancer risk in the 10^{-4} – 10^{-5} range, considerably exceeding the US guideline (D'Souza et al., 2009).

Many VOCs are not classified as carcinogenic agents by the International Agency for Research on Cancer (IARC); nonetheless, quite a few of them have an important toxic potential. Exposure to VOCs can cause such acute and chronic effects as respiratory damage, and can therefore increase, for example, the risk of asthma. The systemic toxic effects of VOCs are also significant. Among these, renal, haematological, neurobiological and hepatic disorders, as well as mucosal irritations, are the most common. Experience of eye, nose or mouth irritation has been reported at 5000 – 25000 µg VOC m^{-3} (Guieysse et al., 2008). They can also affect the nervous, immune and reproductive systems. Classic neurological symptoms associated with VOCs are feelings of fatigue, headaches, dizziness, lethargy and depression (Ras et al., 2009).

Finally, bad odours should necessarily be included among the adverse effects of VOCs, as they can cause diverse indirect health effects such as bad mood, nausea and vomiting, hypersensitive reactions, loss of appetite and even alterations in the respiratory model. In fact, odour nuisance is so annoying that some authors have estimated reductions in house prices of up to 30 % in properties situated one mile away from the odour source (Estrada et al., 2011).

Aromatic hydrocarbons account for 20 – 40 % of the total ambient VOCs in modern urban environments, with benzene, toluene, ethylbenzene, and *meta*-, *para*-, and *ortho*-xylene (BTEX) being the most common VOCs continuously monitored in EU member states (Zalel and Broday, 2008).

Different studies have shown that BTEX compounds are the most abundant fraction of VOCs in car exhaust gases. As an example, concentration of toluene inside cars varies from 13 to 145 µg m^{-3}, reaching levels up to 1000 times higher when the car is at a petrol station (Esteve Turillas et al., 2007). In many cities, BTEX concentration values exceed the limit recommended by the European Union (Iovino et al., 2009). They are also the predominant compounds found in chemical and petrochemical industrial zones in European regions (Ras et al., 2009). Ciarrocca et al. (2012) observed that the personal exposure to urban BTX concentrations (i.e. benzene, toluene and xylene) was high enough for the implementation of preventive measures in pregnant female workers.

In 2010, operating facilities in the US required to report the release of toluene, ethylbenzene and *para*-xylene emitted these compounds into the atmosphere at a rate of 13.2 kt y^{-1}, 1.1 kt y^{-1} and 0.4 kt y^{-1}, respectively (TRI Program, 2011).

In the EU, at a global scale, the continental air emissions of toluene and ethylbenzene were 976 kt y^{-1} and 79 kt y^{-1}, respectively (EURAR-T, 2003; EURAR-E, 2007), which account for 3 % and 0.02 %, respectively, of total NMVOC (non-methane volatile organic compound) emissions.

Although it might seem that these three isolated substances (toluene, ethylbenzene and *para*-xylene) overall make only a small contribution to total air pollution, their contribution alongside hundreds of other different VOCs is significant.

The use of BTEX in industry has persisted despite its toxic properties because of the range of applications involved. The Clean Air Act Amendments (CAAA) of 1990, proposed by the United State Environmental Protection Agency (USEPA), places special emphasis on the handling and

use of BTEX compounds, which are among the 188 Hazardous Air Pollutants (HAPs) on the recognized list (EPA, 1990).

These compounds are likewise included in the Priority List of Hazardous Substances in the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA). This list, which arranges in order of priority substances that are most commonly found at facilities on the US National Priorities List (NPL) and which are considered to pose the most significant potential threat to human health due to their known or suspected toxicity and potential for human exposure, places **Toluene** in position 71, **Ethylbenzene** in 99 and ***para*-Xylene** in 185 (USEPA, 2007).

In Europe, these contaminants are listed in the European Pollutant Release and Transfer Register (E-PRTR) (European Union, 2006).

Given that air quality is a public good, governments worldwide have started to implement clean air policies in recent decades. The European Commission, considering VOC pollution to be a major environmental problem, adopted Directive 1994/63/EC on the control of VOC emissions resulting from the storage of petrol and its distribution from terminals to service stations.

Other Directives adopted by the European Commission are as follows:

- **Directive 1999/13/EC**, which sets tight limits for VOC emissions caused by the use of organic solvents in certain activities and a range of industrial processes in order to reduce photochemical pollution. This Directive was revised and amended by Directive 2004/42/EC, which limits the emissions of organic solvents in certain paints and varnishes and vehicle coating products.
- **Directive 2001/81/EC**, which limits the emissions of acidifying and eutrophying pollutants and ozone precursors in order to improve the protection in the EU of the environment and human health against risks of adverse effects from acidification, soil eutrophication and ground-level ozone by establishing national emission ceilings, taking the years 2010 and 2020 as benchmarks.
- **Directive 2002/3/EC**, which regulates the standard levels of tropospheric ozone. It also recommends measuring a whole list of VOC ozone precursors so that trends can be analyzed, the efficiency of emission reduction strategies checked and sources of emission determined.
- **Directive 2008/50/EC**, which promotes the merging of most of the existing legislation on ambient air quality into a single directive, with no changes to existing air quality targets.

The positive consequences of emission control measures in Europe have led to a significant decrease in anthropogenic VOC concentration trends. For instance, VOC emissions in France have decreased to 3.8 % y^{-1} over the period 1997 – 2006 (Sauvage et al., 2009).

For the case of China, anthropogenic VOCs emissions are expected to rise from 19.4 t in 2005 to 25.9 in 2020 (Wei et al., 2011). However, a new scenario applying recommendations in Euro Directive 1999/13/EC revealed that national VOCs emissions per year would remain at about 20 t. The projections of this study estimated VOC emission abatements of 1.4 t, 2.5 t and 3.9 t,

respectively, in 2010, 2015 and 2020 if exhaust after-treatment systems were installed in new factories (built after 2005) for the main industrial sources.

1.1.2. Treatment technologies

1.1.2.1. Physical, chemical and biological treatments

Air pollution treatment technologies are classified into physical/chemical (adsorption, incineration, catalytic oxidation, absorption, condensation, UV/photochemical oxidation, etc.) and biological (biofilters, biotrickling filters, bioscrubbers, two-phase partitioning bioreactors, membranes, etc.). Physical/chemical technologies have been widely used because of their low empty bed residence time, extensive experience in design and operation, and rapid start-up. Their major drawbacks are high energy consumption, generation of hazardous wastes, high risk for operators, and high reagent/material consumption.

On the other hand, the growing emphasis on sustainability over the past twenty years has boosted the application of biological processes for waste gas treatment, turning them into a robust and feasible alternative for the removal of air emission streams with relatively low pollutant concentrations at high flow rates.

In fact, vapour-phase biotechnologies have been classified as the best available technologies (BATs) for the reduction of VOC emissions in the chemical sector by the European IPPC Bureau (European Commission, 2003).

Among the different biotechnologies, biofilters and biotrickling filters can be defined as maturing technologies, taking into account that the number of full-scale plants has rapidly increased over the past 20 years. For instance, the US biofiltration market in 1996 was estimated to be worth about $10 million, while by the year 2000 the market was expected to record a turnover of more than $100 million (Wang et al., 1996). In 2005, 5 of the 20 domestic wastewater treatment plants in Beijing used biofilters to reduce odour emissions (van Groenestijn, 2005). In Europe, more than 600 chemical processing industries now use biofilters for the deodorization and treatment of VOCs (Mudliar et al., 2010).

Moreover, several papers have reported the successful adaptation of full-scale chemical scrubbers into biotrickling filters. In Gabriel et al. (2004), a ten-step procedure to retrofit chemical scrubbers into biotrickling filters was described in detail.

Prado et al. (2009a) provided a description of the conversion of an odour-treating industrial chemical scrubber into a biotrickling filter treating a gas flow rate of around 50000 $m^3 h^{-1}$, with an empty bed residence time of about 0.9 s, and average concentrations of VOCs, NH_3 and H_2S, of around 10, 2 and <1 ppmv, respectively. Notable elimination capacities of around 18 g C m^{-3} h^{-1} and 3 g N m^{-3} h^{-1} were obtained during the five-month operating period.

Both the investment and operating costs involved in biological air-treatment systems are generally considerably lower than in classical physicochemical techniques, but they differ greatly depending on the type of airstreams (Delhoménie and Heitz, 2005). Therefore, results comparison is troublesome.

For example, in the control of VOC emissions from a panel board press, greenhouse gas emissions can be reduced by 60 – 80 % and operating costs by 90 % by replacing a conventional thermal oxidizer (RTO) with a biotreatment system (Boswell, 2009).

Estrada et al. (2011) evaluated a group of different waste gas treatment techniques applied in wastewater treatment plants (WWTP) in terms of environmental performance, process economics, and social impact. These technologies were as follows: biofiltration, activated sludge diffusion, biotrickling filtration, chemical scrubbing, activated carbon adsorption, regenerative incineration, and a hybrid technology of biotrickling filtration coupled with carbon adsorption.

The comparative analysis showed that physicochemical technologies presented higher environmental impacts than their biological counterparts in terms of energy, material and reagent consumption and hazardous waste production.

Among biological techniques, the main impact was caused by the high water consumption required to maintain biological activity. Besides, the biofilter had a high surface area and material requirements due to its low design height (1 – 1.5 m) and the short lifespan of its packing material.

From a process economic viewpoint, technologies with the highest investments (biofiltration and biotrickling filtration) recorded the lowest operating costs. This shows that the initial investment cost should never be used as a reliable economic selection criterion.

1.1.2.1.1. Waste gas treatment biotechnologies: potential biohazard?

Different studies have been carried out to analyze the additional advantages of biological treatment systems; i.e. the reduction of germ emissions.

Bearing in mind the “physical” function of a biofilter, it should “remove” or “hold” particulate matter, including airborne microorganisms and dust from the waste gas stream. Nevertheless, regarding the biological activity into the bed, the bioreactor could also be a microorganism emission source.

Schlegelmilch et al. (2005a) concluded that biofilters commonly used at composting facilities were effective to reduce airborne emissions. Thus, the biological waste gas systems were able to retain potentially pathogenic microorganisms which were fed into the bioreactor together with the waste inlet gas. On the contrary, non-pathogenic secondary emissions were released after treatment.

These authors proved that biofilter media was the main parameter influencing on the reduction efficiency, but not in the range of materials suitable for odour degradation. A variation of operating parameters like the moisture content and the air-load did not show a significant influence. Consequently, biofilters may well be operated at optimal conditions for odour degradation purposes and still reduce the number of airborne microorganisms in the waste gas.

Tymczyna et al. (2007) tested the effectiveness of three organic and organic-mineral biofilter media used in purifying ventilation exhaust from a chicken hatchery room. These authors observed that all of them were highly effective in removing gram-negative bacteria (RE of 100 %), moderately effective in reducing dust levels (RE of 85 %), and only slightly effective in removing endotoxins (RE of 26 %).

Tymczyna et al. (2011) also demonstrated the validity of a biofilter fitted to the outlet of the ventilation system of a litter-bed pig house to remove air microbial contaminants. Thus, mean bacterial reduction of 77 % and fungal reduction of 69 % were obtained for inlet concentrations of $8.3 * 10^6$ CFU m^{-3} and $1.9 * 10^5$ CFU m^{-3}, respectively. After the biotreatment process, complete absorption/reduction of *Rhodococcus*, *Brevibacterium*, *Neisseria*, *Pantoea*, *Pseudomonas* bacteria and *Scopulariopsis*, *Mucor* and *Paecilomyces* fungi was achieved.

As far as biological systems acting as a source of microorganisms are concerned, Li et al. (2012) found that 1897 ± 164 CFU m^{-3} of bacteria and 577 ± 65 CFU m^{-3} of fungi were released from a biofiltration column packed with polyurethane foam cubes for styrene treatment. Zilli et al. (2005) obtained emission values in the range of $1.5 – 4 * 10^3$ CFU m^{-3} for three lab-scale biofilters when comparing the performance of an indigenous bacterial consortium and an inoculum of a cell suspension of pure benzene-degrading strain of *Pseudomonas*. This range is similar to bacterial airborne emissions measured in wastewater treatment plants (140 – 4580 CFU m^{-3}) (Sánchez-Monedero et al., 2008) and lower than the occupational threshold limit value proposed for bacterial emissions (10^3 to 10^4 CFU m^{-3}) by Health and Safety Executive (2003).

1.1.2.2. Biological systems: bioreactor alternatives

The effectiveness of biological technologies lies on the ability of certain microorganisms to degrade contaminants. Thus, biological systems use different bioreactor configurations or designs to bring biodegradable pollutants into contact with microorganisms. Four general configurations are the predominant types used: biofilters, air-biotrickling filters, bioscrubbers and two-phase partitioning bioreactors. Nevertheless, other alternatives can be also proposed, as explained later.

Four general configurations, biofilters, air-biotrickling filters, bioscrubbers and two-phase partitioning bioreactors, are the predominant types used in biofiltration.

1.1.2.2.1. Conventional Biofilter (BF)

Conventional biofilters are the oldest and most common of the four general configurations aforementioned. A schematic of a biofilter is presented in Figure 1.1. The contaminated inlet gas is conditioned (i.e. humidified and heated/cooled) before it is uniformly forced to pass through the biofilter bed. The bacteria are attached to the porous packed biofilter bed.

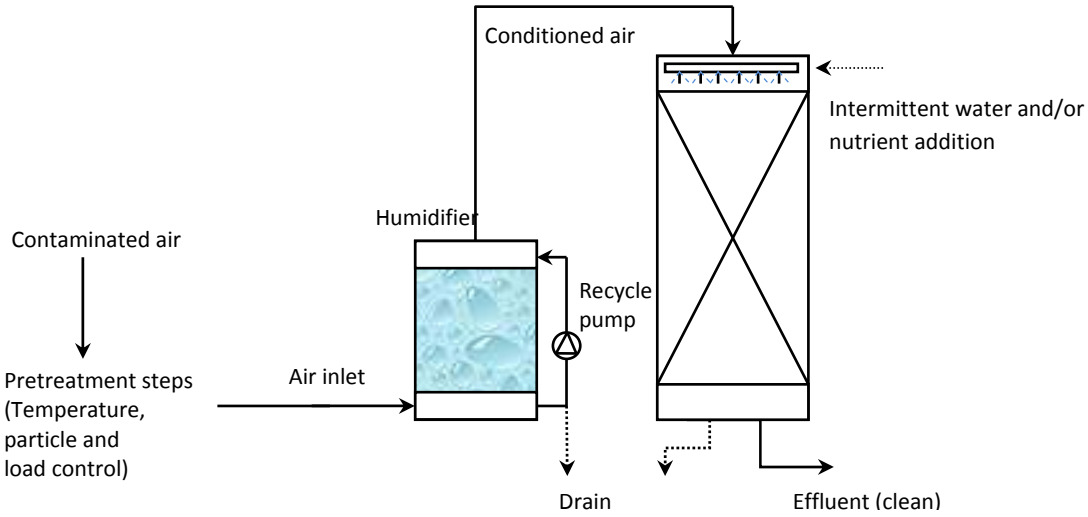

Figure 1.1. Scheme of a conventional closed biofilter.

Biodegradable volatile compounds are adsorbed/absorbed by the bed material and the biologically active biofilm that grows on the porous packed bed particles. Subsequently, the bacteria biologically oxidize contaminants into new biomass and innocuous substances like CO_2, H_2O, NO_3^- and SO_4^{2-}.

There are two biofilter configurations (Delhoménie et al., 2005):

- Open biofilters: They require large areas and, consequently, they are installed outside the VOC/odour generating units. They are exposed to the elements, including rain, snow and temperature extremes and they use up-flow mode.
- Closed biofilters occupy smaller areas than outdoor ones, so they are often installed in closed rooms, and they can use either up-flow or down-flow mode.

Biofilters usually have a height of 1 or 2 m to prevent excessive air velocities through the media, which easily results in high-pressure drop or airflow preferences. Compared with biotrickling and bioscrubber reactors, the footprint of a biofilter is normally much larger, since they usually contain a more open packing that can be over 2 m high (Kraakman, 2005).

Biofilters are mainly recommended for the treatment of waste gases with concentrations below 5 g m^{-3}. They are also more suitable for the treatment of relatively low or moderate flow rate: loads do not normally exceed 500 m^3 m^{-2} h^{-1} (Kennes et al., 2009).

In contrast with other media-based biotechniques (such as biotrickling filters or bioscrubbers), in conventional biofilters there is no continuous feed of a liquid phase. Therefore, this bioreactor is especially suitable for the treatment of hydrophobic and poorly water-soluble compounds with a Henry's constant of up to about 1, and it provides the highest removal efficiencies for moderately hydrophobic pollutants (Iranpour et al., 2005).

However, its effectiveness is limited by operating parameters such as moisture content, the structural weakness of most conventional organic packing materials, medium acidification or

the accumulation of toxic metabolites due to the biodegradation of chlorinated or sulphured organic compounds. For instance, Kennes et al. (2009) confirmed that conventional biofilters treating dichloromethane only achieved an elimination capacity close to 10 g m^{-3} h^{-1}, whereas biotrickling filter were able to reach an elimination capacity higher than 100 g m^{-3} h^{-1}.

These factors are difficult to control as there is no continuous aqueous phase involved in the bioprocess, which requires the frequent replacement of the packing material.

This bioreactor type has been enriched and optimized by several new improvements, as summarized below.

1.1.2.2.1.1. Fungal biofilters

Fungal biofilters have recently been studied as an alternative to traditional bacterial cultures for the elimination of hydrophobic VOCs. Some fungal strains studied in biofilter systems include *Exophiala*, *Scedosporium*, *Paecilomyces*, *Cladosporium*, *Cladophialophora*, *Fusarium* and *Phanerochaete Chrysosporium* (García-Peña et al., 2009).

The use of fungi solves the problem of the transport limitation of hydrophobic VOCs through bacterial aqueous biofilm due to their hydrophobic surface and extended gas exchange area associated to aerial hyphae growth.

Aerial growth and surface hydrophobicity are closely related to hydrophobins, which are small proteins (≈ 10 kDa) with biosurfactant activity, capable of self-assembly at hydrophilic-hydrophobic interfaces. These hydrophobins protect the fungal aerial structures from both excessive cytoplasmic water evaporation and wetting (Vigueras et al., 2009).

Special attention should also be paid to other fungal advantages, such as the fungi's capacity to colonize unoccupied space with the aerial hyphae, the ability to penetrate the solid support increasing nutrient availability and the resistance to grow on low pH and low moisture content (Estévez et al., 2005). For instance, water activity (a_w), defined as the amount of required water that is free at a given environment, is 0.6 for fungi, whereas it is higher than 0.9 for bacteria (Zamir et al., 2011a).

Nevertheless, there are also certain disadvantages: fungi grow slower than bacteria, thus requiring longer start-up times. Vergara-Fernández et al. (2011) sought to reduce the prolonged start-up of fungal biofilters by using more readily available carbon sources as germination and growth promoters. Although they managed to decrease the start-up time, they also recorded lower elimination capacities.

Additionally, fungi filamentous growth could promote pressure drop increase and, under certain conditions, they may produce spores that could become a health hazard if not contained (Prenafeta-Boldú et al., 2006). For instance, Badali et al. (2011) reported an association between the fungal metabolism of aromatic hydrocarbons and "opportunistic pathogenicity" in *Cladophialophora immunda* and *Cladophialophora saturnica* strains, as well as in the related genus *Exophiala*, particularly *Exophiala oligosperma* and *Exophiala xenobiotica*.

Gervais et al. (1988) demonstrated the existence of an optimal water activity value for fungal sporogenesis. The sporulation was greatly lowered above this value. As optimal water activity values for fungal mycelia growth are higher than for sporulation, these authors justified the regulation and adjustment of a minimum water activity value in fungal cultures.

Vergara-Fernandez et al. (2012) calculated an average fungi-spore emission of $1.8 * 10^3$ CFU m^{-3} from a vermiculite biofilter treating *n*-pentane. Sánchez-Monedero et al. (2003) measured a maximum airborne *Aspergillus fumigatus* (one of the most common *Aspergillus* species to cause disease in individuals with an immunodeficiency) concentration of $1.2 * 10^3$ CFU m^{-3} from biofilters applied in seven different commercial composting plants. These values are slightly higher to fungal emissions measured in organic waste treatment facilities ($0 – 2.3 * 10^3$ CFU m^{-3}) (Nadal et al., 2009) or feedstuff-manufacturing factories (83 – 328 CFU m^{-3}) (Kim et al., 2009) and appreciably lower than fungal airborne concentrations reported in industrial working areas like coffee-processing plants (> $1.2 * 10^4$ CFU m^{-3}) (Alonso et al., 2008), piggeries ($1.9 * 10^5$ CFU m^{-3}) (Tymczyna et al., 2011) or the occupational threshold limit value proposed for fungal-flora bioaerosol ($5 * 10^3$ to 10^5 CFU m^{-3}) by Health and Safety Executive (2003).

Elimination capacities of around 50 – 125 g m^{-3} h^{-1} for toluene and xylene have been reported by different authors using fungal biofilters (Estévez et al., 2005; Dorado et al., 2008; García-Peña et al., 2009; Jorio et al., 2009; Zamir et al., 2011a). Even though some authors have occasionally reported elimination capacities exceeding 250 g m^{-3} h^{-1} (Aizpuru et al., 2005), it can be considered the exception rather than the rule.

1.1.2.2.1.2. Monolith biofilters

The natural organic carriers often used in conventional biofilters tend to decay over time, causing compaction, clogging, short-circuiting and increased pressure drop across the bed. Thus, the monolith bioreactor has been proposed as an alternative support matrix for biomass growth. Monolith reactors combine good mass transfer characteristics with low-pressure drop, the principle factors affecting the cost effectiveness of industrial processes (Fang and Govind, 2007).

A monolith bioreactor is a biofilter in which the biomass is attached onto a ceramic, plastic or metal structure with uniform parallel channels separated by thin walls. Bubble-train or Taylor flow occurs in monolithic channels: gas bubbles and liquid slugs move with constant velocity through the monolith channels approaching plug flow behaviour. The gas is separated from the catalyst by a very thin liquid film, and during their passage through the channels the liquid slugs show internal recirculation (Rene et al., 2010a).

The monolith has been marketed as a relatively inexpensive and lightweight bioreactor that uses an inert packing material with a high specific surface area. The open structure without bends hardly obstructs the flow, and the pressure drop is one or two orders of magnitude lower than in classical packed beds with a similar packing surface area (Ebrahimi et al., 2006).

Fang and Govind (2007) tested an activated carbon-coated monolith reactor under diffusive and convective flow conditions to treat gas-phase toluene at low concentrations. The biomass

was attached onto a porous cordierite material with porous channel walls. Although the maximum elimination capacity (EC) was low in both conditions, the convective flow-type recorded a higher EC (7 g m^{-3} h^{-1}) than the diffusive flow-type configuration (3 g m^{-3} h^{-1}). Jin et al. (2006b) obtained removal efficiencies of around 90 % at different gas flow rates. Nevertheless, this value gradually dropped when the inlet load was increased above 30 g m^{-3} h^{-1}.

Clogging after long-term operation has been revealed as the major drawback (Jin et al., 2006b). Ebrahimi et al. (2006), who studied the formation of the biofilm using a monolith reactor as a suspended-cell bioreactor, attained biofilm removal by rinsing the bioreactor with tap water.

1.1.2.2.1.3. Biofilters using surfactants

A surfactant is a surface-active agent; it is a compound with a hydrophobic group attached to a hydrophilic segment, typically a hydrocarbon chain or fatty acid. The main advantage of using surfactants in biofilters is that this agent decreases the surface tension of a liquid by altering the molecular forces at the liquid-gas or liquid-liquid interfaces, thereby enhancing the solubility of organic contaminants in aqueous solutions.

Surfactants are normally classified by the nature of their head group as cationic, anionic, non-ionic and dual charge. Non-ionic surfactants do not form ions in aqueous environments and have a low toxicity. Their water solubility is due to the functional water affinity of units contained in the molecule chain. Since non-ionic surfactants do not ionize in water, they do not form salts with metallic ions. For this reason, non-ionic surfactants can be used in water containing salts without changing their effect, and they can be added to the nutrient solution for biofilters.

Additionally, some authors have proven that they could promote a microbial attachment inhibition to the biofilm (Mireles et al., 2001). Thus, the surfactant may play a significant role in the event of an excess of biomass accumulation.

Non-ionic and anionic surfactants have been intensively studied to enhance the mobilization of hydrophobic compounds in contaminated soils and sediments. Few studies, however, have been conducted for gas treatment bioreactors. Among them, Woertz and Kinney (2004) reported that a non-ionic surfactant (Tween 20) enhanced toluene degradation in a fungal biofilter by simulating bud formation in *E. lecanii-corni*, achieving toluene elimination capacities of up to 150 g m^{-3} h^{-1}.

Chan and You (2009, 2010) studied the dissolution capacity of toluene in the biofilm and the biodegradation of toluene in a composite bead biofilter as the non-ionic Brij30 and Brij35 surfactants were added. The results showed that toluene solubility was enhanced by the addition of surfactant. Besides, the addition of surfactant into the packing material also inhibited microbial growth and the biochemical reaction. The maximum elimination capacity (≈ 24 g m^{-3} h^{-1}) decreased with increasing surfactant content.

Ávalos-Ramirez et al. (2012) studied the biofiltration of methane adding non-ionic surfactants to the nutrient solution. They observed that the removal efficiency increased from 35 % without surfactant to 65 % when the biofilter was operated under non-ionic Tween 20 surfactant at 0.5 % (w/w). These authors also reported that the detergent feature of non-ionic surfactants detached excess biomass. Thus, the addition of non-ionic surfactants to the nutrient solution was suggested to be a useful biomass control strategy.

1.1.2.2.1.4. Biofilters using hydrophobic solvents

The partial coating of the bed packing in a biofilter with an organic non-volatile and non-water miscible solvent before biofilm development is an alternative to improve the performance of the systems for the removal of hydrophobic organic compounds.

The cellular hydrophobicity of the microorganism culture has been shown to increase in the presence of an organic solvent (Hejazi et al., 2010). These authors stated that an increase in hydrophobicity due to silicone oil addition influenced the composition of the microorganism community growing on the carrier surface.

Fazaelipoor et al. (2009) developed a mechanistic model for evaluating the usefulness of amending an organic hydrophobic solvent to a biofilter. They showed that biodegradation enhancement was possible when sufficient contact occurred between the biofilm and solvent layers and when the process was not reaction limited.

On the other hand, they determined that application of the solvent would be justified only if the difference between the solubility of the pollutant and the aqueous phase is large enough. As the addition of a solvent introduces an additional mass transfer resistance, the specific area for mass transfer between the solvent and water must be large enough to compensate accordingly. The most commonly organic solvent/packed-bed ratio used ranges from 5 % to 20 % (Arriaga et al., 2006).

Hejazi et al. (2010) validated this biofiltration alternative by comparing two perlite biofilters for the treatment of α-pinene-contaminated air with and without the addition of silicone oil. The results showed that the silicone oil-coated filter performed better at a flow rate of 2.5 l min^{-1} and a maximum elimination capacity of 20 g m^{-3} h^{-1}, compared with the elimination capacity of 15 g m^{-3} h^{-1} for the filter without oil.

1.1.2.2.1.5. Biofilters coupled with phytoremediation

This system involves the venting of contaminated gas streams through a porous packed solid medium in which plants can grow. The operation of the biofilter relies on both the bioremediation capacity of microorganisms and the purifying capacity of plants (CO_2 consumption, VOC setting). The cultivated soil is expected to sustain a rich and diverse rhizosphere microflora that degrades many pollutants (VOCs, NO_x) using them as a substrate or energy source.

The advantage of using plants lies not only in their ability to simultaneously take up many gaseous pollutants (NO_x, CO_2, CO, O_3 or suspended particles), but also in their great aesthetic value. This new type of biofilter could contribute to the improvement of air quality within urban areas, especially in sites surrounding traffic routes or confined environments, such as car parks and garages (Gawronski et al., 2009).

Rondeau et al. (2011) reported a TEX removal efficiency of 70 % for a filtering bed height of 40 cm for operating conditions corresponding to realistic conditions of car park air treatment (100 – 200 µg TEX m^{-3}).

Another example is The Canada Life Environmental Room (CLER), an experience involving a prototype biofilter designed to improve Indoor Air Quality (IAQ) through the removal of VOCs. It was located in a 160 m^2 boardroom in the Canada Life Assurance Building (Toronto, Ontario). The CLER was a complex collection of higher plants, mosses and microbes in a recirculating hydroponic system, where indoor air was drawn through a 12 m^2 wall constructed from a porous backing and covered in moss and a variety of higher plants.

The CLER did not rely on an inert packing material to maintain the microbial population; instead, the microbial degraders were grown on living moss. The use of moss as a packing material had several advantages over conventional materials. As a living substrate, moss had the ability to both regenerate decomposing substrate and form symbiotic associations with microbial communities.

The researchers responsible for this project found that the biofilter easily removed formaldehyde and toluene, even though the system was subjected to 10 g of toluene every day. However, it did not cope well with trichloroethylene.

1.1.2.2.2. Biotrickling filter (BTF)

The description of a typical biotrickling filter is provided in Figure 1.2. In such a filter, the gas flows through a fixed-bed, which is continuously irrigated with an aqueous solution containing the nutrients (macro- and micro-nutrients in balanced concentrations) required by the biological system.

Microorganisms are immobilized on the packing material. The pollutants are initially absorbed by the aqueous film that surrounds the biofilm. Subsequently, biodegradation takes place within the biofilm that gradually develops on the bed particles.

Inert or synthetic packing materials are used in biotrickling filters. In this case, the carrier has to fulfil a number of essential requirements (Delhoménie and Heitz, 2005):

- Facilitate gas and liquid flows through the bed and gas/biofilm transfers.
- Favour the development of the microflora. In any case, inoculation is a vital step due to the nature of the filter bed.
- Resist crushing and compaction.

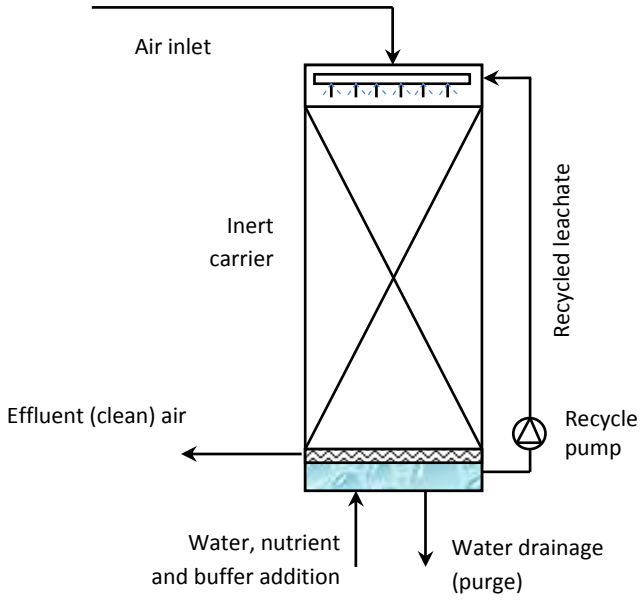

Figure 1.2. Scheme of a biotrickling filter.

The biotrickling filter packings that best meet these specifications are structured plastics (PVC pall rings, glass Raschig rings, polypropylene spheres, polyethylene rings, etc.), silicate-based materials (molecular sieves, perlite, celite, lava rock, etc.), activated carbon, polyurethane foam or porous ceramics (He et al., 2009).

The traditional packing materials used in biotrickling filtration have a lower specific surface area (100 – 300 m^2 m^{-3}) than biofilter ones, which make them unfit for the treatment of poorly water-soluble compounds. However, the use of new synthetic materials (polyurethane foam cubes or porous ceramic rings) with specific surface area values between 500 and 900 m^2 m^{-3} has corrected this drawback. Regarding the gas phase residence time, typical values for the removal of aromatic VOCs such as toluene are between 15 s and 1 min (He et al., 2009; Kennes et al., 2009).

Van Groenestijn and Hesslink (1993) defined biotrickling filters as an intermediate design between biofilters and bioscrubbers. As in bioscrubbers, a nutrient-liquid flow is sprayed onto the packed bed and continuously recirculated. In contrast to bioscrubbers, the absorption and biodegradation of the target compounds are combined in one column.

Compared with conventional biofilters, the two major differences are the existence of a liquid trickling phase and the use of inert packing materials in all cases. The presence of this mobile aqueous phase allows for much easier pH and temperature control and the removal of accumulated metabolites. This bioreactor is likewise better adapted to the elimination of quite soluble VOCs: solubility specifications are less drastic than for bioscrubbers (Henry coefficient < 0.1), and the VOC inlet concentrations are generally lower than 0.5 g m^{-3} (Mudliar et al., 2010).

Biotrickling filters are typically operated in either co-current or counter-current mode. However, Jin et al. (2005) showed that the co-current flow had the advantage over counter-

current flow of a mostly uniform H_2S removal and biomass growth in each section, whereas the latter removed only 40 – 80 % of the H_2S load near the inlet section.

The flow rate and the recycling rate of the liquid phase through the filter bed are the most critical parameters of biotrickling filters. In a study conducted with toluene (Pedersen and Arvin, 1995), the application of a high superficial liquid flow rate was recommended, which resulted in a low gas/liquid ratio (The superficial gas velocity ranged from 16 to 78 m h^{-1} and the water recirculation rate from 3.3 to 4.7 m h^{-1}). This would ensure an optimum degree of wetting and a greater pollutant phase transfer via elevated turbulence, resulting in a high reactor's performance. However, there are energy costs associated with increased pumping that offset these liquid flow gains.

Additionally, pollutant biodegradation and a continuous nutrient solution feeding will lead to biomass growth on the packing material. If biomass concentration becomes too high, pressure drop will increase to such an extent that it will lead to clogging problems and eventually reactor failure. In fact, the accumulation of excess biomass in the filter bed is the major drawback of biotrickling filters (Delhoménie and Heitz, 2005).

Kim and Sorial (2010) discussed the relationship between nutrient addition and BTF performance. These authors showed that high nitrogen amounts were required to sustain high pollutant removal. Thus, when substrate loadings increased, nitrogen availability enhanced energy production and denitrification rather than biomass yield as cell synthesis.

1.1.2.2.2.1. Biotrickling filters using hydrophobic solvents

The application of a non-aqueous phase liquid (NAPL) to the biotrickling medium has only recently been explored. In these modern reactors, a mixture composed of a liquid organic solvent (normally silicone oil at 10 % to 50 % and flow rates ranging from 8.5 to 21 L $L_{reactor}^{-1}$ h^{-1}) and a water/nutrient medium is continuously trickled over the packed bed, while the contaminated air stream flows either co-currently or counter-currently to the liquid. The gas phase pollutant is then absorbed in the organic phase (oil-phase), transferred to the microorganisms at sub-inhibitory levels and then biodegraded (Bailon et al., 2009; Rene et al., 2011).

1.1.2.2.3. Bioscrubber (BS)

Bioscrubbers for waste gas treatment can be defined as a two-reactor unit and a water flow recirculating system between the two reactors (Figure 1.3). The two-reactor unit involves a combination of a scrubber and a suspended growth bioreactor.

In such a system, polluted air is first fed to the absorption tower (scrubber). By doing so, the pollutants are absorbed in the water phase. Gas and liquid phases flow counter-currently within the column. Gas flow/liquid flow ratios typically range between 300 and 500 and gas

residence times do not exceed a few seconds (Kennes et al., 2009). Schlegelmilch et al. (2005b) recommended a superficial air velocity in the range of 0.5 – 2.5 m s^{-1}.

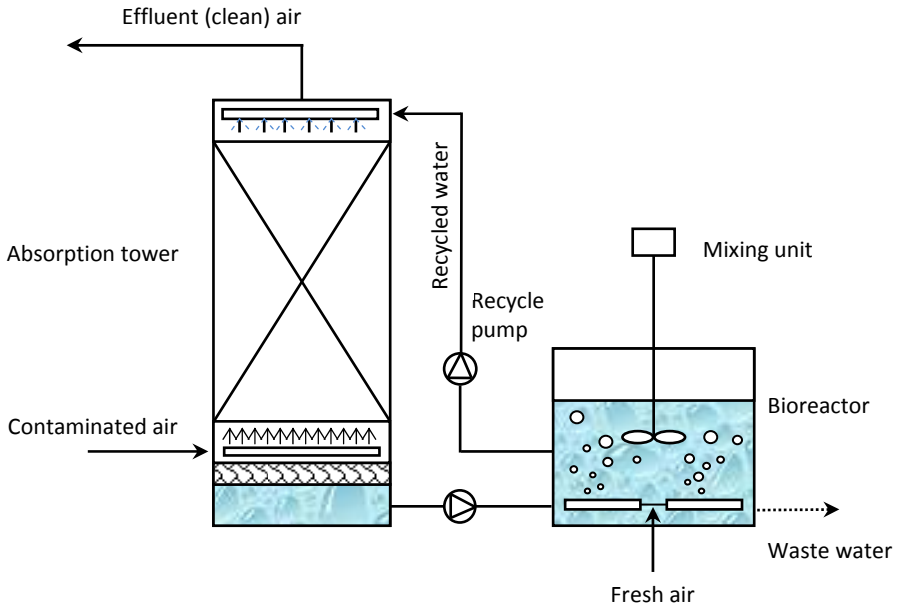

Figure 1.3. Scheme of a bioscrubber.

Subsequently, clean air is released into the atmosphere from the top of the scrubber, whereas the separated contaminated liquid phase (water) is pumped to a stirred, aerated bioreactor. In this second unit, the pollutants are degraded by microorganisms. These microbial strains are immobilized on a packing or suspended in the water containing the essential nutrients for their growth and maintenance (Mudliar et al., 2010).

Absorption can take place either in a bubble column or in a packed bed column. In order to remove poor water-soluble pollutants, packed-bed absorption towers are often recommended. Most often, the absorption tower is randomly packed with a plastic packing material that maintains a minimal pressure drop, but structured packings are also suitable. Ideally, biofilm growth should not take place inside the scrubber, although avoiding this is not always easy in practice. High liquid flow rates and the use of packing materials with a high void space allow minimizing biomass growth in packed scrubbers. As a rule, packed towers operate at liquid irrigation surface rates of around 20 – 60 m^3 m^{-2} h^{-1} of packing surface (Schlegelmilch et al., 2005b).

The scrubber may also be placed on top of the bioreactor, and thus, the water phase reaches the bioreactor by gravity. Clean water is pumped out the bioreactor and it is reused as absorbent in the scrubber (Delhoménie and Heitz, 2005).

The residence times for aqueous solutions in the bioreactor unit range between 20 and 40 days. After a treatment step (filtration and sedimentation of biomass), part of the waste solution may be recycled in the absorption unit while part of the sedimented biomass may be reintroduced into the bioreactor (Kennes et al., 2009).

The presence of a mobile liquid phase allows easy control of certain parameters (pH and nutrient content). Since pH may affect the optimization of both the scrubbing process and the biodegradation stage, the addition of acid or alkali may be required in either the scrubbing tower or the bioreactor, or both.

Compared with biofilters and biotrickling filters, bioscrubbers have the lowest space requirements, the highest operational stability and process control, and the greatest permeability to gas flow (Janni et al., 2001). In addition, bioscrubbers can be operated at higher gas loads of up to 3000 – 4000 m^3 m^{-2} h^{-1} (Kennes et al., 2009). However, van Groenestijn (2005) has predicted that the bioscrubber market will shrink over the coming years, being overtaken by biotrickling filters.

Bioscrubbers and biotrickling filters do not require an upfront humidifier, as biofilters do, to increase inlet air humidity to a preferable 100 %. No medium change-out is required either, since an inert packing material is used and process conditions can be better controlled. Operating costs can be reduced with bioscrubbers or biotrickling filters, since up to 40 % of the operational cost of a biofilter is typically related to the medium replacement (Kraakman, 2005).

An important disadvantage of bioscrubber reactors is their greater complexity for building and operating. The start-up of bioscrubbers and biotrickling filters is also more complicated, since the inert medium itself does not contain active biomass. Additionally, drainage of the process water might wash out the microorganisms; this problem is encountered with the full-scale operation of bioscrubbers.

When biotrickling or bioscrubber reactors are used for the treatment of pollutants such as hydrogen sulphide, ammonia or chlorinated compounds, the degradation produces acid by-products in the drain water, which requires further processing.

On the other hand, since the bioreactor is typically a suspended growth reactor, it will not normally suffer clogging problems, contrary to what occurs in fixed film reactors.

Bioscrubbers are suitable for the treatment of pollutants that are relatively soluble in water, as biodegradation is performed by suspended biomass in the bioreactor. Besides, poor water solubility would also negatively affect the absorption rate and efficiency. Therefore, this technology is recommended mainly for pollutants with a Henry's coefficient not exceeding approximately 0.01 (e.g. alcohols and ketones) (Delhoménie and Heitz, 2005).

To enhance the capture of slightly soluble compounds, organic emulsifying agents such as silicone oil or phthalates can be added to the aqueous solution. Yeom and Daugulis (2001) operated with n-hexadecane and octadecene at percentages of approximately 33 % to remove benzene from a gaseous effluent.

An advantage of bioscrubbers is that they can also be used for the anaerobic biodegradation of volatile pollutants. This may be of interest in the case of pollutants that are hardly biodegradable, or even not biodegradable at all, under aerobic conditions, such as the highly chlorinated organic compound tetrachloroethylene (PCE).

Several authors have sought to compare the performance of bioscrubbers with biofilters and biotrickling filters under the same operating conditions. Koutinas et al. (2005) studied the efficiency of a bioscrubber and a biotrickling filter for the removal of ethyl acetate vapour from a waste gas stream. It was necessary to feed the bioscrubber with pure oxygen in order to achieve similar removal efficiencies in both systems. By contrast, the bioscrubber system was easier to operate and control than the biotrickling filter: several incidents of channelling and blockage of the spray nozzle occurred due to biomass excessive growth within the biotrickling filter.

Le Cloirec et al. (2001) studied the performance of a biofilter and a bioscrubber for treating a synthetic waste gas stream contaminated with ethanol according to different operating conditions (residence time of the gas phase and pollutant loads). In both cases, complete deodorization was successfully attained. However, the biofilter appeared to be adversely affected by ethanol inlet concentration: acidification of the filter bed was recorded at ethanol concentrations greater than 1.3 g m^{-3}.

Different groups have worked on modelling bioscrubber systems. Humeau et al. (2004) developed a model for predicting volatile compound removal efficiencies according to operating conditions (gas and liquid flow rates and washing solution characteristics), the hydrodynamic parameters of each flow (liquid holdup in the column, hydrodynamic behaviour of the liquid flow and axial dispersion of the gas flow) and contact mode (spray column or packed bed). The theory was used for the sizing and optimization of a suspended-growth bioscrubber for the deodorization of a waste gas emitted from a wastewater low-lift station.

1.1.2.2.4. Two-liquid phase bioreactor (TPPB)

This bioreactor was originally developed in Europe (Germany) around the early 1990s (Jin, 2006). Poppe and Schippert (1992) showed the advantage of adding water-immiscible organic solvents to the liquid phase of a bioscrubber for the elimination of hydrophobic VOCs.

The concept of a two-liquid phase bioreactor unit came up because the application of conventional bioscrubbers was limited to the treatment of pollutants that were readily soluble in water. Therefore, the addition of an organic solvent to the water phase (10 – 30 % of the total volume) can solve this problem by enhancing the biodegradation of more hydrophobic compounds and facilitating the elimination of a range of hydrophilic and hydrophobic compounds (Figure 1.4). Thus, as the pollutant is gradually degraded in the aqueous phase, a driving force allows for the continuous and slow release of more pollutant from the highly concentrated organic phase to the poorly concentrated water phase (Kennes et al., 2009).

Besides improving bioavailability, organic solvents reduce the toxicity of contaminants: bearing in mind that microorganisms are present mainly in the water phase, they will never be exposed to high, inhibitory, pollutant concentrations accumulated in the organic reservoir (Mudliar et al., 2010).

The organic phase should meet the following criteria (Quijano et al., 2009):

- Biocompatible
- Non-biodegradable
- High affinity for the target compound
- Low emulsion-forming tendency
- Non-hazardous
- Available in bulk quantities
- Low cost
- Immiscible with water
- Low vapour pressure
- Low viscosity
- Density different from the density of water
- Odourless
- Resistant to autoclaving
- Good hydrodynamic characteristics

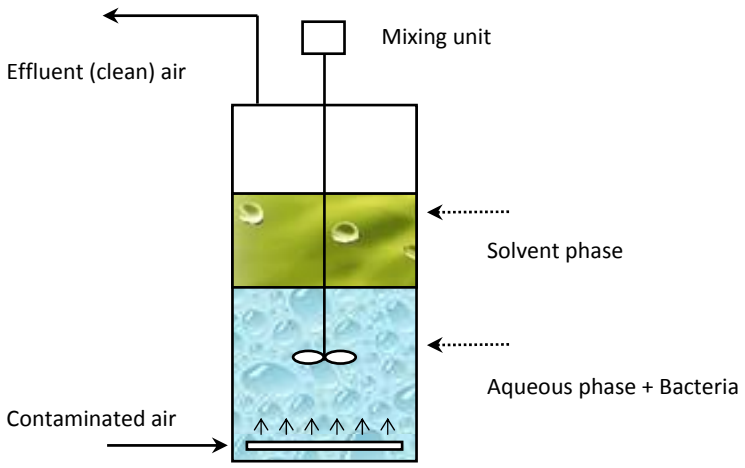

Figure 1.4. Scheme of a two-liquid phase bioreactor.

It should be highlighted that the performance of a TPPB provided with a single non-aqueous phase (NAP) for treating complex mixtures of pollutants depends basically on the characteristics of each pollutant. If the properties of the pollutants, such as polarity or Henry's law, differ significantly from each other, more than one NAP must be selected for optimizing the biodegradation process.

Solvents such as silicone oil, paraffin oil, dibutyl phthalate, di-n-octyl phthalate, di-n nonyl phthalate and pristine are good candidates for this application, and among them silicone oil has been found to be the best for two-liquid phase systems.

Severe limitations such as high energy requirements, high costs of some NAPs, foaming and pollutant sequestration hinder the full-scale application of this technology (Quijano et al., 2009). Recently, the introduction of solid NAPs into this research field has opened a promising pathway for the future development of TPPBs: solid polymers based on copolymers of polyurethane, vinyl acetate, and ethylene (e.g. Kraton, Elvax or Desmopan) have gained increasing acceptance due to their lower cost (up to 250 times less expensive than liquid organic solvents), facilitating separation and recycling in the process.

Regarding BTX compounds, Collins and Daugulis (1999) studied the simultaneous biodegradation of a mixture of benzene, toluene and *para*-xylene by *Pseudomonas sp.* ATCC 55595 in a TPPB consisting of 1 l cell-containing aqueous medium phase and 0.5 l immiscible organic phase (Adol 85 NF, an industrial-grade biocompatible solvent). The system degraded 4.0 g of benzene, 20.2 g of toluene, and 4.2 g of *para*-xylene within 144 h and with the aid of oxygen enriched air.

Most recently, a TPPB consisting of an aqueous phase containing a bacterial consortium and a polymeric phase of silicone rubber pellets (solid volume fraction 0.1) was used to treat a gaseous waste stream containing benzene, toluene, ethylbenzene and *ortho*-xylene (Littlejohns and Daugulis, 2008).

The function of the solid polymer phase was to absorb/desorb the gaseous VOCs providing a buffering effect to protect the cells from high transient loadings and sequester the BTEX for subsequent degradation. Thus, the polymer beads (10 % of the total working volume of the reactor) accounted for up to 70 % of the total BTEX present in the working volume for an inlet loading of 360 g m^{-3} h^{-1}. An overall elimination capacity of 230 g m^{-3} h^{-1} (RE of 81 %) was achieved during a dynamic operating period of 313 min.

1. UP-TO-DATE TECHNOLOGICAL DEVELOPMENT

1.2. Biofiltration of TEX

1.2.1. Conventional biofilters fundamentals and design

A better understanding of the basic mechanisms that affect the operation of a biofilter will help enhance its performance. The efficiency of a conventional biofilter is dependent upon many features and requires a number of technical considerations: these include design parameters such as filter medium, microorganism selection, role of nutrients, gas flow rate effects, moisture content, temperature and pH adjustment, pressure drop and associated problems, filter construction and sizing, etc. A review on each of the aforementioned topics is presented in the following sections.

1.2.1.1. Design parameters of biofilters

Design parameters commonly used in biofilter engineering to describe biofilter performance are: removal efficiency (RE), empty bed residence time (EBRT), pollutant inlet load (IL) and elimination capacity (EC). They are calculated as follows:

- Removal efficiency (RE):

$$RE\ (\%) = \frac{C_{in} - C_{out}}{C_{in}} * 100$$

where C_{in} is the inlet contaminant concentration (g m^{-3}) and C_{out} is the outlet contaminant concentration (g m^{-3}).

RE expresses the fraction of contaminant removed by biofilter. It is an incomplete descriptor of biofilter performance because it varies with contaminant concentration, airflow, and biofilter size and reflects only the specific conditions under which it is measured. For a given set of off-gas composition and filter conditions, removal efficiency or maximum outlet concentration allowed by regulations dictates a minimum empty bed residence time.

- Empty bed residence time (EBRT):

$$EBRT\ (s) = \frac{V * 3600}{Q}$$

where V is the volume of the biofilter (m^3) and Q is the volumetric flow rate ($m^3\ h^{-1}$).

- Elimination capacity (EC):

$$EC\ (g\ m^{-3}h^{-1}) = \frac{(C_{in} - C_{out}) * Q}{V}$$

EC is the mass of the contaminant degraded per unit volume of filter material per unit time. The EC is normalized with respect to volume by definition and allows for direct comparison of the results of two different biofilter systems.

- Inlet load (IL):

$$IL\ (g\ m^{-3}h^{-1}) = \frac{C_{in} * Q}{V}$$

IL defines the amount of contaminant entering the biofilter per unit area or volume of filter material per unit time. It is related to the elimination capacity and the removal efficiency by:

$$EC = RE * IL$$

1.2.1.2. Features affecting biofilter performance

1.2.1.2.1. Packing material

The proper selection of the packing material used in a biofilter is an important decision for achieving high removal efficiencies and maintaining an optimal performance in long-term operation. The support media's main function is to provide contact between the gas-phase contaminants and active microbial cultures attached to the material's surface as a biofilm. Other functions of the media are to distribute the gas flow evenly within the bed's cross-sectional area with minimal gas-phase pressure drop, distribute any liquid nutrients sprayed onto the bed surface, and prevent biomass accumulation, which would eventually lead to undesired channelling of the gas and liquid (Dumont et al., 2008).

Packing materials that can be used during VOC biofiltration are grouped into two main categories: organic and inorganic materials. The latter can be divided into natural inorganic materials or entirely synthetic materials.

Organic materials, generally considered by several authors as the preferred materials, include peat, soil and compost, although wood bark, sugarcane bagasse and peanut shells are also used. The main advantages of these materials are that they are readily available and naturally contain contaminant-degrading microorganisms. Another advantage is that they provide nutrients, such as nitrogen and phosphorous, which are necessary for microorganism growth. However, these materials cause bed compression, increasing pressure drop and thus decreasing biofilter efficiency. They have a low specific surface area and need to be replaced after 2 – 5 years, being difficult to regenerate (Delhoménie et al., 2005).

Inorganic materials were first used as additives to improve the mechanical properties of organic material based biofilters. This group includes natural and manufactured materials such as lava rocks, ceramic rings, glass beads, polyurethane foam, activated carbon, perlite and vermiculite, among others. When used during biological processes, they offer several advantages, such as good mechanical resistance in comparison to organic materials. Their physical properties (e.g. porosity and specific surface area) can be more easily adjusted according to the requirements of the bioprocess. However, their main disadvantage is that they do not provide any nutrients for the biomass and, in some cases, there are unaffordable.

The importance of the packing material properties depends heavily on the characteristics of the biofiltration system and its operation, which implies that a material may be suitable in certain conditions but inappropriate in others. In general terms, the packing material must provide a favourable environment as far as moisture, temperature, pH, nutrients and oxygen supply are concerned. A perfect material should have the following characteristics:

- A suitable particle size, void fraction (0.5 – 0.9) and specific surface area (≥ 300 $m^2 m^{-3}$), enabling the filter bed to benefit from a high biofilm surface and facilitating the transfer of the VOCs contained in the air. If the size of the particle is too small, a large specific surface area is available, although resistance to the gas flow is increased; by contrast, if it is too large, it favours gaseous flows, but the number of potential sites for the microbial activity is lower. A threshold diameter-value higher than 4 mm (minimal pellet size) has been suggested by Leson and Winer (1991). Delhoménie et al. (2002) concluded that pellet size and specific surface area were the major limiting factors for the biodegradation process.
- A high water-holding capacity is desirable to maintain the optimal activity of the immobilized microorganisms. Gutiérrez-Acosta et al. (2010) were able to modify the hydrophobic character of polyurethane foam using a tertiary amine as an additive. This organic compound, containing hydroxyl groups, increased the water retention capacity of the synthetic material without the total loss of its hydrophobicity. The water retention capacity of the composite was 34 % w/w, while for the non-modified polyurethane it was 12.5 % w/w. Such a modification contributed to significant biomass growth and attachment and to the establishment of better interactions with pollutant compounds. The time required for the complete biodegradation of hydrocarbons with the modified polymer was at least 30 % shorter than that obtained with the non-modified polymer. Table 1.1 shows the water-holding capacities of different packing materials used in the literature.

Table 1.1. Water-holding capacities of various packing materials.

Material	Water-holding capacity (%)	Material	Water-holding capacity (%)
Towel scrap[a]	301.2	Sawdust[a]	245.4
Perlite[b]	42.8	Polyurethane + Tertiary amine[c]	34.0
Polyurethane[b]	33.3	Activated carbon powder[a, d]	31.7
Earthworm casting[a]	31.7	Activated carbon granule[a, e]	31.4
Activated carbon powder[a, f]	30.5	Polypropylene[b]	21.7
Crab shell[a]	18.1	Zeolite[a]	5.4
Waste tyre scrap[a]	1.3	Soil[a]	0.9
Scallop shell[a]	0.4		

Notes: (a) Kwon et al. (2009); (b) Gutiérrez-Acosta et al. (2012); (c) Gutiérrez-Acosta et al. (2010). This material was made of a modified polymeric foam based on polyurethane with an organic compound (tertiary amine) containing hydroxyl groups; (d) from coconut; (e) from coconut; (f) from sawdust.

- The bacteriological characteristics favourable to bacterial development are high inorganic and organic nutrient content, high buffering capacity avoiding large pH fluctuations and the absence of inhibiting compounds.
- A high adsorption capacity in order to buffer intermittent loads. Inlet concentrations of contaminant fluctuate throughout the daytime, the operating schedule or even when an eventual emergency situation occurs. The packing material should act as a buffer to adsorb and soften the pollutant load on the biofilter. Table 1.2 shows the toluene adsorption capacity of different packing materials.

  Bioreactors have recorded better yields under stationary conditions. However, industrial air emissions usually present variable and discontinuous concentrations that could hinder the performance of full-scale biofilters. Operating problems have been reported when the adsorption capacity of packing material is not high enough to achieve effective pollutant load equalisation under cyclic or discontinuous operation (Sempere et al., 2010).

  Several researchers have set out to resolve this problem by means of activated carbon filters preceding the biological reactor, which could buffer the sudden variations in the concentration, spikes and unstable loads of contaminants. Additionally, these pre-filters could be desorbed during downtimes to reduce the intensity of the starvation periods.

  Sempere et al. (2009) evaluated the performance of a granular activated carbon filter (GACF) installed, as per legal requirements, prior to a bioreactor treating the waste gases typically found in flexographic printing facilities.

  The GACF, which had a volume 25 times lower than the bioreactor, proved to be capable of buffering the oscillating concentrations of the inlet stream, reaching an average daily outlet emission concentration lower than the emission limit imposed in the European Council Directive 1999/13/EC (< 100 mg C Nm^{-3}).
- Mechanically resistant, chemically inert and stable. A relatively constant volume of pores is advisable to avoid bed compaction and ensure a uniform airflow through the filter bed.
- Low bulk density in order to ensure better hydrodynamic properties and avoid bed compaction. Table 1.3 shows the bulk density of different packing materials.
- The presence of diverse indigenous microorganisms (including bacteria, actinomycetes, fungi, yeasts, algae and protozoa) excludes the need for inoculation and ensures shorter start-up periods. Nevertheless, this microflora should be carefully monitored, as pathogenic species can also be found (Borin et al., 2006).

Table 1.2. Toluene adsorption capacities of various packing materials (Kwon et al., 2009).

Material	Adsorption capacity ($mg_{toluene}\ g^{-1}_{material}$)	Material	Adsorption capacity ($mg_{toluene}\ g^{-1}_{material}$)
Activated carbon powder[a]	56.9	Activated carbon powder[b]	56.6
Activated carbon granule[c]	56.3	Crab shell	15.7
Waste tyre scrap	11.5	Zeolite	8.2
Earthworm casting	6.6	Soil	5.7
Used briquette	4.3	Sawdust	4.2
Chalk	3.5	Styrofoam	3.2
Sand	2.7	Towel scrap	2.2
Pine needle	1.6	Scallop shell	1.1
Rice straw	0.2	Leaf mould	0.1
Wood	0.0	Newspaper	0.0
Pine cone	0.0		

[a] From coconut; [b] From sawdust; [c] From coconut

Table 1.3. Bulk density of various packing materials.

Material	Bed bulk density ($g\ ml^{-1}$)	Material	Bed bulk density ($g\ ml^{-1}$)
UP–20[a]	0.92	Compost-Polyethylene[b]	0.825
Hydroballs[c, e]	0.27	Polypropylene[d]	0.262
Bark chips[c]	0.24	Peat[c]	0.21
Vermiculite[c]	0.15	Perlite[d]	0.115
Polyurethane[d]	0.073		

Notes: (a) Gaudin et al. (2008). UP–20 was a packing material based on urea phosphate. (b) Wu et al. (2009). It was mixture of mature pig compost and a new packing material made of polyethylene. (c) Oh and Choi (2000); (d) Gutiérrez-Acosta et al. (2012); (e) Hydroball was a horticultural product manufactured by Haeran Co. (Korea).

- Low cost: the price of the packing material has a significant impact on overall costs, not only because of the high volumes usually required for biofilter construction, but also because packing material replacement is mandatory due to its limited lifespan. Commercial biofilter packing materials typically last from 1 to 15 years, with the longest lifespans found for nutrient-enriched inert materials, and from 1 to 3 years for conventional organic materials (Estrada et al., 2011). Table 1.4 shows the price of several packing materials.

Table 1.4. Price of common packing materials used in biofiltration.

Packing material	Material cost (€ m^{-3})	Estimated lifespan (years)	Annual material cost (€ m^{-3} $year^{-1}$)
Coconut fibre[a]	200 – 240	2	100 – 120
CAC[a, c]	450 – 500	10	45 – 50
Lava rock[b]	215 – 250	10	45 – 50
Lava rock[a]	40 – 50	15	3 – 4
SMP[b, c]	125	5	25
Peat with heather[a]	40 – 50	2	20 – 25
Advanced material[a, d]	300 – 360	15	20 – 24
Pine leaves[a]	25 – 35	2	12 – 17
SBC[a, e]	70 – 90	10	7 – 9
Lignite[a]	40 – 50	10	4 – 5
Ceramic Rashig ring[b]	350 – 375	10	3 – 4
Compost[a]	5 – 10	2	2 – 5
PUF[a]	25 – 35	15	2 – 3
Ceramic Pall ring[b]	250 – 315	10	2 – 3

Notes: (a) Price of packing materials according to 2009 prices on the Spanish market published by Dorado et al. (2010); (b) Price of packing materials according to the Chinese market published by Sun et al. (2011); Economical data presented herein have been converted to euro (€) currency, considering that 1 € = 0.125 ¥; (c) SMP was comprised of coral rock, bark, ceramisite, charcoal, and compost (160:120:100:60:15, w:w).The SMP structure was reinforced using a urea-formaldehyde resin to enhance bed porosity and specific surface area; (d) The advanced material is based on a thin layer of compost over a clay pellet; (e) CAC is a commercial activated carbon; (d) SBC is based on a sludge-based carbon.

1.2.1.2.2. Microorganisms source: inoculation

The biomass responsible for the degradation of the gaseous contaminant fed into the biofilter can be supplied by the support material itself. Nevertheless, depending on the type of medium (i.e. organic or inorganic) being used and the nature of the contaminant to be degraded, the biofilter medium may need to be inoculated with some sort of microbial suspension.

If organic filter beds are used and the pollutant is a quite readily biodegradable substrate (e.g. H_2S), suitable indigenous microorganisms will generally be present and will easily develop in the biofilter. In the case of inorganic packing materials or if more recalcitrant pollutants are going to be treated (e.g. MTBE or some halogenated compounds), the inoculation of specialized biocatalysts with more rapid degradative capabilities may become necessary.

As an example, Pandey et al. (2009) only started rendering high removal efficiency of diethyldisulphide (DEDS) in a biofilter packed with compost and wooden chips after it was inoculated with DEDS adapted microorganisms. After inoculation, those microorganisms grew rapidly in the packed bed of the biofilter, and the specific count of the microorganisms increased from $0.5 * 10^2$ to $2.5 * 10^2$ CFU g^{-1} within a period of 10 days.

Similarly, Andrès et al. (2006) calculated that inoculation with an adapted biomass suspension of a biofilter used for the treatment of a waste gas containing methyl-ethyl-ketone (MEK), ethanol, dichloromethane (DCM) and toluene reduced the acclimatisation period by a factor of 2. This kind of microbial consortium supported stable biodegradation capacities near 100 % for ethanol and 50 % for DCM, MEK and toluene with loading concentrations between 7 and 35 g m^{-3} h^{-1}.

It is generally accepted that for the most easily biodegradable compounds (e.g. ketones and alcohols), a period not exceeding 20 days of acclimation is required after the process start-up of the process is required (Nikiema et al., 2007). On the other hand, in the case of poor biodegradable compounds or complex gas mixtures, several weeks to several months are often required to obtain adapted inoculums with high removal capacity.

If adapted inoculums were available at any time, biofiltration technology would be more competitive compared to other treatment methods for VOCs (Chen et al., 2010). Kraakman (2003) suggested that an active culture of appropriate organisms should be maintained on a regular basis, so that a system in which the microorganisms have been inadvertently killed could be immediately reinoculated through the irrigation system.

However, enriched/prepared liquid microbial inoculums may be difficult to preserve over extended time periods. Ridgway et al. (1990) and Leddy et al. (1995) observed that prolonged biomass exposure to target contaminants resulted in stressed subpopulations that continue growing with a selective loss of catabolic functions. Arcangeli and Arvin (1992) observed that the active fraction of a biofilm exposed to toluene over an extended period accounted for only 5 % of the total, while nearly all the cells were active during the initial attachment phase.

Prado et al. (2005) maintained the inoculation aerobic sludge for not more than 4 – 5 weeks in the laboratory at room temperature, with continuous supply of air, in order to minimize biomass decay and avoid anaerobic growth. After that time, unused sludge was replaced with a fresh one.

Chen et al. (2010), attempted to solve this problem by employing a microbial consortium bound to a solid carrier as an alternative to traditional liquid inoculums. These authors reported that the solid inoculum could be preserved at 4 °C for at least 6 months without running the risk of significantly losing metabolic potential.

1.2.1.2.2.1. Inoculum sources

A variety of sources for obtaining the adapted degrading microorganisms is mentioned in the literature. Those sources are:

- **Pure cultures or consortia of specialized microorganisms (purchased from trade catalogues)**:

An advantage of pure strains is their known biological properties. The relation between activity and physiological conditions (e.g. pH, temperature or substrate concentration) can be determined, and the reactions catalyzed by the organism(s) can be revealed. Excretion of possible intermediates and final products can be determined as a function of environmental conditions, and biodegradation can thus be optimized.

Morales et al. (1998), using a stable population made up five bacteria and two yeasts pre-adapted to toluene degradation to report an adaptation period of approximately 6 h inside the biofilter. After this period, during which no removal was observed, the elimination capacity started to increase rapidly up to a maximum of 190 g m^{-3} h^{-1} (100 % efficiency) after 21 h.

In order to compare these results, it should be noted that studies on the biofiltration of toluene vapours with non-adapted microbial populations reported that steady states were reached in about 1 – 3 weeks. Bhaskaran et al. (2007) reported a start-up stage of 7 days for a biofilter where an unacclimated aerobic sludge was added to the indigenous toluene-degrading bacteria living in the packing material (coir pith).

In the case of *para*-xylene, Jeong et al. (2006) isolated and satisfactorily used a *para*-xylene-degrading bacterium *Pseudomonas sp*. NBM21. These authors listed other xylene-degrading bacteria: *Pseudomonas putida* 39/D, *Xanthomonas maltophilia, Sphingomonas strain* ASU1, *Rhodococcus sp. strain* DK17, and transformed *Escherichia coli*. However, these strains were not applied to non-sterile biofilters.

Ethylbenzene was degraded by pure cultures of *Stenotrophomonas maltophilia* T3-c or *Sphingomonas sp.* D3K1 by biofilters with rock wool-compost media as packing material (Cho et al., 2009).

By contrast, as biofilters are open systems operated for long periods, it is difficult to maintain a pure microbial population throughout the experiment. Qi and Moe (2005) observed that a biofilter initially inoculated with only *Cladosporium sphaerospermum*, presented multiple additional fungal species (*Penicillium brevicompactum, Exophiala jenselmei, Fusarium oxysporum, Fusarium nygamai, Talaromyces flavus,* and *Fonsecaea pedrosi*) growing attached to the packing medium by the end of experiment.

The emergence of species not detected in the inoculum could proceed from:

- The inoculum wherein their abundance was below the detection limit of the analytical method.
- The endogenous diversity reservoir constituted by the packing material itself.
- The polluted gaseous effluent or aerosols through immigration mechanisms.

Besides, the operating conditions can be adjusted to favour the development of a defined population. For instance, Hwang et al. (2003) sterilized the packing material to avoid microbial community competition. Additionally, the influent air can be filtered and nutrient solutions supplied so the column can be autoclaved.

- **Adapted biomass isolated from other bioreactors**:

Material from biofilters that already eliminate poorly degradable or xenobiotic compounds can be mixed with the packing material from the new filter. In this way, the old material serves as an inoculum.

Prado et al. (2002) observed immediate toluene removal following this strategy. Removal efficiency close to 100 % was maintained for more than 2 weeks for inlet loads around 10 g m^{-3} h^{-1}.

- **Active or mixed cultures collected from locations contaminated with the target pollutant**:

This latter alternative provides a consortium culture capable of surviving in selective contaminated environments, thereby ensuring a naturally adapted biomass. For industrial application, a mixed culture would be preferred since there is less of a need to prevent contamination with unwanted microorganisms, and the microbial diversity allows for greater flexibility with respect to substrates.

Moe and Qi (2005) explained the way to inoculate a biofilter using an enrichment culture derived from a mixture of composted wood waste and composted municipal wastewater sludge.

1.2.1.2.2.2. Preliminary acclimation

The preliminary acclimation of the biomass to the pollutant and the correct inoculation of the microbial consortia into the biofilter are recommended strategies for shortening the start-up period of biofilters and for ensuring longer successful operation.

Acclimation is a period required for the development of the optimum population of substrate consuming microorganisms (inoculum) before they start vigorous biofiltration. Failures in this ripening period have been considered as the second major limitation of biofilters (Aldric et al., 2008).

Prado et al. (2005) proved that the prior biomass adaptation of the inoculum dramatically affected the start-up and performance of conventional biofilters treating methanol during the

first stages of operation. Thus, a fast start-up was obtained when using adapted inoculum (100 % removal efficiency was achieved after 3 days), while non-adapted inoculum proved to be much less efficient, reaching a highest RE value of around 50 % one week after inoculation, decreasing later on to values below 25 %.

In a similar way, Torkian et al. (2003) achieved removal efficiencies reaching 90 % for toluene and close to 100 % for xylene in less than 5 days. These authors attributed the shorter acclimatization period to the fact that prior to the start-up of the two biofilters, the bed media (80:20 w/w ratio of compost-wood chip) was mixed with high concentrations of these pollutants.

On the other hand, many studies briefly describe this step and use arbitrarily a nutrient solution and/or an easily degradable carbon source (e.g. glucose or saccharose) and the target pollutant to acclimate/activate the biomass for a variable period of time.

As an important change is expected in microbial diversity in the inoculum at the end of the acclimation procedure when one specific compound is fed (Steele et al., 2005), the addition of an easily degradable carbon source (different from the target contaminant) could damage or inhibit the microorganisms of interest (Vergara-Fernández et al. 2010).

In fact, in the case of complex or undetermined biomass, prior activation/adaptation with glucose is recommended only when indiscriminate (unspecialized) biomass is to be developed. Otherwise, the acclimation to a specific contaminant may be delayed because glucose is an easily degradable carbon source for a wide variety of microorganisms.

For instance, Singh et al. (2006) supplied glucose (5 g day^{-1}) during the initial phase of acclimation of the microbial inoculum culture obtained from the activated sludge taken from a local WWTP. However, these authors gradually replaced glucose by toluene as the only carbon source, increasing the colony of desirable toluene-degrading microorganisms in the mixture.

García-Peña et al. (2008) sought to enhance benzene degradation with the filamentous fungus *Paecilomyces variotii* through the presence of an initial alternate substrate or nutrient. For this reason, glucose, ethanol, yeast extract, and phenol was initially added. The concurrent presence of these compounds did not promote or reduced benzene consumption in batch assays, and a similar degradation level was observed.

In other assay, *P. Variotii* was grown with toluene as the only carbon and energy source and with a mixture of toluene and glucose. Crude enzyme extracts from biomass with toluene and with both glucose and toluene were used to induce benzene degradation. Results showed that enzymatic induction was partially repressed by the presence of glucose, and higher activity was observed when the fungus was grown with toluene as the sole carbon source.

Vergara-Fernández et al. (2010) set out to reduce the start-up period of an n-hexane degrading biofilter inoculated with *Fusarium solani* comparing the performance of four carbon sources as germination and growth promoters: n-hexane, glycerol, 1-hexanol and wheat bran. The last three substances were known to be less hydrophobic than the target pollutant (n-hexane), rendering them into more available carbon sources for the inoculum.

The results showed that the adaptation period was reduced from 35 days when the target pollutant was used to 24 days with glycerol, 14 with 1-hexanol and 8 with wheat bran.

In contrast, this decrease in start-up time has to be evaluated considering also that lower elimination capacities were obtained when the three alternative substrates were employed; thus, a maximum EC of 225 g $m^{-3} h^{-1}$ were attained with n-hexane, while values of 205, 200 and 165 g $m^{-3} h^{-1}$ were obtained with glycerol, 1-hexanol and wheat bran, respectively.

Besides, even if the acclimation process is carried out using the pollutant(s) of interest, it is not clear which is the appropriate experimental-procedure to complete the enrichment/acclimation period.

For example, Sercu et al. (2005) compared two protocols for a biotrickling-filter removing dimethyl sulfide (DMS). A biotrickling filter (HBF–1) was then filled with rings that were submerged in a nutrient medium containing the strain *Hyphomicrobium VS* fed with DMS; another biotrickling filter (HBF–2) was similarly filled with rings that were also submerged in nutrient medium but continuously supplied with actively growing *Hyphomicrobium VS* and fed with methanol.

Accordingly, the main differences between both protocols were:

- The carbon source, which was DMS in the first protocol and methanol in the second.
- The inoculation reactor, operating in batch mode in the first protocol and continuously supplied with actively growing bacteria in the second protocol.

Bearing in mind the degradation-capacity, the maximal DMS elimination capacity for HBF–1 was 7.2 g $m^{-3} h^{-1}$ after 30 days of operation (RE of 90 %). The elimination capacity decreased, however, when the inlet loading rate exceeded 15 g $m^{-3} h^{-1}$ (200 ppmv inlet concentration). The performance of HBF–2 was much better, with an elimination capacity of 8.3 g $m^{-3} h^{-1}$ (RE of 90 %) after 2 days of operation, increasing to a maximum of 57 g $m^{-3} h^{-1}$ at RE of 92 %.

From a microbial point of view, two to three times more *Hyphomicrobium VS* cells were still present on the rings in HBF–2 compared to HBF–1. Microbial community characterization showed very different microbial communities in both biotrickling filters. Moreover, the decreased DMS elimination capacity of HBF–1 at higher influent loading rate corresponded with a drastic change of the microbial community on the rings.

Likewise, Bayle et al. (2009) compared a further two different feeding methods during the acclimatization period using two reactors filled with an identical activated sludge suspension. Hence, one reactor was supplied with an effluent containing a complex VOC mixture (i.e. oxygenated, aromatic and halogenated compounds) for which the concentration of oxygenated compounds varied with incubation time. The other was supplied with an effluent gradually enriched with chlorinated, aromatic and oxygenated compounds.

The results suggested that the microbial communities in both reactors were affected differently in response to the treatment but developed a similar capacity to remove VOCs at the end of the treatment period.

In conclusion, the experimental strategy used could lead to different enrichments in terms of functionality and microbial diversity.

1.2.1.2.2.3. Impact of inoculation on biofilter long-term operation

As mentioned above, the choice of the inoculum and the acclimation-inoculation process can potentially influence the removal of pollutants and reactor stability. It has also been shown that this strategy is beneficial for biofilter operation and efficiency early on, i.e. start-up and the first weeks of operation.

In addition, Hernández et al. (2010) suggested that inoculation with a specific pre-enriched microbial culture might slow uncontrolled biomass growth and postpone clogging problems.

The question therefore arose as to whether the initial strains persist in the biofilm or if they are replaced by other strains inoculated from the inlet air or from residual populations present in the filter material. Jorio et al. (1998) showed that none of the inoculating strains [*Pseudomonas putida* (ATCC 31483), *Pseudomonas putida* biotype A (ATCC 39213), *Rhodococcus sp.* (ATCC 21499) and *Arthrobacter paraffineus* (ATCC 15590)] remained dominant in the biofilm after several weeks' operation in a biofilter packed with commercially conditioned peat.

These authors suggested that the inoculating species helped the natural selection and establishment of efficient species for the biodegradation of toluene and xylene, even though some of the species disappeared from the biofilter after a while.

Moreover, Sercu et al. (2007) showed that the effect of adding strains to a complex sludge matrix largely disappeared eventually for the removal of dimethyl sulfide (DMS) in a biotrickling filter. These authors observed that after several months of operation, the differences in DMS removal efficiencies were very small, and roughly equal levels of the DMS degrading bacteria developed. *Hyphomicrobium VS* and *T. thioparus* strains were abundant in all reactors, even in those in which they were not manually added.

Cabrol and Malhautier (2011) affirmed that the final structure of a biofilter's microbial community is influenced by four factors, namely, the endogenous community on the packing material (if it exists), the inoculum, growth conditions within the reactor (e.g. attachment on the carrier and the hydrodinamics of gas and water flows/streams) and contaminant feeding.

These authors defined the start-up period as extremely dynamic because of the selection of the most fitted populations emerging from the packing material itself (when an endogenous community is present) as well as from the inoculum. During this critical period, a decrease in microbial diversity is expected due to community specialization, competitive exclusion, the toxic effect of the contaminants and/or reduction in resource variety. For that reason, dynamics during start-up and divergence from the original inoculum have been reported frequently, even when the inoculum had previously been acclimatized to the contaminants.

In order to corroborate their hypothesis, Cabrol et al. (2012) studied the spatial and temporal dynamics of a microbial community structure and function in duplicated wood-chip biofilters treating a gaseous mixture composed of ammonia and six VOCs. Both bioreactors were operated under constant conditions for 231 days.

Characterization of the biofilm after 42 days of operation revealed the limited impact of inoculum compared to the greater persistence of the endogenous wood-chip community. The

long-term performance of the biofilter community was influenced mostly by contaminant loading.

These results are in accordance with Omri et al. (2011), who reported significant differences in the microbial community structure from the downside to the upside in a peat biofilter treating H_2S in downflow mode, with a sharp decrease in species diversity in the inlet gas zones.

1.2.1.2.3. Role of nutrients

The most commonly accepted formula for biomass composition is $C_5H_7NO_2$ (Kennes et al., 2009). Microbial cells are not only formed of carbon, hydrogen and oxygen atoms, but also of nitrogen, as well as other elements, such as phosphorus, that do not appear in the simplified formula. Thus, in order for biomass growth to take place and optimize microbial enzymatic activities, the presence of macro and micro-nutrients (e.g. nitrogen, phosphorus and trace elements) is compulsory in bioreactors.

The availability of these macronutrients (N, P, K, S) and micronutrients (vitamins, metals) must be guaranteed either by the filtering material or by external nutrient-solution addition.

It is obvious that biofilters using inorganic or composite packing material require an external nutrient supply for microbial growth. However, in early 1990s natural organic packing materials (e.g. compost or peat) were generally thought to contain a sufficient nutrient supply for biomass maintenance. The effectiveness of nutrient addition to natural media biofilters remained a highly debated issue.

Nowadays, several studies have shown that the long-term use of organic-based beds leads to progressive exhaustion of the intrinsic nutritive resources. This is particularly clear with nitrogen, which is a major constituent of microorganism proteins and nucleic acids insofar as it can make up approximately 15 % of the microbial cell dry weight.

Ramirez and Kinney (2000) found that approximately 75 % of the original nitrogen content was lost from the packing material after 30 days of operation of a compost biofilter treating xylenes and toluene, compromising elimination capacity.

Hwang et al. (2007) experienced nitrogen limitation when two organic biofilters packed with pig manure compost and food waste compost, respectively, were operated without adding a supplemental nitrogen source. These authors were finally forced to add an aqueous solution of 20mM nitrate fortnightly in order to achieve elimination capacities of around 20 g $_{para\text{-xylene}}$ m^{-3} h^{-1} for an operation period of 150 days.

The above-mentioned works have shown that, whatever the filtering material used, the steady addition of nutrients is necessary to sustain a satisfactory degrading activity.

Nonetheless, although low nutrient levels in the biofilm lead to unreliable biofilter performance (particularly at high VOC loadings), an excessively high concentration of nitrogen in the nutrient solution may also adversely affect microbial activity and bioreactor behaviour.

Delhoménie et al. (2005) confirmed the existence of nitrogen-limitation and nitrogen-optimum regions. In this case, when an optimum nitrogen level near 3.0 g_N l^{-1} was applied, toluene elimination capacities as high as 135 g m^{-3} h^{-1} were achieved. For nitrogen concentrations between 3.0 and 6.0 g_N l^{-1}, nitrogen was in excess and acted as an inhibitor of toluene degradation. The elimination capacity decreased significantly to 100 g m^{-3} h^{-1}.

These authors attributed the decline in performance to the colonization of the bioreactor by nitrifying species such as Nitrosomas or Nitrobacter bacteria, to the detriment of degrading species.

On the other hand, Mathur et al. (2007) suggested that a high salt concentration in the nutrient solution may have inhibited the activity of the aromatic degrading microorganisms. Thus, when the nitrogen-containing components were temporally removed from the nutrient solution, the ionic strength of the solution was greatly reduced and an initial improvement was noticed in the aromatic hydrocarbon degrading capacity of the biofilters.

Finally, when nitrogen is abundant in biofilm systems, operational problems such as excessive biomass accumulation, rapid clogging and system failure could also happen (Song et al., 2003).

Theoretically, a carbon to nitrogen (C/N) ratio of 4.3 is required in accordance with stoichiometric demand. In fact, the "threshold" amount of nitrogen required in the media depends on the VOC loading rate to the biofilter.

For instance, Sempere et al. (2011) decided that a mass ratio (C/N) between 15 and 20 had to be supplied to remove satisfactorily styrene from a gaseous emission by biotrickling filtration. By contrast, Smith et al. (1996) showed that operating at a C/N ratio of 14.6 led to a rapid biomass accumulation in two biofilters treating high loads of toluene.

Maestre el at. (2007) succeeded in increasing the elimination capacity of both a peat biofilter (from 6.2 to 71.6 g m^{-3} h^{-1}) and a compost biofilter (from 17.0 to 89.9 g m^{-3} h^{-1}) when the amount of nitrogen was increased by a factor of 15 to reach a C:N:P ratio of 136:9.7:1. Originally, a nutrient solution with an initial C:N:P ratio of 123:0.6:1 was fed.

Six et al. (2006) stated that fungal:bacterial ratio changes, as does the (C/N) ratio. Low quality substrates (high C/N) favour fungi development, while high quality substrates (low C/N) favour bacterial growth.

However, these nitrogen ratios give no indication of the real availability and form of the nutrients to the microorganisms in the media.

Nitrogen is present in the packing material in both organic and inorganic forms (Figure 1.5) (Gribbins and Loehr, 1998). The organic forms are in the media itself (if an organic filter is used, of course), while the inorganic soluble forms (i.e. nitrate, nitrite, and ammonia) are present in the water surrounding the media particles. Organic forms of nitrogen are less available for microbial uptake, and the soluble forms of nitrogen are considered the available nitrogen.

Soluble nitrogen (i.e. ammonia) is formed by the mineralization of organic nitrogen in the media and recycling of nitrogen when microorganisms die, lyse, and mineralize. Subsequently, ammonia could be volatilized, nitrified, assimilated into new cell growth, or denitrified to

nitrogen gas and/or ammonia in local anoxic or anaerobic zones. In general, unless resupplied, readily available nutrients are eventually depleted along the length of a biofilter.

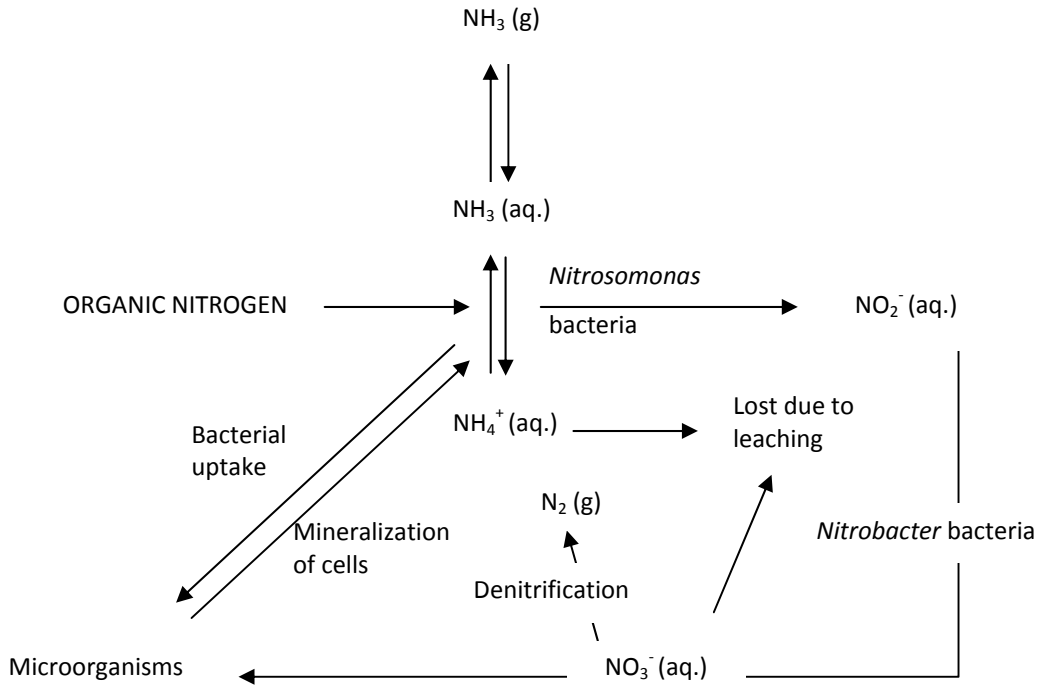

Figure 1.5. Nitrogen cycle in an organic filter media (Gribbins and Loehr, 1998).

Although the impact of a nutrient limitation may be mitigated by the gradual release of nutrients from the packing media or from the biomass generated, nitrogen addition to the media through fertilization is the best way to replenish soluble nitrogen that is lost.

Indeed, when organic nitrogen serves as the source of nitrogen, as it does when natural organic packing media are used, a relatively slow rate of production of readily available nitrogen limits the rate of contaminant destruction (Moe and Irvine, 2001).

Gribbins and Loehr (1998) recommended a soluble nitrogen concentration in the media of more than 1000 mg kg^{-1} as N (dry weight) for the biofilter to operate optimally. In this case, a biofilter packed with a mixture of compost and perlite was used for a toluene loading of 30 g m^{-3} h^{-1}.

This suggestion is in agreement with Ramirez and Kinney (2000): a tenfold increase in the removal efficiency was reported after nitrogen addition (1500 mg kg^{-1}) in a compost biofilter treating xylenes and toluene. Besides, according to these authors, an increase in nitrogen concentration from 1500 to 3500 mg kg^{-1} in the biofilter medium has no effect on elimination capacity.

Oh et al. (2009) also supported the "threshold" value proposed by Gribbins and Loehr (1998). These authors observed that when the available nitrogen (i.e. ammonia and nitrate) along the biofilter column declined from an original value of 2520 to 396 mg kg^{-1} (day 50 of operation), there was a breakdown in the toluene removal efficiency.

1.2.1.2.3.1. Available nitrogen forms. Which is best ammonia or nitrate?

Smith et al. (1996) studied the nitrogen effect (nitrate or ammonium) on the biofiltration of toluene. They found that the addition of nitrogen in the form of nitrate instead of ammonia helped to reduce biomass production and prevented excess biomass accumulation in the filter bed. Jorio et al. (2000) observed that higher styrene elimination rates (up to 141 g m^{-3} h^{-1}) were reached in a biofilter supplied with ammonia as the major nitrogen source in comparison to the lower elimination performance (up to 50 g m^{-3} h^{-1}) obtained when nitrate was provided.

Sempere et al. (2011) reported a better performance by using urea instead of nitrate, recording a 50 % higher peak elimination capacity working at an EBRT of 60 s and with lower biomass content. On the other hand, Jin et al. (2007) indicated that nitrate could be a most advantageous nitrogen source compared to ammonia for fungal biofilters treating α-pinene emissions.

1.2.1.2.3.2. Nutrient supply modes

Nutrient supply is performed either in the solid form, directly inserted into the filter bed, or as mineral salts dissolved in aqueous solutions, which is the most frequently used method. Nevertheless, several authors have tried different alternatives to improve this system.

Kwon and Yeom (2009) tried a leaf-mould solution as an alternative nutrient, instead of a defined chemical medium, for reducing the operating costs. This substitution turned out to decrease the removal efficiency by 10 – 20 %. However, the authors were convinced that the results were good enough to consider the possibility of using leaf-mould solution as a nutrient for biofilter operation.

Chan and Lin (2006) preferred to prepare a synthetic filter material (poly(vinyl alcohol) (PVA)/peat/KNO_3 composite bead) containing the nutrients itself. In this study, the composite bed worked for 230 days without the further addition of nutrients. The percentage of removed ethyl acetate remained at more than 98 % at all times.

1.2.1.2.4. Oxygen availability

The role of oxygen in biofiltration is to act as the main agent in aerobic breathing for microbial growth. Oxygen can be obtained by microorganisms from VOCs or can be taken from polluted air as it passes through the biofilter.

Jin et al. (2006a) investigated the influence of the oxygen within the biofilter, by comparing α-pinene degradation at 21 % (air) and 45% oxygen (air enriched with pure oxygen) in the inlet gas (V/V). These authors concluded that the maximum degradation rate at high α-pinene concentrations was limited by the availability of oxygen. Contrary to what happened with normal air, where the elimination capacity levelled off when reaching 125 – 130 g m^{-3} h^{-1}, such a plateau was not found in presence of 45 % O_2, suggesting that elimination capacities exceeding 190 – 200 g m^{-3} h^{-1} could reasonably haven been reached if higher loads had been applied.

Due to the importance of oxygen concentration in air (21 % V/V) and the thinness of the biofilm in a normal operation scenario (e.g. low pollutant concentration), oxygen transfer is relatively easy.

Nevertheless, oxygen transfer in the biofilm could be limited in some cases. Oxygen deprivation is undesirable because it could lead to partial or total anaerobic conditions, causing nuisance odours and system upset (Jin et al., 2006a).

A reason for oxygen limitation is that most of the oxygen is in the gas phase rather than dissolved in the liquid phase. Moreover, biofilm strongly influences mass transfer by limiting the diffusion of oxygen within the biofilm. If the biofilm thickness exceeds the depth of oxygen penetration, an "inactive" area could appear in the inner biofilm layer. Devinny and Ramesh (2005) reported that biofilm-beds farther than 75 – 100 µm from the surface became inactive. Regarding biofiltration mathematical modeling, Álvarez-Hornos et al. (2009) mentioned that a usual value for the effective biofilm thickness is about 50 – 100 µm.

In contrast, Pineda et al. (2000), indicated that no oxygen limitation existed in a biolayer of 133 µm for a toluene concentration in the range of 0.2 – 3.7 g m^{-3} (EBRT between 30 – 150 s) for a biofilter packed with vermiculite.

Dorado et al. (2008) confirmed that oxygen consumption was not a limiting process in the degradation of toluene for a reactor operated for 100 days at an EBRT of 60 s and an average inlet load of 1.3 g m^{-3}. These authors calculated that oxygen concentration in the biofilm was higher than 5.5 g m^{-3} under the maximum oxygen consumption rate.

Fungal biofilters partially solve the problem caused by an excessive thickness of the biofilm: while the biofilm thickness of the bacterial biofilters ranges from 30 to 390 µm (Pineda et al., 2000; Vergara-Fernandez et al., 2008), values between 2.1 – 2.6 µm have been reported for fungal biofilters (Arriaga and Revah, 2009; Vergara-Fernandez et al., 2008). The biofilm thickness of the fungal bioreactors is considered the average of different measurements of hyphae diameters.

1.2.1.2.5. EBRT, feed conditions and composition

EBRT is defined as the empty bed volume of the reactor divided by the volumetric flow rate of air through the system. This parameter is critical for microbial degradation performance as it is directly related to the reaction time allowed for the microbial degradation of pollutants.

Previous studies on the characteristic timescales of the various physical, chemical, and biological processes occurring in a biofilter have found that the diffusion rates of pollutants are slower than the microbial reaction rates (Jung et al., 2011). Therefore, EBRT should exceed the time required for diffusion processes, as is the case for low operating flow rates. If the flow rate is too high, the contact time between microorganisms and gas is too short and, consequently, the biodegradation reaction cannot be completed.

On the other hand, a longer EBRT for better removal efficiencies requires larger filter bed volumes. Thus, determination of an optimum EBRT as a function of the filter bed volumes is necessary for a better performance of a biofilter system.

As the gas flow rate is generally set by the operating conditions of the facility, EBRT is the main factor to be considered when sizing a bioreactor. It has a huge influence on the bioreactor investment and filter replacement costs (Prado et al., 2009b).

Furthermore, EBRT depends on the type of pollutant or mixture of pollutants to be treated, and on the concentration and removal efficiency required. Figure 1.6 shows the typical EBRT ranges that ensure high removal efficiencies for a series of compounds typically treated in conventional biofilters.

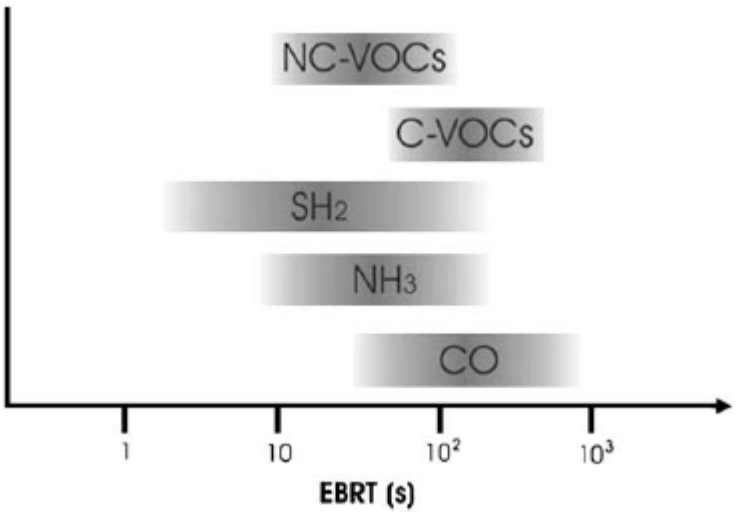

Figure 1.6. Common EBRT ranges for the treatment of some of the most typically degraded substrates in conventional biofilters. NC-VOCs: non-chlorinated volatile organic compounds. C-VOCs: Chlorinated volatile organic compounds (Prado et al., 2009b).

Regarding BTEX compounds, Jung et al. (2011) evaluated the effect of the EBRT on removal efficiency and elimination capacity at the same inlet load for toluene degradation in a biofilter packed with ceramic beads. These authors indicated that when the EBRT was lower than 20 s, the diffusion rate was lower than the reaction rate; on the other hand, when EBRT was over 20 s, the diffusion rate was higher than the reaction rate. Consequently, the highest elimination capacity values were obtained when EBRT was optimized at 20 s.

Gabaldón et al. (2006) studied the influence of EBRT on the removal efficiency for toluene and ethylbenzene degradation at a constant inlet load (50 g m^{-3} h^{-1} for the toluene experiment and 18 g m^{-3} h^{-1} for the ethylbenzene experiment). The results showed that almost complete ethylbenzene elimination was observed for an EBRT higher than 50 s, and the removal efficiency decreased almost linearly to around 60 % for residence times of 25 s. Similar results were obtained in the toluene experiment. A decrease in EBRT from 90 to 50 s caused a decrease in removal efficiency from 90 % to 80 %. However, for residence times shorter than 50 s, a sharp linear decrease was observed, achieving a removal efficiency of 50 % at 16 s.

These authors suggested that a minimum EBRT of about 60 s may be required to reach adequate removal efficiencies, and an EBRT of 90 s could be considered safe to ensure the absence of limitations associated with contact time.

The range of EBRT employed by different authors in recent years for the treatment of TEX compounds has been summarized in Table 1.5. As predicted by Gabaldon et al. (2006), when EBRTs between 60 and 90 s were set up, removal efficiencies higher than 80 % were achieved when inlet loads up to 150 g m^{-3} h^{-1} were assayed.

1.2.1.2.5.1. Residence Time Distribution (RTD) analysis

EBRT is a relative measure of the residence time of the gas stream in the biofilter and is used to compare system performance and loading conditions. However, calculation of the real residence time of the gas within the biofilter would require taking into consideration the porosity of the packing. Differences between both values could be appreciable.

As an example, Hort et al. (2009), using sewage sludge and yard waste compost as biofilter media, calculated a true residence time of the gas around 25 s, while EBRT was assumed to be around 60 s.

Residence Time Distribution (RTD) analysis is an interesting tool that provides information on fluid behaviour inside the column, such as true residence time, dead zones, fluid mixing intensity and dispersion. Salazar et al. (2012) observed that the dispersion increased at lower gas superficial velocities. Regarding the channelling, the dead zones reduce the useful volume of the biofilter and, therefore, the real residence time of the gas and operating efficiency tend to decrease.

RTD analyses are measured by introducing a non-reactive tracer into the system at the inlet. The concentration of the tracer is changed according to a known function and the response is found by measuring the concentration of the tracer at the outlet.

The selected tracer should not modify the physical characteristics of the fluid (equal density, equal viscosity) and the introduction of the tracer should not modify the hydrodynamic conditions. Additionally, the tracer should not become metabolized by the microorganisms involved. Tracers like hydrogen, butane, methane or helium have been employed (Morgan-Sagastume et al., 2003; Singh et al., 2006; Prenafeta-Boldú et al., 2008; Salazar et al., 2012).

Prenafeta-Boldú et al. (2008) defined RTD technique as a precise quantitative method for diagnostic the packing media condition. These authors proved that, in practical applications and under relatively short retention times, the air mixing regime in biofilters packed with synthetic materials can be acceptably assimilated to an ideal plug flow.

Correspondingly, Singh et al. (2006), using RTD analysis, confirmed a plug flow behaviour inside a biofilter packed with maize cobs.

These two examples provide evidence to confirm the hypothesis of most theoretical studies, which consider that biofilters are based on the idealized plug flow pattern.

Table 1.5. Influence of the EBRT in conventional biofilters treating toluene, *para*-xylene and ethylbenzene as single contaminants.

Pollutant	EBRT (s)	EC_{MAX} (g m^{-3} h^{-1})	Packing material	Reference
Toluene	94	≈ 40 (≈ 85 % RE)	Cow-dung compost	Dixit et al., 2012
Toluene	68	≈102 (> 80 % RE)	Polyurethane	Singh et al., 2010a
Toluene	18	73 (97 % RE)	Crab shell	Kwon and Yeom, 2009
Toluene	14	47 (95 % RE)	Cattle Bone Porcelite	Sakuma et al., 2009
Toluene	57	93 (80 % RE)	Peat	Álvarez-Hornos et al., 2008a
Toluene	118	97 (80 % RE)	Coir-pith	Bhaskaran and Hima, 2007
Xylene	25	≈ 43 (20 % RE)	Sieved compost + ceramic beads	Rene et al., 2010b
Xylene	59	58 (49 % RE)	Wood chip	Jorio et al., 2009
Xylene	42	67	Commercial press mud (sugar industry waste)	Saravanan and Rajamohan, 2009
Xylene	150	61 (93 % RE)	Peat + mineral additive with binding capacity	Elmrini et al., 2004
para-Xylene	68	236 (66 % RE)	Conditioned peat	Jorio et al., 2002
Ethylbenzene	60	45 (35 % RE)	Granular perlite	Hinojosa-Reyes et al., 2012
Ethylbenzene	90	84 (84 % RE)	Macadamia nutshells	Volckaert et al., 2011
Ethylbenzene	48	34 (62 % RE)	Composite rock wool – compost	Cho et al., 2009
Ethylbenzene	127	120 (> 95 % RE)	Fibrous peat	Álvarez-Hornos et al., 2008b
Ethylbenzene	60	26 (100 % RE)	Celite	Gunsch et al., 2007
Ethylbenzene	60	25 (> 90 % RE)	Harwood saw dust + WWTP sludge	Son and Striebig, 2003

1.2.1.2.6. Moisture conditions

The humidification of the biofilter bed is a critical control parameter for keeping the availability of water for microbial growth at an acceptable level. Water activity, which is defined as the amount of required water that is free in a given environment (Zamir et al., 2011a), is responsible for both the type (bacteria vs. fungi) and level of the activity of the microbial community present. This activity also determines the long-term structural stability of the packed bed (e.g. compaction or formation of preferential pathways).

Cercado et al. (2012) studied the loss of performance caused by drying of the bioactive support. These authors artificially dried biofilms from a microbial consortium adapted to toluene to obtain final water activities of 97 %, 84 %, 75 % and 32 %. They observed that biofilm activity presented a water activity critical point between 0.84 and 0.97. Accordingly, usual biofilm re-humectation strategies (i.e. contact with a 100 % relative humidity atmosphere or water spraying) were not effective to recover toluene consumption capacity in samples with a_w lower than 84 %.

A bed moisture content that is too high leads to a reduction in the specific surface available for gas/biofilm exchanges that is detrimental for the elimination of relatively insoluble substrates due to mass transfer limitation phenomena. Likewise, a packing deterioration caused by bed compaction takes place, favouring a sharper pressure drop and the creation of anaerobic zones.

By contrast, limited watering is beneficial for a more economical and simple operation, particularly in medium- and small-scale biofilters, which require minimal intervention and the generation of leachates. Low water content has also been related to the enhanced mass transfer of hydrophobic pollutants from the air to the biofilm, where they are metabolized. Even so, overly low water content has been related to the inhibition of biological activity, negatively affecting the biodegradation process and, ultimately, biofilter performance (Fu et al., 2011).

Sakuma et al. (2009) reported that the maintenance of an adequate moisture content in the packing material (a bioreactor packed with Cattle Bone Porcelite (CBP) treating toluene vapours) provided better environmental conditions for the microbial community, which involved a higher biomass density, a larger fraction of active biomass and, finally, an enhanced pollutant removal. The results showed that the rate of toluene elimination in the biofilter with an adapted irrigation system was 1.2 – 1.7 times greater than the rate of toluene elimination in the control biofilter.

Besides, biofilter operation under water-limited conditions has been reported to promote the development of fungal biomass in the biofiltration of BTEX monoaromatic hydrocarbons (Prado et al., 2002). These authors observed that filamentous fungi gradually became dominant in a downflow biofilter packed with perlite and used to treat toluene-polluted air and irrigated with a nutrient medium just once every fortnight.

Fungi have the advantage that their aerial mycelia form a larger surface area in the gas phase than bacterial biofilms, which is suggested to facilitate the uptake of hydrophobic volatile

compounds (Kraakman et al., 2011). In any case, undesired fungi-spore emissions could be also happened due to exceedingly reduced water content (Vergara-Fernández et al., 2012).

The control and estimation of the appropriate moisture content in the packing material is compulsory and complex, as a large number of factors need to be considered: the moisture content of the gas stream entering the biofilter, the frequency and flow of external irrigation, the exothermicity of pollutant mineralization, the organic nature of the packing material, the biomass distribution profile within the biofilter and the water retention capacity of each packing material (Kennes, 2001). In fact, inefficient control of the moisture content has been reported as the cause of 75 % of biofilter failures (Auria et al., 1998). Therefore, there are several published studies devoted to the understanding and optimization of moisture content distribution within biofilters: Morales et al. (2003) used a dynamic drying study to estimate the critical water content for a peat bioreactor treating toluene. They found that for moisture content below 58 % there was an insufficient flux of water to maintain an optimum elimination capacity. Shareefdeen et al. (2010) likewise observed that a peat biofilter performed efficiently when the water content was around 50 %.

As a rule, moisture content in the range between 30 % and 60 % wt/wt (wet based) has been recommended by Nikiema et al. (2007) for optimum biodegradation activity in organic filter media. However, this moisture content range has not been applied in all cases: Table 1.6 shows the moisture content of a variety of packing materials for treating toluene, *para*-xylene and ethylbenzene.

Consequently, water activity in biofiltration systems must be carefully studied on a case-by-case basis in order to ensure consistent VOC and odour treatment efficiencies, especially when using novel organic packing materials.

Table 1.6. Moisture content of packing material from biofilters treating toluene, *para*-xylene and ethylbenzene.

Compound	Packing material	Moisture (%)	Reference
Toluene	Cow-dung compost	60 – 70	(Dixit et al., 2012)
Toluene	Peat	60 – 80	(Álvarez-Hornos et al., 2008a)
Toluene	Peat	75 – 85	(Álvarez-Hornos et al., 2007)
Toluene	Coir-pith	60 – 80	(Bhaskaran et al., 2007)
Toluene	Compost	70 – 80	(Maestre et al., 2007)
Toluene	Compost + ceramic (1:1, v:v)	60	(Znad et al., 2007)
Toluene	Cattle bone porcelite	13 – 32	(Sakuma et al., 2006)
Toluene	Ceramic Rasching ring	19 – 47	(Aizpuru et al., 2005)
Toluene	Compost + Organic binder (90:10, v:v)	55 – 60	(Delhoménie et al., 2005)
Toluene	Ceramic pellets	45 – 60	(Song and Kinney, 2005)
Toluene	Compost + wood chips (80:20 ratio)	60 – 65	(Torkian et al., 2003)
Toluene	Vermiculite-GAC	60	(García-Peña et al., 2001)
Toluene	Sillicate pellets	30 – 40	(Woertz et al., 2001)
Toluene	Peat	65	(Auria et al., 2000)
Toluene	Peat + glass beads	50 – 60	(Zilli et al., 2000)
Toluene	Peat	55 – 80	(Bibeau et al., 1997)
Toluene	Chaff-compost	45	(Tang et al., 1995)

Table 1.6. (Continuation) Moisture content of packing material from biofilters treating toluene, *para*-xylene and ethylbenzene.

Compound	Packing material	Moisture (%)	Reference
19% (v/v) *meta*-xylene, 65% *para*-xylene, 16% *ortho*-xylene	Conditioned peat	50 – 70	(Jorio et al., 1998)
19% (v/v) *meta*-xylene, 65% *para*-xylene, 16% *ortho*-xylene	Conditioned peat	16 – 70	(Jorio et al., 2002)
para-Xylene	Food waste compost + oyster shell + polyurethane foam	40 – 60	(Hwang et al., 2007)
para-Xylene	Biosol (glass & cardboard)	45	(Jeong et al., 2006)
para-Xylene	Manure pig compost-forest soil – polyethylene	50	(Wu et al., 2006)
Xylene (mixture of isomers)	Sieved compost + Ceramic balls	55 – 60	(Rene et al., 2009a)
Xylene	Commercial press mud (sugar industry waste)	50	(Saravanan and Rajamohan, 2009)
Xylene	Compost-wood chip	60 – 65	(Torkian et al., 2003)
Ethylbenzene	Fibrous peat (95% organic content)	74 – 81	(Álvarez-Hornos et al., 2008b)
Ethylbenzene	Mixture of composting material (hardwood sawdust and municipal wastewater sludge	45 – 50	(Benitez et al., 1995)
Ethylbenzene	Composite rock wool-compost	40 – 60	(Cho et al., 2009)
Ethylbenzene	Soil amendment composed of high mineralized peat (35% organic content)	35 – 57	(Gabaldón et al., 2006)
Ethylbenzene	Fibrous peat (95% organic content)	80	(Son et al., 2001)

1.2.1.2.7. pH

This pH has an important influence on biofiltration efficiency. Above or below an optimum pH range, microbial activity is severely affected. The optimal pH of a filter bed corresponds to that of the specific microorganisms developing in it. The growth range for most bacteria is around neutrality, a pH between 6 and 8, whereas fungi can withstand a pH ranging from 2 to 7 (Mudliar et al., 2010).

The pH of a biofilter bed determines the type of microorganisms that will prevail in it. In those cases where multiple species with diverse pH requirements for growth are capable of degrading a contaminant, the pH level at which a biofilter is operated will impose a selective pressure on the microbial community. Microbial species with the highest growth rates under the conditions imposed will increase in relative abundance over time, and the relative abundance of other species will decrease. Hence, pH control at a constant level in the system is very important for bioreactor function.

A weak basic environment (pH = 7.0 – 8.0) has been reported as the most suitable pH range for BTEX biodegradation. Lu et al. (2002) observed that BTEX removal efficiency increased as the pH of the nutrient feed increased in the pH range of 5.0 – 8.0 for a trickle-bed air biofilter packed with coal, while the opposite trend was observed for a pH between 8.0 and 8.5.

Mathur and Balomajumder (2011) who isolated the bacterial strain *B. Sphaericus* from a biofilter effectively used to remove BTEX from polluted air streams, stated that *B. Sphaericus* cultivated in a pH range from 3 – 11. However, the percentage removal of BTEX in batch assays peaked at pH 7.0, specifically between the optimum pH of 6.0 and 8.0. These authors confirmed that the hindrance effect of super acidity and super alkalinity damaged the activity of intracellular enzyme of *B. Sphaericus*.

Lee et al. (2002) reported a pH of 7.0 to be optimal for BTEX degradation. Singh et al. (2010b) also observed a very favourable growth of the microbial strain *P. putida* (MTCC 102) when the pH variation of the leachate stabilized around 7.0 in a wood charcoal biofilter treating toluene.

Alternatively, several authors have achieved feasible TEX biodegradation at extremely low pH values. At a low pH, fungi are preferentially selected over bacteria as dominant degrading species. The relatively better growth of fungi at a low pH compared with the average bacterial species is a well-known fact. Van Groenestijn et al. (2001) found that toluene degrading perlite biofilters fed with a pH 4 nutrient solution recorded markedly higher contaminant removal rates than biofilters fed with a pH 8 solution. They also found that pH 2.5 – 4.0 biofilters were dominated by fungi, while pH 6.5 – 8.0 biofilters were dominated by bacteria.

In a similar way, Qi and Moe (2006) showed that fungal-dominated biofilters successfully treated a paint VOC mixture that included ketones (acetone and methyl ethyl ketone) and aromatic compounds (toluene, *para*-xylene and ethylbenzene) at a pH value of 3.0. Likewise, Liang et al. (2007) used a biologically activated carbon as a novel biofilter packing medium for the simultaneous treatment of hydrogen sulphide and toluene at a pH value in the range of 1.0 – 3.0.

However, low pH conditions contribute to organic medium degradation, which creates channels in some areas and compaction in others, reducing removal efficiency (Chitwood et al., 2009).

In order to control medium pH, buffer materials and alkaline chemicals may be added, such as calcium carbonate, dolomite, oyster or crab shells (Kwon and Yeom, 2009). The pH can also be controlled by bed irrigation with nutrient solutions that contain pH buffers; for example, a sodium carbonate solution (Rodrigues et al., 2010).

Jover et al. (2012) tested two methods for pH control; by increasing the phosphate buffer capacity of the mineral medium (method 1), and by adding solid $CaCO_3$ (method 2) to the packing material (sugarcane bagasse) at the upper inlet of the biofilter treating H_2S. As far as method 1 is concerned, pH increased gradually along the bed (from the bottom to the top), from a constant value of 3.0 to 7.0. Regarding method 2, pH was constant (2.4 ± 0.8) along the bed, although a sudden increase (7.1) was observed at the inlet section.

However, the spent minerals and acid-degraded organic packing materials often form small particles and contribute to biofilter clogging. In practical applications, it is difficult and expensive to control the pH, and the medium is often replaced when alkalinity is completely exhausted.

1.2.1.2.8. Temperature

Operating temperature is an important factor that influences biofilter performance. Temperature directly affects the growth and metabolic activity of microorganisms, playing an important role in the evaporation of water from the biofilter bed. For example, Zamir et al. (2011b) used sensitivity analysis to prove that temperature was a more effective factor than pollutant inlet load for influencing a compost biofilter performance treating n-hexane.

Microbial activity tends to increase with temperature to a given value, after which activity gradually tails off. The temperature of the biofilter is influenced mainly by the temperature of the inlet air stream and, to a limited extent, by the exothermic biodegradation reactions in the filter bed.

According to laboratory and pilot research assays, the range of optimum operating temperatures is between 10 and 30 °C (also called mesothermal range) (Nikiema et al., 2007). Several authors have reported that fluctuations in that temperature range do not have any apparent influence on odour removal and overall process performance (Clark et al., 2004; Lebrero et al., 2010).

However, many industrial waste gases are emitted at high temperatures, e.g. waste gases from the tobacco, pulp and paper and food industries. In these cases, additional cooling of the contaminated hot waste air stream effluent (50 – 60 °C) is required before biological treatment.

Nevertheless, cooling these gases to below 40 °C prior to biological treatment is not viable because the gas must be water saturated, thereby increasing capital and operating costs. This has hindered the use of biofiltration for high temperature industrial waste gases. The use of microorganisms adapted to high temperatures (thermophilic range) has been presented as an alternative to avoid gas effluent cooling.

In the mid–1990s, preliminary experiments were carried out in laboratory and pilot-scale biofilters working at temperatures between 50 and 70 °C. Hence, two full-scale thermophilic biofilters (60 °C) were satisfactorily installed at a cocoa factory (van Groenestijn and Kraakman, 2005). Furthermore, Cho et al. (2007) and Mohammad et al. (2007) confirmed that hot mixed gases of BTEX compounds could be degraded in thermophilic biofilters.

Thermophilic biofilters should be inoculated with thermophilic microorganisms. The metabolic rate of the thermophilic bacteria increases with temperature, doubling with each 10 °C increase to 65 – 70 °C (Moussavi et al., 2009). These biofilters can eliminate the same type of volatile compounds as non-thermophilic biofilters, albeit with a few exceptions (e.g. ammonia). Another advantage of thermophilic operation is a decrease in biomass yield and its accumulation in the biofilter bed, which normally causes bed clogging and pressure drop throughout the bed.

On the other hand, high temperature biofilters have several operational drawbacks that are normally not encountered at lower temperatures. The transfer of the more hydrophobic VOCs is negatively affected by the increase in temperature (lower VOC solubility). Additionally, high operating temperatures accelerate the degradation of the packing material if an organic filter bed is used, causing bed compaction, air short-circuiting and preferential paths (Mohammad et al., 2007). Offensive odours from compost biofilters operated at high temperatures have been also reported. These odours were very different from the usual pleasant earthy smell from ambient temperature biofilters (Cox et al., 2001).

1.2.1.2.9. Pressure drop and associated problems

Pressure drop across the filter bed is an important parameter because it can give an indication of the compaction state of the filter bed and excess biomass accumulation, which has been related to an increase in airflow resistance in the bed.

In full-scale biofiltration operation, an air-blower is required to pump the contaminated air stream through the biofilter. Therefore, pressure drop is the main factor that influences biofilter equipment and operating costs due to air-blower energy consumption. Prado et al. (2009b) expressed blower cost as a function of the gas flow rate required by means of the following equation:

$$\text{Air-blower cost (€)} = 0.095 * Q\ (m^3\ h^{-1}) + 1226.5 \quad (1)$$

Likewise, these authors calculated the power of the blowers as a function of the gas flow rate fed into the biofilter by means of the empirical relation:

$$P\ (kW) = 3.64 * 10^{-4}\ Q\ (m^3\ h^{-1}) \quad (2)$$

Estrada et al. (2011) estimated that biofilter operation at pressure drops of 200 Pa m^{-1} would result in energy consumptions of 2.5 MJ $(m^3 h^{-1})^{-1}$ $_{air\ treated}$ in a biofilter packed with a mixture of organic and inorganic material (bed height of 1 m and material density of 300-600 kg m^{-3}) and operating at EBRTs ranging from 50 to 70 s for an air emission of 3000 $m^3 h^{-1}$ (293 K, 1 bar, 40 % relative humidity).

Thus, it is clear that eventual increases in pressure drop across the bed directly affect the energy requirements of the air-blower and, accordingly, the operating costs.

Commonly reported pressure drop values in pilot-plant biofiltration systems are around 50 – 600 Pa m^{-1} (Vergara-Fernández et al., 2007; Ramirez-Lopez et al., 2010; Singh et al., 2010a). Le Cloirec et al. (2005) recommended using a pressure drop value ranging from 0.1 to 1 m H_2O.

The pressure drop is generally affected by the structure and void fraction of the packing material and the moisture content. It increases with the superficial velocity of the air flowing across the cross-sectional area of the medium. Additionally, the pressure rises as the packing medium ages or degrades.

Ramirez-López et al. (2010) thus defined packing material density as a key factor in pressure drop behaviour from a design point of view. They suggested that the biofilter medium should have high porosity, sufficient moisture-holding capacity and resistance to compaction for minimizing pressure drop.

Thus, Delhoménie et al. (2002) described the sizing of the bed pellet as a fundamental characteristic. If too small, this sizing provides for large specific surface areas, available for essential gas/biolayer exchanges, but it also creates some resistance to gas flow, while if it is too large, it favours gaseous flows but reduces the number of potential sites for microbial activity. Leson and Winer (1991) suggested a minimal pellet size of 4 mm in order to minimize pressure drop throughout the bed.

From an operational point of view, the gradual accumulation of biomass over long-term operating periods results in the uneven distribution and excess accumulation of biomass in filter beds, causing process problems, such as channelling of the gas stream, clogging and excessive head loss.

Morgan-Sagastume et al. (2000) reported that biomass accumulation was greater at the inlet sections of biofilters. These authors showed that the highest pressure drops were caused by layers of biomass with a high concentration of extracellular polymeric substances that retained a very high amount of moisture. Hence, throughout the experimental period, around 90 % of the pressure drop took place in the first half of the biofilter, where more biomass accumulated and where up to 95 % of the incoming methanol was removed.

Along these lines, Xi et al. (2006) suggested the use of a critical biomass concentration for maintaining stable biofilter performance. The critical biomass concentration differs depending on the properties of the packing materials. A critical biomass concentration of 20 – 24 kg $_{dry\ cell\ weight}$ m^{-3} was found for a biofilter packed with porous silicate pellets (Song and Kinney, 2002). A polyurethane biofilter recorded a relatively high critical biomass concentration of 30 – 31 kg $_{dry\ cell\ weight}$ m^{-3} due to its high porosity and larger surface area (Ryu et al., 2010).

The processes for excess biomass removal are derived from four main types: physical/mechanical, chemical, biological and operational. All the treatments applied should be as innocuous as possible and economically viable too.

Physical treatments consist of filter media stirring and mixing (Znad et al., 2007), backwashing of the bed medium with a high water flow rate (Kim and Sorial, 2007) and air sparging (Odín et al., 2011).

Morgan-Sagastume et al. (2003) proved that filter medium mixing was a good technique that assured a constant medium quality throughout operating time, favouring filter bed oxygenation and pressure drop control. Nevertheless, the investment and operational costs, as well as the engineering required to carry out medium mixing in full-scale biofilters, must be taken into account and evaluated prior to its application.

Odín et al. (2011) considered air sparging to be the most efficient physical treatment. However, it is also the most expensive one, requiring expensive electrical equipment (high-pressure air compressors, water recycling pumps, electrical dehumidification filters and water recycling systems).

Chemical treatments use chemical solutions or reagents (e.g. NaOH or NaOCl) that dissociate the chemical bonds between the biofilm and the supporting solid surfaces or directly degrade the biomass to a removable condition (Chen and Stewart, 2000; Odín et al., 2011). Carbon/nutrient supply control methods have also been widely used.

Thus, Delhoménie et al. (2003) created a nutrient deficiency, alternating solutions enriched with nitrogen and solutions without or poor in N. The resulting performance of this chemical method revealed that the overall microbial degrading activity was significantly reduced, but biofilm accumulation could not be avoided through such nutrient control.

Sakuma et al. (2006) halted the nutrient supply for 21 days after excessive pressure drop was observed in a biofilter treating toluene, operating in this period as a biofilter without any nutrient supply. This mode of operation significantly reduced the pressure drop but, in contrast, it resulted in lower pollutant removal because of nutrient starvation.

Odín et al. (2011) established that 1 or 2 weeks was the time period required by the biofilter to recover original/previous performance values after a NaOH treatment was applied.

The main downside of the biological treatments is also the worsening in biofilter performance. In this case, biological treatments use microorganisms to degrade the biomass accumulated throughout the bed.

Organisms such as predatory mites (Prado et al., 2002), fly larvae (Won et al., 2004) and a grazing fauna composed of rotifers, nematodes and tardigrades (Bhaskaran et al., 2008) have been reported to control the microbial biomass in biofilters and biotrickling filters.

Woertz et al. (2002) observed a pressure drop decline from 3.9 to 1.3 cm H_2O after five days of mite addition in a reactor packed with perlite. Correspondingly, van Groenestijn et al. (2001) studied the introduction of mite predation in fungal biofilters. After one hundred days of operation, the biofilter without mites had a gas pressure drop of 400 Pa, with a tendency to

increase. In the biofilter with mites, conversely, the pressure drop was only 130 Pa and decreased with time.

However, in many cases the use of these species was not applied on purpose. Prado et al. (2002) and Won et al. (2004) referred to the appearance of the predators as a natural invasion. Therefore, the drop in biofilter performance due to the loss of biomass, including the active contaminant degrading microorganisms, could not be properly regulated. Won et al. (2004) reported that an unwanted reduction from 455 to 113 kg m^{-3} of wet biomass content occurred in 2 – 4 days. Figure 1.7 shows an invasion of mites in a biofilter packed with scoria in a biofilter treating toluene (unpublished data from our research).

Figure 1.7. Scanning electron micrograph of a "dried" mite invading a biofilter packed with scoria in a biofilter treating toluene.

Finally, improved operating modes, such as switching the inlet position and split feeding gases, have been tested as pressure drop control systems.

Song and Kinney (2000) investigated the frequency of the directional switching mode. They reported that a one-day switching frequency could hinder biofilm development and bioreactor stability. On the other hand, with a seven-day switching frequency, a period of 48 h was required to fully restore biodegradation capacity in the inlet bioreactor section. Thus, a three-day switching frequency was recommended bearing in mind reacclimation requirements and biodegradation activity losses.

Woertz et al. (2001) used a directionally switching operation (alternating the inlet feed position every 3.5 days) to obtain a more even distribution of biomass across the entire length of a black yeast biofilter. Although the pressure drop across the bioreactor remained relatively low (< 200 Pa m^{-1}) until day 90 of operation, the clogging of the bioreactor could not be avoided due to a filamentous fungi invasion.

Lee et al. (2011) evaluated the performance of a polyurethane biofilter over 25 days of operation using two operating modes: unidirectional flow and flow directional switching under

transient loading conditions: on/off (8 h on/16 h off per day), and short-term shutdown (2 days off per week). The flow direction was switched every day and a gas mixture containing benzene, toluene and xylene was employed as a model gas. Results indicated that an even distribution of pollutant degraders was obtained in the directional switching filter, which contributed to a better removal performance. Moreover, the pressure drop in the unidirectional biofilter (118 – 147 Pa m^{-1}) was twice that in the directional switching biofilter (69 – 118 Pa m^{-1}).

1.2.1.2.10. Biofiltration of BTEX mixtures

As explained in a previous section, benzene, toluene, ethylbenzene, and xylene isomers (*ortho-*, *meta-* and *para*-xylene), collectively known as BTEX, are one of the major causes of environmental pollution because of widespread instances of leakage from petroleum and fuel storage tanks and spills at petroleum production wells, refineries, pipelines, distribution terminals and landfills.

The ability of bacteria or fungi for BTEX simultaneous degradation under aerobic and anaerobic conditions, or in extreme circumstances like growth limiting conditions, dry and/or acidic soil, etc. has been studied for several decades (Badali et al., 2011; Seyedmousavi et al., 2011). For instance, Berlendis et al. (2011) reported evidences of BTEX aerobic degradation by halophilic or halotolerant bacteria (pure cultures of *Marinobacter*) in diverse hypersaline effluents.

Besides, the metabolism of each BTEX compound by microorganisms has been well reported through physiological, biochemical, and molecular investigations of their degradation pathways (Smith 1990; Pieper et al. 2004). However, studies on the simultaneous biodegradation of mixtures of these hydrocarbons have become important because the individual compounds are rarely found alone, without the other ones.

The biodegradation of the different BTEX components in a mixture can be affected by the presence of others components. Many reports have singled out the antagonistic effects (inhibition) or beneficial ones (enhancement or co-metabolism) of mixed substrates.

Although substrate interactions between BTEX compounds in biofilters often vary with microbial culture and culture conditions, several conclusions can be drawn:

In many BTEX-treating biofiltration studies, the removal of xylene isomers was always less efficient in comparison with other gas streams pollutants. On the other hand, toluene has generally been reported as the most favorable or biodegradable component.

Song et al. (2012) studied the substrate interactions during the biotransformation of a mixture of quaternary substrates, benzene, toluene, para-xylene, and styrene using a single isolated *Pseudomonas* bacterial strain which was grown using gaseous toluene as a sole carbon and energy source. Styrene was biodegraded at the highest rate amongst the four compounds, followed by toluene, benzene and *para*-xylene. *para*-Xylene showed no biodegradation activity as a single compound, and just underwent cometabolic degradation by the *Pseudomonas* culture when present with other substrates.

Mathur and Balomajumder (2011), who isolated a new bacteria strain called *B. sphaericus* from a bioreactor operating for six months for the biodegradation of BTEX, showed that the biodegradability sequence was as follows: toluene in first place, followed by benzene, ethylbenzene and finally, xylene.

Chen et al. (2010) reported that under different variable operating conditions (EBRTs of 90, 60, 45 and 30 s and total loading rates ranging between 8.30 and 154.80 g m^{-3} h^{-1}) the removal efficiencies for benzene and toluene were greater than those for *ortho*-xylene.

Cho et al. (2009) assessed the capability of previously isolated *Sphingomonas sp*. D3K1 to degrade mixed BTEX substrates (benzene, toluene, ethylbenzene and *ortho*-xylene) by biofilters with rock wool-compost media as packing material. These authors proved that *Sphingomonas sp*. D3K1 toluene removal was generally the highest, followed by benzene, ethylbenzene, and then by *ortho*-xylene.

Gabaldón et al. (2006) reported that ethylbenzene and toluene were degraded more easily than *ortho*-xylene using a commercial peat as filter-bed material. These authors verified the absence of inhibitory effects due to the presence of multiple substrates.

Ortiz et al. (2003) proved that a microbial culture accustomed to the consumption of toluene and then acclimated to BTX vapours adapted faster to toluene, then to benzene and finally to xylene isomers. After acclimation, toluene, benzene, *para*- and *meta*-xylene attained were fully degraded, while the rate of *ortho*-xylene elimination was 85 %.

Ortho-xylene is considered to be biologically the least degradable xylene isomer due to its high toxicity (Maliyekkal et al., 2004). Regarding the removal of xylenes in a mixture with 19 % *metha*-xylene, 65 % *para*-xylene, and 16 % *ortho*-xylene, these authors found that *ortho*-xylene was the most resistant to biodegradation. The removal efficiency of *ortho*-xylene was lower than 50 % at a maximum inlet loading rate of 4.5 g m^{-3} h^{-1} with EBRT of 102 s.

These results are in agreement with those presented by García-Peña et al. (2008). These authors, who inoculated the filamentous fungus *Paecilomyces variotii* in vermiculite biofilters, classified the degradation activity of the fungal biofilter in the following order: toluene > ethylbenzene > benzene > *metha*- and *para*-xylene > *ortho*-xylene. Accordingly, Jorio et al. (1998) stated that the order of reactivity for xylene isomers was as follows: *metha*-xylene > *para*-xylene > *ortho*-xylene.

Nevertheless, the results regarding the recalcitrant performance of xylene isomers in mixtures vary and are often contradictory. Mohammad et al. (2007) reported that *para*-xylene degradation was low in comparison with the other two isomers' degradation when they were fed together to a mesophilic (20 °C) and a thermophilic (50 °C) biofilter.

Nikolova and Nenov (2005), who studied the potential of *Cladophialophora sp*. and *Cladosporium sp*. for BTEX degradation, showed that neither of these fungal strains was able to degrade *para*-xylene completely in any case. On the contrary, *ortho*- and *metha*-xylene were fully assimilated as single substrates or in mixtures with toluene.

Likewise, the decrease in benzene degradation performance when fed together with the other compounds is generally accepted.

Song et al. (2012) who studied biodegradation kinetics of binary mixtures of BTXS (benzene, toluene, *para*-xylene and styrene) by a *Pseudomonas* indicated that the degradation of benzene was slightly inhibited in the presence of *para*-xylene, and substantially inhibited in the presence of toluene.

Lee and Cho (2009) investigated how interactions between the components of BTEX affected each other's degradation by *Rhodococcus sp*. EH831. These authors examined the degradation of pure compounds, and the results were compared with those obtained in binary, ternary, and quaternary mixtures.

In binary mixtures, benzene was inhibited by all the other components, and its degradation was initiated after the degradation of toluene was completed when both were present. Based on the time for complete degradation, the inhibition of benzene in binary mixtures gradually increased in presence of toluene < xylene << ethylbenzene.

García-Peña et al. (2008) also stated that benzene degradation was also negatively affected by both toluene and ethylbenzene.

Lee et al. (2002) isolated the strain *Stenotrophomonas maltophilia* T3-c from a biofilter for the removal of benzene, toluene, ethylbenzene and xylene. These authors proved that the presence of ethylbenzene in binary mixtures inhibited benzene degradation. The presence of more than three kinds of substrates also inhibited the specific degradation rate of benzene.

du Plessis et al. (2001) studied the BTEX catabolism interactions in a toluene-acclimatized biofilter. These authors found that toluene competitively inhibited the catabolism of all the other compounds, including benzene.

Moreover, the degradation of benzene was especially compromised by the high loading of benzene (Cho et al., 2009; Mohammad et al., 2007). This could be explained by its high toxicity, since the threshold limit value for benzene is much lower than for other benzene-compounds.

1.2.1.2.11. Biofilters modelling

Due to the complexity of the several steps involved in pollutant elimination, biofilters have often been considered mysterious "black boxes" where pollutants vanish because of the action of capricious microbes. When observations of different parameters corresponding to biofilter operation are available, any modelling effort represents a strategy to describe, with a certain degree of accuracy, the underlying rules that govern that process.

There are basically two ways to model a process, namely, a deductive manner using laws of nature (also called mechanistic modelling) or a statistical manner using data acquisition (also called black-box modelling).

Traditionally, the performance of biofilters has been predicted using process based models derived from laws of nature (mechanistic modelling). The main advantage of these process models is that they are based on the underlying physical process and the results obtained generally provide a good understanding of the system.

Nevertheless, the determination of several parameters that decisively influence the substrate biodegradation process is not an easy task, and such data are frequently taken from the literature, assumed or determined by batch tests (Elías et al., 2006). The main uncertainties/challenges are focused on:

- Biofilm mass distribution. The assessment of biofilm mass distribution is complicated by several factors, such as non-uniformity of biofilm coverage over media and the presence of inactive biofilm in contaminant transfer and degradation.
- Determination of biodegradation kinetic parameters. The estimation of kinetic parameters for the biological reactions taking place in the biofilter is not straightforward. Experimental data from batch reactors was used to easily obtain microbial kinetic information in biofiltration. However, it is well known that the biofilm formation of continuous reactions is often different from that of batch reactions.
- Use of air/biofilm distribution coefficients instead of the usual air/water distribution coefficients.

The successful development and implementation of these mechanistic models relies largely on the availability of good process information and on the accurate determination of these parameters. Arriaga and Revah (2009) stated that the majority of the published models include parameters that were not measured independently by experimentation, but were obtained by fitting experimental data, masking their real influence, as they are generally lumped together in the equations. These authors evaluated the importance of some of the most significant parameters including kinetic constant, partition coefficient in the biofilm, biofilm thickness, superficial area, and effective diffusivity.

1.2.1.2.11.1. Black-box modelling

On the other hand, a model of a process can also be obtained in a statistical manner by means of data acquisition. This second approach is especially recommended when, as in the case of biofilters, certain constants needed to develop a mechanistic modelling approach cannot be easily measured. Hence, the approach of considering the bioreactor as a system with a set of inputs and outputs (data-driven approach) is an alternative tool for modelling purposes. Regardless of the many mechanisms involved and the existence of immeasurable variables, equations relating inputs and outputs (on-line handy information) will be fitted if sufficient observations are available.

Experience has proved that the development of empirical models using numerical approximations can be fruitfully applied to biofiltration cases with a remarkably predictable performance: Elías et al. (2006) used a multilayer perceptron (MLP 2–2–1) model with two input variables (unit flow and concentration of the contaminant fed into the biofilter) to estimate the removal efficiency of a biofilter treating hydrogen sulphide (H_2S). These authors represented a response surface that provided an illustrative and easy tool to predict what the H_2S removal efficiency in the bioreactor would be 24 hours after changes in the unit flow and/or contaminant concentrations were carried out (Figure 1.8).

Rene et al. (2009b) proposed an Artificial Neural Network (ANN) model to predict the performance of an immobilized-cell biofilter treating ammonia vapours in terms of removal efficiency and elimination capacity, where inlet concentration, loading rate, flow rate and pressure drop were input parameters. Chairez et al. (2009) designed a continuous neural network to calculate toluene vapours elimination capacity in a fungal biofilter using carbon dioxide production and pressure drop as input information. Zamir et al. (2011b) were the first authors to apply an ANN to simulate the effect of operating temperature and intermittent inlet loading rate on the behaviour of a compost biofilter for hexane removal from polluted air.

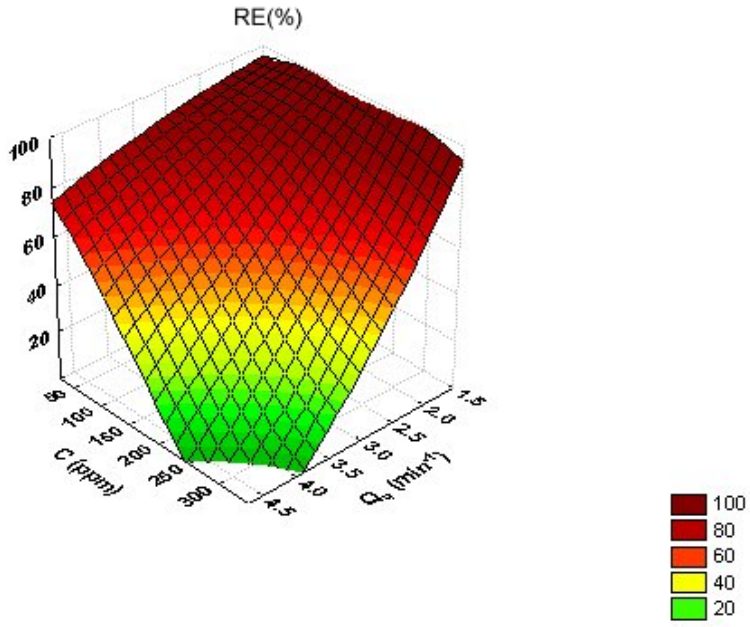

Figure 1.8. A response surface of the multilayer perceptron 2-2-1 model relating unit flow, H_2S concentration and removal efficiency (Elías et al., 2006).

1.2.1.2.11.2. Mechanistic modelling

No single model has become a generally accepted standard; each research group has developed its own approach, often specific to the experiments being performed. The first attempt was made by Ottengraf and van den Oever (1983). The strength of Ottengraf's model was the availability of an analytical solution. This model simply dealt with a conventional biofilter at stationary state.

It was based mainly on mass balance for all the phases involved, and removal efficiency could be determined depending on the phenomena (reaction or diffusion) ruling the process. Two different equations were proposed; one for the reaction limitation area and the other for the diffusion limitation area. The transition between the two conditions was ruled by the Thiele number.

One pollutant only was considered, and the biodegradation kinetics of the single pollutant by the microorganisms on the biofilm was described by a Monod-type expression. Oxygen was always in excess and did not affect the kinetics. Biofilm thickness was negligible in relation to the diameter of the carrier particles and its value was constant across the biofilter.

Over time, new elements of complexity have been introduced, so their solution can be obtained by numerical methods alone:

- Shareefdeen and Baltzis (1994) proposed a new model taking into consideration also transient conditions and adsorption effects on removal efficiency. Oxygen diffusion throughout the biofilm and its availability for the biomass were also included. The mass balance was solved using dimensionless constants, assuming only a quasi-steady-state condition for the biofilm phase. This model solution can be obtained only by numerical calculation. They used a Haldane-type dependence to describe the effect of self-inhibition due to the high concentration of methanol and Monod-type dependence on oxygen consumption.

- Deshusses et al. (1995) developed a model under dynamic conditions to describe the transient performance of compost biofilters treating methylethyl-ketone and methyl-isobutyl-ketone as single pollutants or as pollutants in a mixture. These authors also considered diffusion inside the biofilm and described three different phases: gas, biofilm and solid. Biological kinetics was the Monod-type without oxygen limitations and cross-inhibition between substrates, and gas-liquid equilibrium was ruled by Henry's law. These models assumed that biofilm thickness and biomass density remained constant with time and was uniform for the whole biofilter. The adsorption of pollutants in the solid was included.

  Park and Jung (2006) evaluated the applicability of the Deshusses model when substrate inhibition phenomenon in biofilters was caused by toxic gases with high concentrations. These authors observed that the use of the Deshusses model to predict toluene vapour concentration at the biofilter outlet resulted in a tendency to underestimate high inlet concentrations (> 2.0 g m^{-3}) and overestimate low inlet concentrations. This tendency appeared to be due to the model's assumption of zero-order reaction prior to substrate inhibition.

- Alonso et al. (1998) proposed a waste gas treatment dynamic model to simulate the changes in biofilter performance with time due to the variation in the amount of biomass, including a dependence of the biofilm's specific surface area on the biofilm growth. The model considered two-phase system, quasi-steady-state process, uniform bacterial population, one limiting substrate, and negligible biomass deactivation. The model accounted for the importance of the changing biofilm surface area and thickness, which are particularly important when biodegradation is controlled by diffusion in the biofilm.

- Ranasinghe et al. (2002) included moisture content and energy balance in their model. Water and biofilm were considered one homogeneous phase, and its equilibrium with the gas phase was ruled by Henry's law. The substrate utilization rate followed Monod's equation with the introduction of two new factors, which are respectively affected by

temperature and moisture level. Energy balance was realized considering the gas phase as an ideal gas and the biofilter as an adiabatic reactor.

- Iliuta and Larachi (2004) considered the formation of an excessive amount of biomass that can lead to the clogging of the biofilter. These authors developed an unidirectional dynamic flow model based on the volume-average mass, momentum and species balance equations coupled with conventional diffusion/reaction equations describing apparent kinetics in the biofilm. Biomass was assumed to grow by filling the void fraction between the packing solids. The impact of the formation of an excessive amount of biomass was evaluated in terms of pressure drop increase as a function of time, as well as in terms of clogging patterns. Pollutant depletion due to adsorption in the biofilm and solid particles was ignored. The Monod rate expression was used to describe culture growth kinetics.
- Vergara-Fernández et al. (2008) argued that the biofilm could not be considered a pseudo-homogeneous phase, specifically in the case of the aerial growth generated by filamentous fungi within the biofilter. Their main objective with this model was to describe accurately the growth of filamentous fungi for the degradation of hydrophobic VOCs. Thus, the model showed the increase in the transport area by the growth of the filamentous cylindrical mycelia and its relation with n-hexane elimination in a quasi-stationary state in a biofilter. These authors assumed an isothermal system with no oxygen limitation and negligible n-hexane adsorption in the support. Additionally, n-hexane biodegradation and nitrogen utilization was done on the fungus surface, and the time of the transport phenomena in the gaseous phase is considerably shorter than the growth time.
- Álvarez-Hornos et al. (2009) developed a dynamic model based on gas transfer, diffusion and biodegradation for predicting the performance of peat biofilters treating pure ethyl acetate, toluene and a mixture of both, with the following assumptions: Haldane-type kinetic expression that included oxygen limitation, the inhibition effect by high concentrations of substrate and the cross-inhibition between substrates. A general axial gradient equation of the biomass density distribution was also considered.
- Fazaelipoor (2012) presented the first model in biofiltration that incorporated heat effects and humidity in modelling in a deterministic way. The model used the principles of energy and mass transfer in the biofilter rather than empirical relations. The effect of temperature variations on all temperature-dependent variables was also incorporated in the model. Thus, it was possible to estimate moisture requirements in biofilters in addition to the estimation of elimination capacities. It was also possible to estimate the rate of carbon dioxide production based on the biomass yield per unit mass of carbon substrate.

1.2.1.2.12. Biofiltration economics

Given manufacturers' reticence to provide economic data on their processes to academic researchers, this issue has traditionally been poorly discussed in the literature. In 1996,

Scotford et al. (1996) suggested that biofilters were still an extensive option in Europe based on information provided by Pearson et al. (1992). Nevertheless, 20 – 30 years of intense lab and field research and development have marketed biofiltration as a current low-cost air pollution treatment method.

Indeed, from a process economics viewpoint, the advantage of any biological treatment over physicochemical techniques relies on savings obtained in operating costs (Gabriel and Deshusses, 2004). On the other hand, technologies with the lowest operating costs usually incur the highest investments costs (Estrada et al., 2011).

Consequently, Estrada et al. (2011) described the net present value (NPV) as the most appropriate economic criterion for technology evaluation and selection rather than the initial investment cost.

Economic data reported by other authors and presented below have been converted to Euros (€), at an exchange rate of 1 € = 1.30 US$.

Biofiltration unit cost itemization

Schmidt et al. (2004) pointed out that both capital/investment and operation/treatment costs are highly variable for a biofiltration unit. Jorio and Heitz (1999) summarized these main influential factors.

1.2.1.2.12.1. Capital/Investment costs

Main factor influencing capital costs are summarized in Table 1.7.

Table 1.7. Main factors influencing both capital/investment costs (Jorio and Heitz, 1999)

- ✓ Biofilter type (open or closed), nature of packing material, available land and initial site preparation costs.
- ✓ Requirements for pre-treatment of the inlet gas flow (removal of particulates, humidification, heating/cooling, etc.)
- ✓ Location of the pollution source.
- ✓ Accessories (pumps, piping, electric, etc.), construction materials, miscellaneous costs, etc.
- ✓ Degree of desired automatic monitoring and sophistication of control equipment.
- ✓ Airflow, nature and concentration of the target pollutants in the effluent and rate of expected removal efficiency.
- ✓ Preliminary costs associated to lab-scale plant experimentation and engineering design of the reactor.

The installation of a closed biofilter (6.9 – 16.2 € for $m^3 h^{-1}$ of treated gas) is more expensive compared with an open-bed biofilter (1.2 – 5.4 € for m^3h^{-1} of treated gas) or just covered with a plastic or metallic cap (1.5 – 8.5 € for m^3h^{-1} of treated gas) (Jorio and Heitz, 1999).

Schmidt et al. (2004) estimated that the installation costs for new-build biofilters on mechanically ventilated livestock facilities would be between 0.07 and 0.12 € for m^3h^{-1} $_{treated\ gas}$.

The investment costs per unit of flow rate treated decrease exponentially with increasing design flow rates. Estrada et al. (2011) calculated capital costs ranging from 5 to 28 € $(m^3/h)^{-1}$ for design airflow rates between 100000 and 5000 $m^3 h^{-1}$.

Kan and Deshusses (2006) developed a rule of thumb for full-scale biofilters designed with an EBRT of 30 s and packed with permanent synthetic media:

- 23 – 28 € per 1 m^3h^{-1} of airflow rates lower than 25000 $m^3 h^{-1}$.
- 12 – 16 € per 1 m^3h^{-1} of airflow rates higher than 25000 $m^3 h^{-1}$.

Given the need to maintain low pressure drops across the packing bed and because of the high EBRT needed for efficient odour treatment, biofiltration has high land requirements. Estrada et al. (2011) projected land requirements of $(1.7 \pm 0.3{*}10^{-2}$ m^2 $(m^3/h)^{-1}$ of air treated for an airflow emission of 3000 $m^3 h^{-1}$ with a composition based upon the characterization of odour pollution from a WWTP located at Stuttgart University. Chou and Li (2010) estimated a required bed area of 140 m^2 (with a typical packed bed height of 1 m), for treating a gas flow rate of 12000 $Nm^3 h^{-1}$ with an average methyl ethyl ketone concentration of 700 mg Nm^{-3}. Prado et al. (2009b), assuming a gas flow rate of 20000 $m^3 h^{-1}$ and an EBRT of 60 s, obtained a total biofilter volume of 400 m^3 (including a 20 % safety factor).

As a rule, Thalasso and Pineda Olmedo (2002) estimated land requirements of between 3 and 20 m^2 per 1000 $m^3 h^{-1}$ of air treated. Thus, when the land available is limited or the price of land is relatively high, an additional cost should be expected. Reversely, due to construction simplicity and the low average density of the biofilters (200 – 500 kg per m^2 of surface area), underused or difficult access zones (e.g. ceilings) could be employed for their installation.

The costs related to the type of filter media and its purchase have already been studied in the chapter “Packing material”. In general terms, Thalasso and Pineda Olmedo (2002) emphasized that the purchase cost of the packing material does not have an excessive influence over the total expense of the biofiltration unit: on average, each m^3 of packing material will deal with around $(1.5 – 8){*}10^6$ m^3 of air treated before being replaced. If a filter media price of 77 € m^{-3} is supposed, packing material contribution to the total cost will range between 0.8 and 5.4 cents of € per 1000 m^3 of air treated.

A large reactor implies a large capital investment, while a small but very effective reactor will be less costly but expensive to operate because of frequent clogging. The specific building costs will decrease by increasing the size of the reactor.

Contrary to most other expenses, monitoring and control costs are fairly independent of reactor size. Hence, their proportion to the total reactor costs will also decrease when increasing the size of the unit.

Nevertheless, Deshusses and Webster (2000) reported that capital charges were a significant part (25 – 45 %) of the total expenses in an 8.7 m^3 full-scale biotrickling filter, showing the importance of a careful design in order to minimize capital expenditures. These authors suggested that over-dimensioning bioreactors (inefficient use of the entire reactor volume) to

allow for starvation or nutrient limitation in order to control biomass growth might be a very expensive solution.

Estrada et al. (2011) estimated the installation costs as 25 % of the final investment costs. Prado et al. (2009b) considered that piping costs, electrical costs, equipment installation costs and engineering design costs represent 10 %, 4 %, 4 % and 12 %, respectively, of the total investment costs. Miscellaneous costs, which include instrumentation, insurances, taxes, spare parts, etc., have been considered by these authors to represent a 5 % of the total investment costs.

Deshusses and Webster (2000) showed that about one-half of the total bioreactor construction costs were for personnel and engineering time. From a commercial point of view, these authors suggested that constructing a bioreactor filter with modular units was probably more cost-effective than a bespoke design. This would drastically reduce the engineering time, simplify the construction phase, and provide more flexibility on the customer's side. The dimensioning would then be reduced to the determination of the type and number of modules required for a particular application. This practice is already commonly used by several biofilter vendors.

To summarise, Prado et al. (2009b) observed that the design and construction costs of an average-sized biofilter may cost around 50000 €. These authors estimated, for example, that total investment costs would be around 56500 € for an average-sized compost-based conventional biofilter with an airflow of 20000 $m^3 h^{-1}$, a biofilter volume of 400 m^3 and an EBRT of 60 s. These results were consistent with those reported by Devinny (1998), who estimated that total investment costs would be between 65000 and 90000 € for an open-bed biofilter treating 17000 $m^3 h^{-1}$ at an EBRT of 70 s.

Kan and Deshusses (2006) estimated at 277000 € the capital costs of a biofilter designed for the treatment of a toluene polluted airflow (1 g m^{-3}) with an EBRT of 83 s, a bed volume of 231 m^3 and an expected removal efficiency of 92 %.

Chou and Li (2010) calculated at 154000 € the cost of constructing a fern-chip packed biofilter for the treatment of a gas flow rate of 12000 $Nm^3 h^{-1}$ (evaluated at 0 °C and 1.0 atm), with an average methyl ethyl ketone concentration of 218 ppm_v and an expected removal efficiency of 98 %. This biofiltration process for the target gas included passing the gas through a water-spray chamber to humidify and cool the gas from 60 °C to 35 °C before it entered the fern-chip bed.

In the local Spanish market, the company "Sistemas y Tecnologías Ambientales, S.A." (STA), which specialises in providing custom-designed process equipment and systems for air pollution treatment, reported a comprehensive example detailing the total costs of a biofilter (Table 1.8) (Nadal, 2008). This author reported total investment costs of around of 2 million Euros. Although no in-depth information about the set-up is given in the reference, the high degree of automation and the presence of a novel, commercial packing material developed by STA that guarantees a lifespan of eight years with a negligible pressure drop may be responsible for this apparently higher cost.

Table 1.8. Detailed breakdown of a full-scale biofilter designed by STA company (Nadal, 2008)

	Unit	STA-Biofilter
Treated airflow	Nm^3h^{-1}	300000
NH_3 concentration	ppm_v	40
H_2S concentration	ppm_v	40
Effluent Odor concentration	uoE Nm^{-3}	30000
Final average Odor concentration	uoE Nm^{-3}	< 1000
Packing material lifespan	years	8
Packing material replacement time	days	30
Maximal removal efficiency guarantee	%	99
CONSUMPTIONS		
Biofilter irrigation water	m^3d^{-1}	30
Prehumidification chamber water	m^3d^{-1}	15
Lixiviate production	m^3d^{-1}	21
Power requirement	kWh	101
Investment cost	€	1986200
Operation cost without filter replacement	$year^{-1}$	97380
Total costs for a service life of 15 years (investment + operation + filter replacement)	€	4656200

1.2.1.2.12.2. Operation/Treatment costs

Estrada et al. (2011) presented biofiltration treatment costs between 0.21 and 0.27 € per 1000 m^3 of air treated. The average operation costs obtained in their study agreed with those published by Prado et al. (2009b), who reported a cost of 0.20 € per 1000 m^3 of air treated, including annualized bed-replacing costs.

Similar values were reported by Boswell (2002), who estimated that operating costs for small biofiltration units (< 850 $m^3 h^{-1}$) ranged from about 77 € to several hundred Euros per month.

The main factors influencing treatment costs are summarized in Table 1.9. As can be observed, operating costs can be calculated as the sum of annual electricity and water consumption costs, labour costs and costs associated with controlling the growth of biomass. All other operating costs can be considered negligible compared to these. Among them, operating and

maintenance costs depend critically on costs for air blowers and packing material replacement (Schmidt et al. (2004)).

Table 1.9. Main factors influencing operation/treatment costs (Jorio and Heitz, 1999)

✓ Energy consumption: air ventilation, pumping of water for the irrigation of the bed, energy consumption for the pretreatment of the waste gas flow.
✓ Nature of the packing material and its lifespan.
✓ Periodic inspections and regular measurements to test the state of the filter bed: temperature, content water, nutrients and pH.
✓ Costs associated to the adjustment and maintenance of the operation conditions.
✓ Quantity and cost of the treatment of the drainage water.
✓ Costs associated to the maintenance of the various equipment of the biofiltration unit.

1.2.1.2.12.2.1. Packing material replacement

Due to packing material compaction and nutrient depletion, packing materials need to be replaced after some time. Prado et al. (2009b) reported that packing material replacement cost is the most important expense in biofilters with a volume higher than 800 m^3. These authors estimated at 16600 € per action the packing material replacement costs for an average-sized compost-based conventional biofilter with an airflow of 20000 $m^3 h^{-1}$, a biofilter volume of 400 m^3 and EBRT of 60 s.

In all the possible scenarios studied, “old” packing material disposal represented around one-third of annualized medium replacement costs, while new medium addition accounted for the remaining two-thirds.

Kan and Deshusses (2006) estimated at 3700 € $year^{-1}$ the average cost for packing replacement based on packing costs of 46 € m^{-3}. Devinny et al. (1999) calculated that 14230 € per action were necessary to carry out the total medium replacement cost, assuming an airflow of 17000 $m^3 h^{-1}$, a biofilter volume of 400 m^3 and an EBRT of 70 s, with a contaminant removal efficiency of 95 %.

Additionally, the final disposal of the residual packing material could pose a new environmental risk due to its impact on soil. However, in most cases, when the biofilters are correctly operated, the waste packing materials from biofilters can be considered non-hazardous biological waste (the packing can be mixed with other disposal materials and/or the pH neutralized).

1.2.1.2.12.2.2. Energy consumption of the air blower

Energy consumption in a biofilter is a substantial part of operating costs. Electrical equipment, such as water pumps, analytical equipment, and computer equipment, will all require some electricity; however, the majority of electrical costs arise from the air blower. Gabriel and Deshusses (2004) estimated that, on average, 99 % of energy costs in a full-scale biotrickling plant were due to electrical power requirements for the air blower.

The majority of pressure drop is generated in the filter bed itself. A large portion of the electrical demand is a function of the porosity, moisture content, and structure of the filter bed. As the material degrades over time, the demand for electricity increases. An increase in pressure drop will lead to a substantial increase in electricity consumption.

Prado et al. (2009b) proved that electricity consumption is the main influencing factor in biofilters with a gas flow rate above 50000 $m^3 h^{-1}$.

Kan and Deshusses (2006) assumed that an air blower with about 20 hp (horsepower) was required to force 10000 $m^3 h^{-1}$ air through the filter bed. This meant an electricity consumption of 18550 € $year^{-1}$. (The electricity cost has been calculated assuming a kilowatt-hour cost of 0.142 €/kWh, which was the electricity price in Spain in 2011 for industrial use when no-time restriction tax was applied). Devinny et al. (1999) considered a 10 hp blower in their study. This meant an electricity consumption of 9275 € $year^{-1}$.

Chou and Li (2010) calculated the electrical energy required for the blower fan in order to force the waste gas flow through a humidification spray chamber and the biofiltration bed, assuming a fan efficiency of 65 % and a total pressure drop of 100 mm H_2O. They obtained a final electricity demand of 8.4 hp, which supposed a cost of 7790 € $year^{-1}$.

Deshusses and Webster (2000) recommended the use of a variable speed blower: as pressure drop increases over time due to biomass build-up, the blower speed can be adjusted so that the airflow rate remains constant while energy consumption is minimized.

1. UP-TO-DATE TECHNOLOGICAL DEVELOPMENT

1.3. Background of the selected packing material

The biofiltration research group of the Chemical and Environmental Engineering Department in the University of the Basque Country (UPV/EHU) started its activity in biofiltration field in 1995. The first challenge to start up a fix bed bioreactor was to select an adequate support material for treating H_2S. Originally, four organic agricultural materials (soil with algae, pig manure with sawdust, horse manure and sludge) were selected due to their environmental and economic advantages: apart from being cost-effective, their use in the industrial application of biofilter system might solve the problem of their disposal or recycling.

These types of packing materials ensured high moisture retention capacity, a sufficient nutrient content to support microbial growth and the presence of native microbes that could be activated to biodegrade H_2S. The last requirement avoided the inconvenience of external inoculation with microbial cultures previously enriched with the targeted pollutants or with specific microbial species recognized for their ability to degrade the contaminants present in the air stream.

The preliminary results showed that the pelletized material obtained by mixing pig manure and sawdust was the most efficient filter medium for degrading H_2S within a concentration range from 0.03 to 0.32 g m^{-3} at a constant gas flow rate of 0.78 m^3 h^{-1}.

This support material was supplied by the Spanish company SLIR (Specialized Engineering in Recycling Agricultural Residues) and its commercial name was ABONLIR™. The material was obtained by mixing pig manure and sawdust, and the pellets were manufactured by mechanical compression without the addition of any chemicals. Arias, R. (2005) summarized the main characteristics of the packing material. The pelletized compost was stored in sealed plastic bags at room temperature to keep the material in its original moist condition.

In order to ensure the minimum size of these cylindrical pellets, the compost was sieved so that only pellets whose diameter was between 6.3 and 8.0 mm were used as packing material (Figure 1.9).

Figure 1.9. ABONLIR™ commercial packing material. The pelletized material is obtained by mixing pig manure and sawdust.

Pellet size and the specific surface area of the filter bed are the key physical properties that influence the VOC mass transfer rate and removal rate (Jin-Ying et al., 2005). In fact, Delhoménie et al. (2002) found those two parameters to be the major factors limiting the VOC removal capacity of compost-based biofilters. These authors reported that EC increased as particle size decreased, or as specific surface area was increased. They showed that toluene elimination rate increased from 45 to 180 g m^{-3} h^{-1} when the specific area of the compost increased from 120 (pellet size 20 mm) to 590 m^2 m^{-3} (pellet size 5 mm).

Accordingly, Leson and Winer (1991) suggested a threshold pellet size of 4 mm in order to minimize pressure drop throughout the bed. Besides, a cylindrical shape was chosen because it was defined as the best geometrical form for biofiltration carrier materials (Shinabe et al., 2000).

More exhaustive studies carried out with this packing material have confirmed its suitability for treating airflows contaminated with H_2S. Elías et al. (2002) corroborate that the packing material supplies the biomass and nutrients required for microorganisms to grow properly and efficiently degrade H_2S for 2500 h. During continuous operation, the contaminant mass loading rate was increased from 10 to 45 g m^{-3} h^{-1} with a superficial gas velocity of 200 m h^{-1}, obtaining removal efficiencies close to 100 %.

During continuous operating time, the packing material turned out to be chemically inert (the leaching solution did not significantly contain contaminants or metal contents) and mechanically resistant (no particle disgregation occurred).

In several studies pH value did not vary significantly (remaining between 8.4 and 6.8) even though sulphur by-products accumulated within the bioreactor, which indicated a good natural buffer capacity of the filter bed (Elías et al., 2000; Barona et al., 2004). Consequently, no additional buffer material was needed for proper pH control.

Pressure drop measurements between 15 and 460 Pa m^{-1} were recorded, which are considered to be usual values in biofiltration pilot plants. Nevertheless, the pore distribution of the fresh packing material before and after use in the biofilter changed drastically, with 93 % of the pores being smaller than 7.5 µm at the end of the experiment (72 % in the original packing material sample).

The good performance of the biofilter might be based on the fact that no nutritive solution or external water irrigation was used for feeding microorganisms over the course of the experimental run. The compacting of the filter bed due to overwatering after extended periods of usage has given rise to high pressure drops (Maestre et al., 2007). Excessive moisture content in the medium can lead to the agglutination and coagulation of particles that form the natural carriers, sharply increasing the pressure drop to values of around 1000 Pa m^{-1} (Morgan-Sagastume et al., 2000).

The fungal biomass developed under such moisture-restricted conditions did not clog the system either (Elías et al., 2000; Gallastegui et al., 2011). Although there are several authors who stated that filamentous fungi may cause higher and faster head losses compared to bacterial systems, this does not happen in all cases. For instance, Jin et al. (2006a) did not

detect any significant pressure drop in a fungal biofilter treating α-pinene, even after 6 months of operation.

Prior to testing the selected filter medium as a potential support material for the treatment of airflows contaminated with TEX, additional specifications such as adsorption capacity in wet and dry conditions were calculated (Barona et al., 2007).

The adsorptive properties of the filtering material provide information about the behaviour of the material when the bed dries and reversible adsorption of the contaminant takes place on the exposed surface of the packing material. In this study, the organic waste material rendered a toluene retention capacity ranging from 168 to 1231 μg $g^{-1}_{material}$ and from 65.2 to 739 μg $g^{-1}_{material}$ in dry and wet conditions, respectively. The results obtained confirmed the poor adsorptivity of typical organic packing materials for hydrophobic compounds such as toluene (Table 1.10). For instance, ground tyre rubber, a low-cost waste-derived inorganic material classically used as bulking media, has an adsorption capacity 18 – 131 times higher than the material used here.

Table 1.10. Toluene retention capacity of different packing materials

Packing material	Dry/Wet	Adsorption capacity (μg $_{toluene}$ g^{-1}_{filter})	Measure/Analysis conditions	Reference
Pig manure + sawdust	Wet	65 – 739	C = 1.1 – 13.5 g m^{-3} Q = 0.1 m^3 h^{-1}	(Barona et al., 2007)
Pig manure + sawdust	Dry	168 – 1231	C = 1.1 – 13.5 g m^{-3} Q = 0.1 m^3 h^{-1}	(Barona et al., 2007)
Coir pith	Wet	330	C = 0.946 g m^{-3} Q = 0.048 m^{-3} h^{-1}	(Bhaskaran et al., 2007)
Compost	Wet	32	C = 1.61 g m^{-3} Q = 0.18 m^3 h^{-1}	(Maestre et al., 2007)
Peat	Dry	263 (BTX mixture)	C = 3.4 g m^{-3} Q = 0.3 m^3 h^{-1}	(Ortiz et al., 2003)
Peat	Dry	4300	Batch mode	(Álvarez-Hornos et al., 2011)
Ground tyre rubber	Dry	22100	Batch mode	(Álvarez-Hornos et al., 2011)

Finally, the behaviour of our packing material has been validated by other authors. Thus, Gracy et al. (2006) have tested its suitability for the removal of acetone, trichloroethylene and toluene. No trichloroethylene (TCE) was biodegraded, acetone was completely removed (13 g $m^{-3}h^{-1}$) and only 85 % of the toluene was eliminated, and the critical mass load was estimated to be 17 g $m^{-3}h^{-1}$. These authors have highlighted the stability of the packing material throughout the entire experimentation (100 days), which has been attributed to the high organic matter content.

1. UP-TO-DATE TECHNOLOGICAL DEVELOPMENT

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2. ACCLIMATION METHODOLOGY

Preliminary acclimation strategies for successful start-up in conventional biofilters (*J. Air & Waste Manage. Assoc. 60, 959 – 967*)

2.1. Introduction

Amongst biotechnologies, biofiltration is a seemingly simple system with minimal energy requirements and low waste production. The ability of biofilters to treat a wide variety of gaseous pollutants (alcohols, phenol, aldehydes, ketones, aliphatics, petroleum fuel vapours, simple aromatic organic compounds, sulphur-containing compounds and ammonia, among others) has been widely proven (Delhoménie and Heitz, 2005; Chen et al., 2009; Sattler et al., 2009). Thus, this technology has been used in pilot-plant studies and in full-scale applications at different facilities, such as wastewater treatment plants, paint shops, pulp and paper industry, dairy farming, soil decontamination or indoor air treatment systems (Kennes, 2001; Iranpour et al., 2005; Civillini, 2006; Barona et al., 2007; Guieysse et al., 2008). There is now no doubt about the viability and reliability of this biological air treatment process that is particularly effective for high volumes of low-concentration pollutants (Datta and Allen, 2005; Revah and Morgan-Sagastume, 2005). Prado et al. (2009) developed a protocol to assess the main costs implied in conventional biofilters based on an empty bed residence time (EBRT) between 5 and 120 s, gas flow rate values between 5000 and 100000 m^3h^{-1} and bioreactor volume between 8.3 and 4000 m^3.

A considerable research effort has been made for this method to become a “low-risk choice” for many buyers or “end users”. Subsequently, many studies focus on the optimization of several relevant factors in biofilters, such as packing material, moisture content, temperature, oxygen content, pH, nutrients, pressure drop, medium depth, control and maintenance, and microbial community (Elías et al., 2002; Gaudin et al., 2008; Chou et al., 2008; Ortiz et al., 2008; Goncalves and Govind, 2009; Jin et al., 2009).

Other studies seek to evaluate biofilter performance through modelling. Thus, different mathematical and statistical models have been proposed in the literature (Devinny and Ramesh, 2005; Elías et al., 2006; Dorado et al., 2007; De Visscher and Li, 2008).

Nevertheless, microbial growth with degrading activity is a wholly undetermined key parameter that governs biofilter “success”. The support material itself can supply the biomass responsible for degradation. Pure strains of microorganisms can be purchased from specialist companies, and biomass from other active biofilters can be extracted or mixed cultures can be obtained from the sludge from wastewater treatment plants or similar sources (Barona et al., 2004; Prado et al., 2005; Borin et al., 2006; Gabaldón et al., 2006, Avalos Ramirez et al., 2009).

Depending on the type of medium being selected and the type of contaminant being treated, the biofilter may need to be inoculated with some sort of microbial suspension. Wastewater treatment sludge is commonly assumed to be a constant and virtually universal inoculant for many biofilters because of the variety of microorganisms present and their collective ability to degrade many different types of contaminants (Devinny et al., 1999; Steel et al., 2005).

The preliminary acclimation of the biomass to the pollutant is a suitable strategy for shortening the start-up period of biofilters and for ensuring longer successful operation (Prado et al., 2005; Kwon and Yeom, 2009). Acclimation is a period required for the development of the optimum population of substrate-consuming microorganisms before they start vigorous biodegradation (Jones et al., 2004). Likewise, Jiang et al. (2009) showed that substrate acclimation was a crucial step when a biofiltration process is applied to the co-treatment of several pollutants. Steel et al. (2005) observed a substantial decrease in microbial diversity at the end of successful acclimation to ethanol in batch experiments. Likewise, Borin et al. (2006) reported that microbial succession occurred in a biofilter using organic compost as packing material and as a biomass source. These authors observed that a natural selection for strains with degrading potential (a decrease in microbial diversity) occurred when the microflora were exposed to low concentrations of benzene. However, a sharp increase in species diversity and quantity surprisingly occurred when the selected microflora were subjected to a high organic load. Consequently, microbial evolution was different for high and low concentrations of pollutant.

However, to the best of the authors' knowledge, no intensive research has been conducted on the biomass acclimation procedure prior to biofilter inoculation or start-up operation. Many studies briefly describe this step and use a nutrient solution and/or an easily degradable carbon source (e.g. glucose or saccharose) and the target pollutant to acclimate/activate the biomass for a variable period of time (Singh et al., 2006; Gaudin et al., 2008; Saravanan and Rajamohan, 2009). Analytical or biological molecular techniques for biomass growth detection and identification are also used because they are essential for understanding the interactions between biomass community and successful biofiltration (Hwang et al., 2003; Steele et al., 2005; Borin et al., 2006; Ortiz, et al., 2008).

Before the inoculation itself, the proper selection, storage, acclimation and activation of the biomass is crucial to ensure the long-lasting and effective operation of new bioreactors. Contrary to the inoculum obtained from other active biofilters, when an acclimation procedure is to be applied to a new pollutant, the question is raised of where and how to obtain the best inoculum. Thus, the aim of this study is to ascertain the relevance of certain simple parameters and steps in obtaining an active inoculum to set up toluene treating biofilters. The answer to several general questions was sought. After sludge sampling, should the whole sample, the supernatant or the semisolid concentrated phase (settled sludge) be used for ulterior acclimation? Is the preliminary addition of an easily degradable carbon source necessary? Is continuous or discontinuous acclimation recommended? Toluene was used in all experiments and was selected as representative of simple aromatic volatile organic compounds. Toluene has been frequently used in literature to study microbial adaptation to chemically similar compounds such as benzene, ethyl benzene, and xylenes. Yeom et al. (1997) found that toluene adaptation increased the final degradation of phenol. Heipieper and de Bont (1994) found that *Pseudomonas putida* S12 was more tolerant to ethanol when pre-adapted to toluene, which was explained by changes in the level of the fatty acid composition of cellular membranes. Thus, toluene was selected as representative of a certain group of compounds for studying preliminary acclimation strategies.

2.2. Experimental work

2.2.1. Characterization of the original sludge samples

Two sludge samples containing original biomass were collected in the aerobic tank of a sewage treatment plant (Muskiz, Bizkaia, Spain). The samples were labelled M1 and M2 because they were collected within two consecutive months in the aerobic tank at the treatment plant.

A preliminary characterization of the two samples was carried out including the following parameters (Standard Methods for the Examination of Water and Wastewater, 2005): sedimentable solid content (SS) using Imhoff cones, pH at 25 $^{\circ}$C measured in a Crison pH-meter, volatile solid content (VS) and total solid content (TS) by drying the samples at 550 $^{\circ}$C and 105 $^{\circ}$C, respectively, in a Leco TGA 500 thermobalance. Additionally, the density of volatile solid content per litre of sludge (ρ_{VS}) was also determined.

To ascertain the original live/dead biomass in the samples, 1 ml of each sludge was sonicated (0.50 W) for 30 seconds to disrupt cell aggregates. Subsequently, 10 µl of sample were fixed to a multi-well microscope slide (Anaspec) after diluting as necessary. DAPI (4', 6'-diamidino-2-phenylindole) stain was used to determine total cell amount. Live/dead cell counts were carried out using LIVE/DEAD BacLight Bacterial viability kits (Molecular Probes, Leiden, The Netherlands) that use mixtures of SYTO® 9 (green) and propidium iodide (red) fluorescent nucleic acid stains. Twenty microscopy fields were counted to determine standard deviation. Samples were examined with a Zeiss Axiovert 200 epifluorescence microscope (Oberkochen, Germany). Cell density was calculated by dividing cell total amount by the previously determined volatile solid content. All the chemicals were of high purity (> 99.5 %, Panreac).

2.2.2. The supernatant, the semisolid phase or the whole sample?

With the sludge sample in the laboratory and before proceeding with activation/acclimation, a decision was taken on the phase to be selected (the supernatant phase after 2 hours, semisolid concentrated phase after 2 hours or the whole sample).

The 250 ml vessels used in these experiments were capped with a Mininert valve (Alltech Associates) sealed with a synthetic rubber septum (Figure 2.1a). The contaminant was added to the vessels by perforating the rubber septum with a syringe (Hamilton).

Before setting up the assays, it was necessary to assess how many times the synthetic rubber septum of the valve could be perforated to add the contaminant before septum replacement (in order to render the system hermetic). Thus, two compounds were selected for these prior leakage containment experiments: toluene, as the target contaminant in this study (vapour pressure 2.9 kPa at 20 $^{\circ}$C) and, acetone, as a highly volatile compound useful for leakage tests (vapour pressure 24 kPa at 20 $^{\circ}$C). The preliminary experiments were performed by adding 1 µl of toluene or acetone to the bottles with a syringe. All the bottles were previously filled with 10 glass balls for gas homogeneity. Each bottle septum was perforated 8, 10, 12, 14, 18 and 20 times with the syringe and possible leakage was determined by weighing every 12 hours.

During these experiments, the Mininert valve was in the open position and the bottles were placed on an orbital shaker (Lab-line Instruments) at 150 rpm. A mass loss greater than 2 % was considered an unacceptable leakage.

To select the phase for further acclimation, three vessels containing 105 ml of the whole sample and 15 ml of nutrient solution were used. The nutrient solution was prepared by adding 0.2 g KH_2PO_4, 0.8 g K_2HPO_4, 0.05 g $MgSO_4 * 7\ H_2O$, 0.02 g $CaSO_{4*}\ 2\ H_2O$, 0.02 g $FeSO_{4*}\ 7\ H_2O$, and 1 g $(NH_4)2SO_4$ to 1 l of water. An amount of 5 ml of another micronutrient solution (containing 2 $gl^{-1}\ FeCl_{2*}\ 4\ H_2O$, 2 g $l^{-1}\ CoCl_{2*}\ 6\ H_2O$, 0.5 g $l^{-1}\ MnCl_{2*}\ 4\ H_2O$, 60 mg $l^{-1}\ CuCl_2$, 50 mg $l^{-1}\ ZnCl_2$, 50 g $l^{-1}\ H_3BO_3$, 2 g $l^{-1}\ NaHCO_3$, 90 mg $l^{-1}\ (NH_4)_6Mo_7O_{24*}\ 4\ H_2O$, 1 g l^{-1} EDTA, 0.1 g $l^{-1}\ Na_2SeO_3$, 0.1 g $l^{-1}\ NiCl_{2*}\ 6\ H_2O$) was added to 1 l of the aforementioned solution to prepare the final salt nutrient solution.

An amount of 2 µl of liquid toluene was injected to yield an initial gas phase concentration in each vessel of 13 mg toluene l^{-1}. The contaminant was injected on a daily basis for 7 days. The same procedure was repeated by using 105 ml of the supernatant (in triplicate) and by using 105 ml of the settled sludge (in triplicate) instead of the 105 ml of the whole sample. The vessels were exposed to the atmosphere every day for 20 min to ensure oxygen supply and avoid carbon dioxide accumulation. After oxygenation, the bottles were capped and a new toluene dose was injected. Control assays were also carried out, albeit with distilled water instead of sludge.

After one week, the samples were transferred to 600 ml glass bottles in order to determine the toluene degradation rate. The degradation rate was the slope of the graph obtained when measuring the remaining concentration of toluene along time after consecutive additions of 2, 4 and 8 µl of toluene. The analytical equipment was a gas chromatograph (Micro-GC CP 4900) equipped with an auto-sampling injector, a 5CB column and a TCD detector. The temperatures of the column and the injector were 80 °C and 110 °C, respectively. Prior calibration was carried out with a calibration cylinder provided by Air Liquide and containing a toluene concentration of 50 ppmv in nitrogen atmosphere. The degradation rate of the three phases was measured immediately after collection and after 7 days of acclimation.

The VS/TS ratio and the cell density (calculated by dividing cell total amount by VS) of the three phases were also measured for comparison purposes.

2.2.3. Prior carbon addition

Twenty-five millilitres of nutrient solution were mixed with 25 ml of the whole M2 sample and transferred to a 250-ml glass vessel capped with Mininert valves (in triplicate).

The experiments were carried out by previously adding glucose to the sample before toluene addition. A dose of 0.03 g of glucose was added to the sample and the consumption of glucose was measured after 24 h using Keto-Diabur Test 5000 reactive strips. A new addition was repeated only when the substrate was fully consumed, and the procedure was repeated for 3 consecutive days.

Subsequently, two sets of new experiments were carried out; one of them used the glucose-added sample and the other set used the original sample without glucose addition. These tests were carried out by injecting 1 µl of toluene (4.3 mg toluene l^{-1} in the headspace) per day into the glass bottles, and 30 min later the toluene content in the upper chamber was measured by gas chromatography at 25 °C, as explained before. The bottles containing the cultures were continuously shaken in an orbital shaker (at 150 rpm) and the toluene content was measured again 24 h after the addition. If the contaminant was completely depleted, the bottle was opened to the atmosphere for 20 min to ensure oxygen supply and avoid carbon dioxide accumulation. After oxygenation, the bottles were capped and a new toluene dose of 4.3 mg l^{-1} in the headspace was added. If the toluene was not completely depleted, the hydrocarbon content was measured again after another 24 h.

2.2.4. Selection between continuous and discontinuous acclimation

Although microcosms or batch experiments are commonly used to grow specific strains or to estimate the degradation rate and the qualitative evolution of the biological activity of the biofilter (Acuña et al., 1999; Ortiz et al., 2008), the preliminary acclimation of the sample to the contaminants can be carried out in discontinuous (batch) mode or continuous mode.

The discontinuous acclimation mode was carried out in 250-ml glass bottles provided with a Mininert valve sealed with a synthetic rubber septum (Figure 2.1a). The assays were conducted at 25 °C by injecting toluene into the glass bottles capped with Mininert valves and containing 105:15 (vol:vol) of the whole sample and nutrient solution. Two assay series were prepared in triplicate. In the first series (three bottles), toluene was added for 7 days on a daily basis of 13 mg toluene l^{-1} in the headspace, and in the second series toluene was added for 1 month (30 days) at the same daily dose. The bottles, containing 12 glass balls for homogenous liquid mixing, were continuously shaken in an orbital shaker (at 150 rpm) during experimentation and exposed to the atmosphere every day for 20 min to ensure oxygen supply and avoid carbon dioxide accumulation. After oxygenation, the bottles were capped and a new toluene dose was injected. Control assays were also carried out with the sludge being replaced by distilled water.

After discontinuous acclimation for 7 and 30 days, the toluene degradation rate was determined as explained previously.

Biomass acclimation in continuous mode was carried out in a 3-l vessel or reactor (in triplicate) with one inlet tube and one outlet tube on the upper part (Figure 2.1b). A 5:1 (vol:vol) ratio between the whole sample and the nutrient solution was used. The inlet tube was submersed in the liquid and used to feed a contaminated airflow (1 l min^{-1}) with a toluene concentration of 100 ppm_v or 0.38 g toluene m^{-3} of incoming air. As far as the rationale for selecting the inlet toluene concentration for continuous additions is concerned, Bordel et al. (2007) found that an inlet mass loading higher than 16 g m^{-3} had an inhibitory effect on the *Pseudomonas putida* F1 pure strain. As opposed to the unspecific biomass used in the study presented here, these authors selected a pure strain that is a well-known toluene degrader. Consequently, and

bearing in mind that the biomass in this study was obtained from a wastewater sludge, a lower inlet concentration of 100 ppm_v (or 0.38 g toluene m^{-3}) was initially selected.

The airflow was previously bubbled into water for saturation before mixing with toluene. The systems were additionally shaken with a Teflon shaker at 150 rpm. The toluene degradation rate was determined after 7, 30, and 90 days and 1 year of acclimation, as explained for the discontinuous mode (i.e. by the consecutive addition of 3.6, 7.2, and 14.4 mg l^{-1}, respectively, for each acclimation period). Nevertheless, this continuous acclimation mode required the weekly extraction of 105 ml of medium for each determination. This volume was replaced by adding 105 ml of nutrient solution so that the final volume in the acclimation system was maintained at 1500 ml.

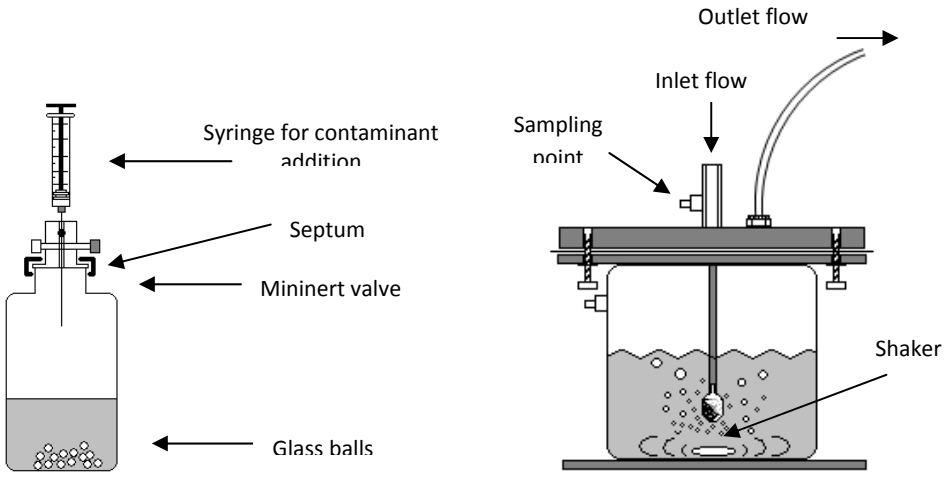

Figure 2.1. Diagram of the experimental system for (a) discontinuous (left) and (b) continuous acclimation (right).

This system was maintained for 1 year at 25 °C because it provided information about the system's evolution (degradation rate) with minimal maintenance. This information is very useful for industrial bioreactors because, according to Kraakman (2003), installing a cultivation system for the continuous growth of microorganisms is an effective way of reducing risk in industrial bioreactors. Thus, the re-inoculation of any full-scale bioreactor becomes possible whenever the removal efficiency (RE) decreases considerably.

2.2.5. Start-up of biofilters

A biofiltration system was assembled under downflow mode (Figure 2.2). Sampling and measurement ports were located along the two polyvinyl chloride modules of the bioreactor. The packing material selected was made up of composted pig manure and sawdust and has already been characterized in previous works (Elías et al., 2002; Barona et al., 2007). An amount of 400 g of the packing material was mixed (wetted) in a tray with 100 ml of the M2

sample, which was previously acclimated for 1 month in continuous mode, as explained before. This operation was repeated with a total amount of 1200 g of support material before filling the biofilter.

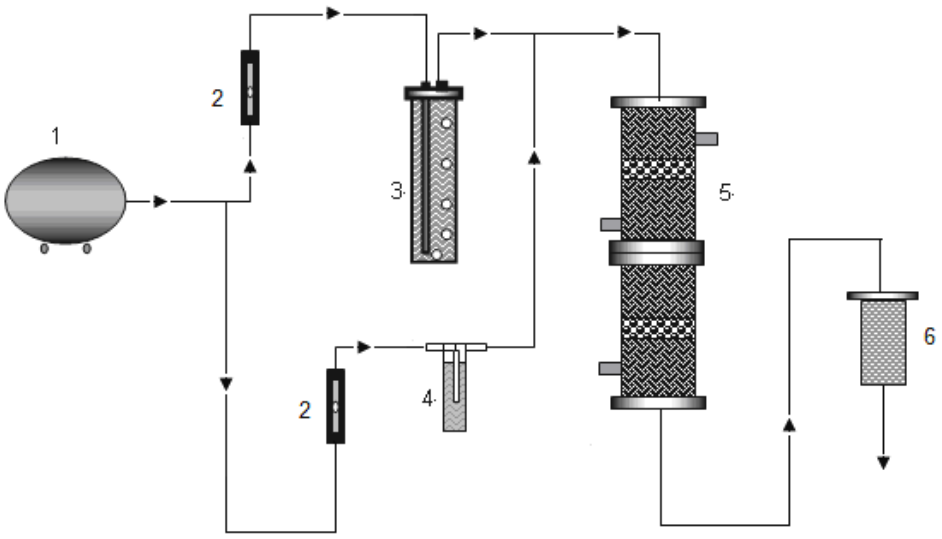

Figure 2.2. Diagram of the biofiltration systems: 1. Air-compressor 2. Flow meter 3. Humidification chamber 4. Contaminant (toluene) vessel 5. Biofilter with two modules 6. Active carbon chamber.

The start-up procedure was carried out in duplicate (called biofilter 1 and biofilter 2), although the operating conditions were not exactly the same because of technical difficulties in accurately controlling the inlet concentration. The contaminated gas flow for one of the biofilters (biofilter 1) was 1.3 l min^{-1} (residence time of 138 s), and for the other biofilter (biofilter 2) it was 1.1 l min^{-1} (residence time of 163 s). The inlet toluene load ranged from 29 to 400 ppm_v for biofilter 1 and from 22 to 370 ppm_v for biofilter 2.

2.2.6. Results selection based on statistical tools

The strategy of applying statistical tools in this study was based on the inherent practical difficulties of collecting representative and homogenous samples of active biomass growing in heterogeneous systems. Thus, discrimination was made between similar or rejectable samples.

In each assay for determining the degradation rate, the slope was calculated by using 10 – 15 data points (the depletion of toluene was monitored for 40 – 60 min in all cases).

Because of the low number of cases (replicates) used, a straightforward comparison of the slopes (degradation rate) obtained might be highly misleading. This applies to the intercomparison between slopes corresponding to the triplicates and to the comparison of slopes between different samples.

If the slopes corresponding to different assays are to be compared, the straightforward use of the values calculated can be helpful for a preliminary assessment. However, if the values of these slopes obtained for two assays are similar, 95 % confidence levels should be estimated to test the null hypothesis that they are not actually different. Ninety-five percent confidence levels of the differences can be calculated using the bootstrap resampling technique. Thus, the null hypothesis of equality of slopes can be properly assessed.

The bootstrap resampling technique samples a dataset with replacement (i.e. a single case may be randomly sampled several times in the bootstrap set). The bootstrap can be applied any number of times to increase accuracy.

The bootstrap operates by constructing artificial data batches using random sampling with replacement from the original data. Finally, the 95 % confidence intervals for the slopes and their differences were defined as the range between the 97.5^{th} and 2.5^{th} percentiles in the intervals obtained.

If the 95 % confidence interval corresponding to the difference between two slopes includes the value 0, the null hypothesis whereby both slopes are the same cannot be rejected at a 95 % confidence level, regardless of what the straightforward comparison of slopes may suggest.

The main features of bootstrap resampling have been widely explained in the literature (Efron, 1979; Emery and Thomson, 2001; Wilks, 2006).

2.3. Results and discussion

2.3.1. About using the supernatant or the whole sample as inoculum

The results in Table 2.1 show that both M1 and M2 samples were similar as far as VS/TS and pH are concerned. Although SS and ρ_{VS} for M1 were higher than for M2, the total cell content was slightly higher for M2. Other samples collected 5 and 10 months later rendered similar results (data not shown), which supported the preliminary conclusion that samples from the wastewater treatment plant were basically similar along time (upon regular operation of the wastewater treatment plant).

Table 2.1. Preliminary characterization of the original sludge samples.

Sample	SS (ml/l)	ρ_{VS} (g_{VS}/L)	pH	VS/TS	Live/dead cell content (%)	Total cell content ($* 10^{11}$ cfu/g_{VS})
M1	333	2.67	7.2	0.77	74.64/25.36	4.2 ± 1.3
M2	267	1.82	7.2	0.69	79.01/20.99	5.2 ± 1.4

Concerning the selection of the most suitable phase (supernatant phase, settled sludge or the whole sample) for ulterior activation/acclimation of biomass, only one sample (M2) was used for this experimentation. The evolution of the VS/TS ratio and the density of VS for the three cases were determined immediately after reception in the laboratory and after 7 days of daily acclimation to toluene (Table 2.2). The results of ρ_{VS} were as expected, ranging from 5.84 g VS l^{-1} for the settled sludge to 0.31 g VS l^{-1} for the supernatant. The VS/TS ratio after 7 days of toluene feeding increased only slightly in the supernatant, whereas it remained constant for the other two cases. Surprisingly, the live/dead cell ratio for the three cases at the beginning of the experiments showed no difference; thus, this value was close to the 79/21 ratio of the whole sample (data not shown).

Table 2.2. Parameter evolution after 0 and 7 days of acclimation for the sample M2.

Sample	Whole sample		Supernatant		Semisolid phase	
Time (d)	0	7	0	7	0	7
VS/ST	0.69	0.70	0.39	0.45	0.75	0.72
ρ_{VS} (g_{VS}/L)	1.82	1.41	0.31	0.38	5.84	4.63

On the basis of these results, no categorical decision about the best sample among the three phases could be reached and, consequently, further research was required.

Before proceeding with the results discussion, the slope value of one replicate (of each case) was compared with the other two values (of the triplicate) by using the bootstrap resampling technique. Thus, this statistical tool showed which replicates were basically similar and which of them should be rejected for average calculation. As an example, Table 2.3 shows that the three replicates of the supernatant were equal at a 95 % confidence level, which can be explained by the homogeneity of this phase at collecting replicates. Nevertheless, the difficulty in collecting representative samples of the settled sludge and whole sample was clearly identified by the fact that one replicate was to be rejected in both cases (WS-1 and SS-3, respectively), although a straightforward comparison of the slopes (degradation rates) did not reveal any differences between them. This procedure was applied to all data in this study and, accordingly, certain replicates were rejected for subsequent calculations.

Table 2.3. Results of the bootstrap resampling technique for detecting samples with the same behaviour in the three replicates of the whole sample (WS), the supernatant sample (SUP), and the settled sludge (SS) (at a 95 % confidence level).

	WS – 1	WS – 2		SUP – 1	SUP – 2		SS – 1	SS – 2
WS – 1		<>	SUP – 1		=	SS – 1		=
WS – 3	<>	=	SUP – 3	=	=	SS – 3	<>	<>

Notes: = equal; <> rejected.

The average degradation rates recorded for the settled sludge and the three toluene additions (3.6, 7.2, and 14.4 mg toluene l^{-1}) were dramatically lower than those for the other two cases

(Figure 2.3). On the basis of these results, and in addition to the handling difficulty, this sample was rejected for subsequent study. As far as toluene consumption rate in these preliminary tests is concerned, there is no advantage in separating the supernatant from the whole sludge before activation/acclimation (or even before direct inoculation) because the degradation rate achieved for the supernatant is approximately 1.8 times lower than the rate obtained for the whole sample.

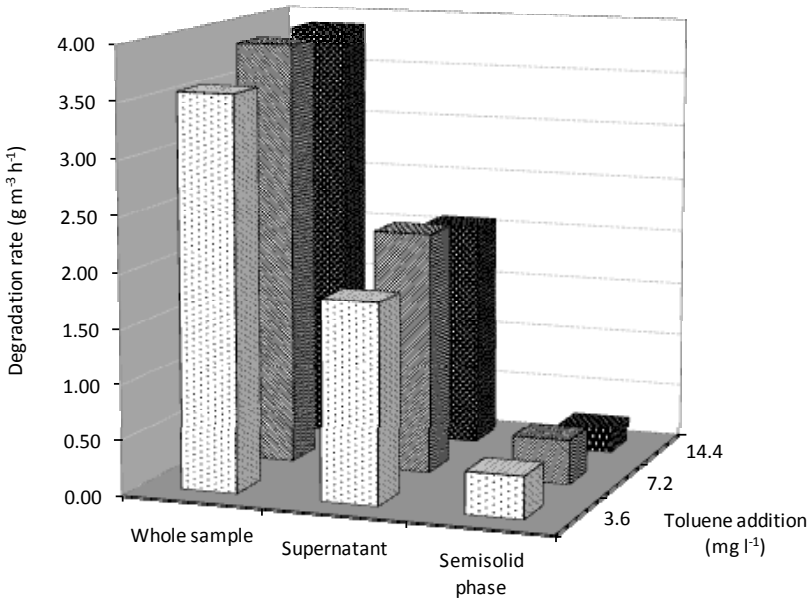

Figure 2.3. Toluene degradation rate for the three phases of the sludge after a 7-day acclimation.

Hence, no obvious advantage was found in separating the supernatant phase of the sludge for subsequent activation/acclimation, and consequently, the use of the whole sample is recommended. In addition, the whole sample does not require further handling.

2.3.2. About adding a prior carbon source

The results concerning the acclimation of the whole sludge sample with and without prior glucose addition are shown in Figure 2.4. Acclimation without glucose rendered a high elimination efficiency 24 h after the first toluene dose addition. Nevertheless, biomass response to the toluene degradation was delayed when glucose was previously added. Thus, in this latter case, the first toluene dose (4.3 mg toluene l^{-1} in the headspace) was depleted in 4 days. From the fourth day onward, both samples had similar degradation behaviour.

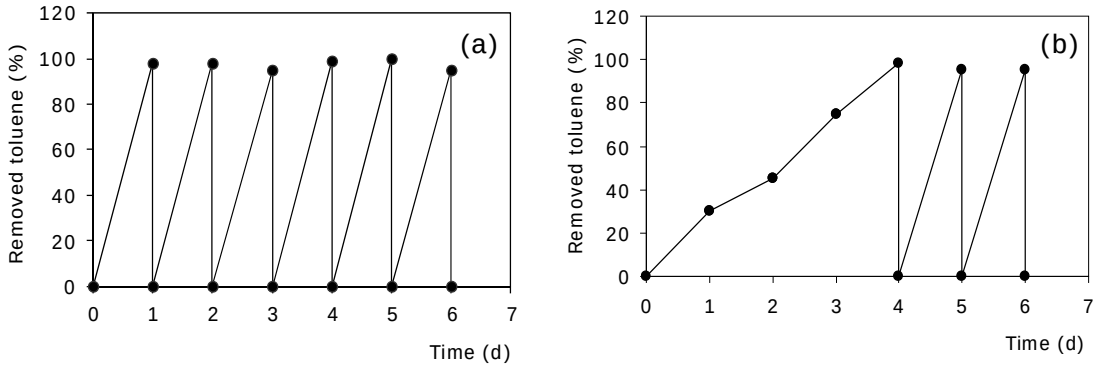

Figure 2.4. Toluene daily consumption (a) without glucose addition and (b) with the previous addition of glucose.

Kwon and Yeom (2009) used a culture medium containing glucose, yeast extract, and other inorganic salts for growing a microorganism that was previously isolated and identified. However, in the case of complex or undetermined biomass, the previous activation/adaptation with glucose is only recommended when indiscriminate (unspecialized) biomass is to be developed. Otherwise, the acclimation to a specific contaminant may be delayed because glucose is an easily degradable carbon source for a wide variety of microorganisms. This result is supported by the conclusions reached by Steele et al. (2005) who found that the microbial community at the end of the acclimation processes substantially differed from the initial community. Thus, an important change in microbial diversity in the inoculum at the end the acclimation procedure is to be expected when one specific compound is fed.

2.3.3. About continuous or discontinuous acclimation

The valve septum in the bottles containing toluene was perforated more than 20 times before unacceptable leakages were detected (an unacceptable leakage was considered when the mass loss was > 2 %). However, the acetone containing bottles could be perforated only up to 12 times, with the mass losses of the compound through the septum remaining less than 2 % for 60 h. This different behaviour was associated with the fact that the molecular weight of toluene is 1.58 times higher than that of acetone (therefore, so is its molecular volume).

As far as the degradation rate for the discontinuous and continuous modes is concerned, Figure 2.5 shows the results obtained after 7 and 30 days of acclimation for an initial dose of 3.6 mg l^{-1}. No relevant differences in the degradation rate between both modes were achieved when the sludge sample was acclimated for 1 week. Two key phenomena may occur during

this adaptation period according to Yeom et al. (1997); that is, microorganisms may induce various enzymes to degrade the substrate and may change their morphology to protect themselves from the toxic pollutant.

Nevertheless, the acclimation operation for 1 month rendered a degradation rate 2-fold greater for the discontinuous mode (1.76 g m^{-3} h^{-1}) than for the continuous one (0.88 g m^{-3} h^{-1}). These results are explained on the basis of the procedure itself because the discontinuous mode supplied "intermittent" contaminant amounts that fed biomass with no toxic conditions. Nevertheless, obvious disadvantages are inherent to discontinuous operation, such as the daily maintenance and the septum replacement.

In the case of continuous acclimation, the live/dead cell ratio was measured after 30 days of acclimation and the results (71/29) showed that the live cell content decreased by approximately 10% (and the dead cell content subsequently increased by the same amount). This finding contrasted with the higher degradation rate shown in Figure 2.5, which can only been explained by the development of more specialized biomass rather than by general biomass growth.

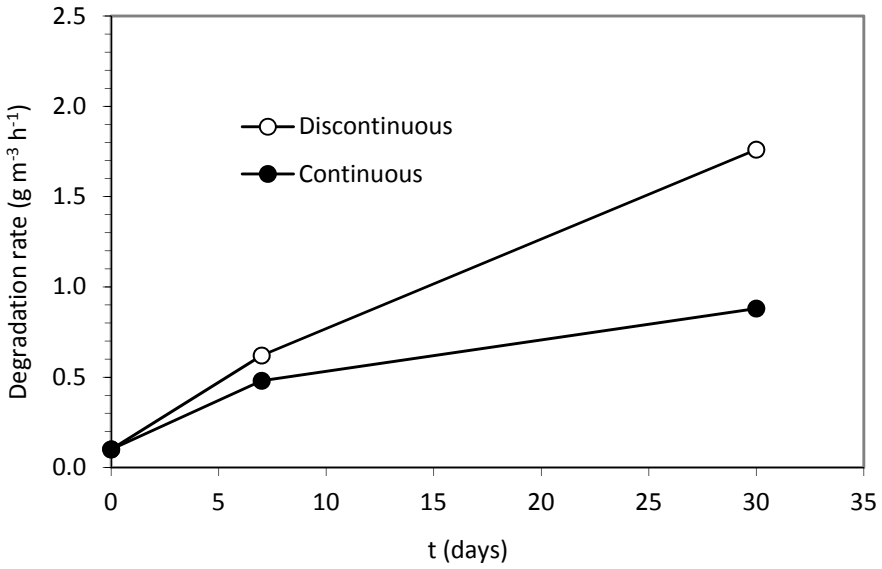

Figure 2.5. Degradation rate after a 7 and 30 day acclimation period by applying the discontinuous and continuous modes (for a toluene addition of 3.6 mg l^{-1}).

In addition to the initial degradation rate for a 3.6 mg l^{-1} toluene addition (2 µl), the subsequent rates for 7.2 and 14.4 mg l^{-1} doses (4 µl and 8 µl) were also determined (Figure 2.6). The comparison between both acclimation modes rendered higher degradation rates for the discontinuous system in all cases. The results obtained for the third addition (14.4 mg l^{-1} or 8 µl) in the discontinuous system were surprisingly high, which may be attributed to a faster selection of active biomass.

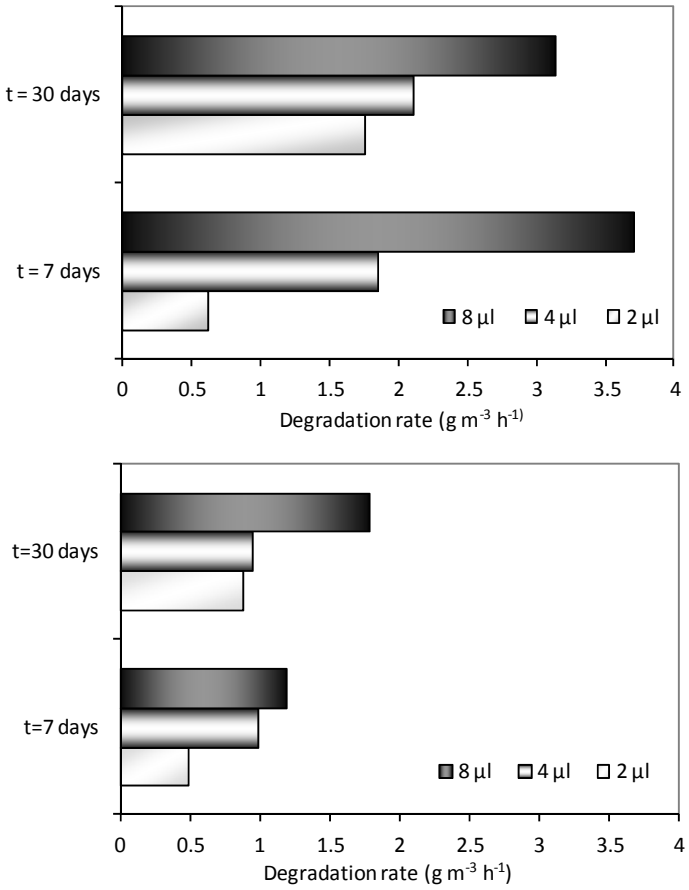

Figure 2.6. Comparison of the degradation rate for discontinuous (top) and continuous (bottom) mode after three consecutive additions of toluene and two acclimation periods.

Concerning the effect of repeated adaptation concentrations, Kwon and Yeom (2009) found that microbial adaptation to phenol required different strategies according to the contaminant dose. These authors found that a relatively low concentration of phenol (100 – 700 mg l^{-1}) required only one preadaptation step whereas a high concentration (1000 mg l^{-1}) did two or more consecutive stepwise preadaptations.

Regardless of the practical difficulties involved in maintaining daily feeding and oxygenation in discontinuous mode, this system rendered the highest degradation rate after 7 and 30 days of acclimation. On the basis of this result, this mode could be recommended for rapidly obtaining acclimated sludge samples by operating the system daily for no longer than 1 month.

For laboratory practice purposes, the operation of the continuous acclimation mode was extended for 1 year because it did not require any further maintenance, and, because installing a cultivation system for the continuous growth of microorganisms is highly recommended for industrial bioreactors (Kraakman, 2003).

The degradation rate for a 3.6 mg l^{-1} dose was measured after 7, 30, 90, and 365 days of operation (Figure 2.7). A dramatic increase in the degradation rate from 0.08 to 2.01 g m^{-3} h^{-1}

(25-fold) was achieved over the first 90 days of operation, whereas a slight increase was detected from that point to the end of the experiment (1 year). This slight increase was attributed to the fact that the system reached the steady state after 90 days of operation. Hence, the toluene disappearance rate increased only approximately 7 % during the last 9 months of performance, peaking at 2.14 g m^{-3} h^{-1}. In short, the sludge was more slowly acclimated in the continuous mode, which may be attributed to the more toxic conditions of feeding the contaminant. As a matter of fact, 60 days would be required for the continuously acclimated sample to reach the degradation rate of the discontinuously acclimated sample at 30 days (degradation of a 3.6 mg l^{-1} concentration).

Nevertheless, the great advantage is that no maintenance is required (apart from regular purges on a weekly basis) and that the activated sample is always ready for inoculation whenever required.

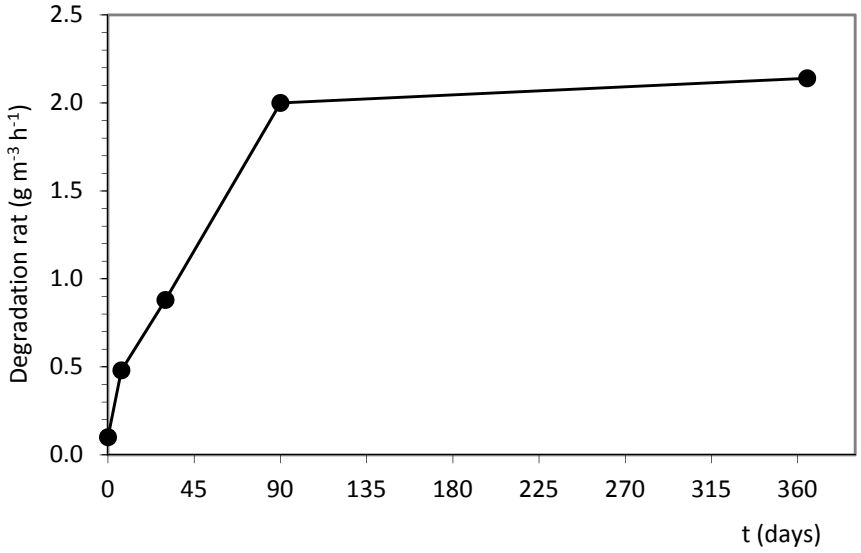

Figure 2.7. The evolution of the degradation rate for 1 year of continuous acclimation performance (for a toluene addition of 3.6 mg l^{-1}).

Once the cultures have been acclimated/adapted, they are expected to be versatile for the treatment of other chemically similar compounds (Yeom et al., 1997). Maliyekkal et al. (2004) found that the growth rates of precultured xylene and benzene cells in batch cultures were much faster when toluene was previously used as the sole carbon source.

2.3.4. About starting biofilters

The inoculation of a previously acclimated biomass is known to accelerate and improve the performance of the start-up step in biofilters. A procedure for obtaining an active inoculum from a wastewater sludge sample was proposed here. Thus, a sludge sample continuously acclimated to toluene was considered to be ready for inoculation when the degradation rate

exceeded the 85 % of the maximum degradation rate. The acclimated sample was used as inoculum for two biofilters. Downflow operation was selected because it allows better drainage (better moisture and biomass distribution) (Prado et al., 2005). The two conventional biofilters were started up and operated for 104 days (Figure 2.8, a and b). The inlet toluene load ranged from 50 to 400 ppm_v (from 0.015 to 0.118 g m^{-3} h^{-1}) for biofilter 1 and from 25 to 370 ppm_v (from 0.006 to 0.092 g m^{-3} h^{-1}) for biofilter 2. Nutrient solution irrigation was carried out once a month.

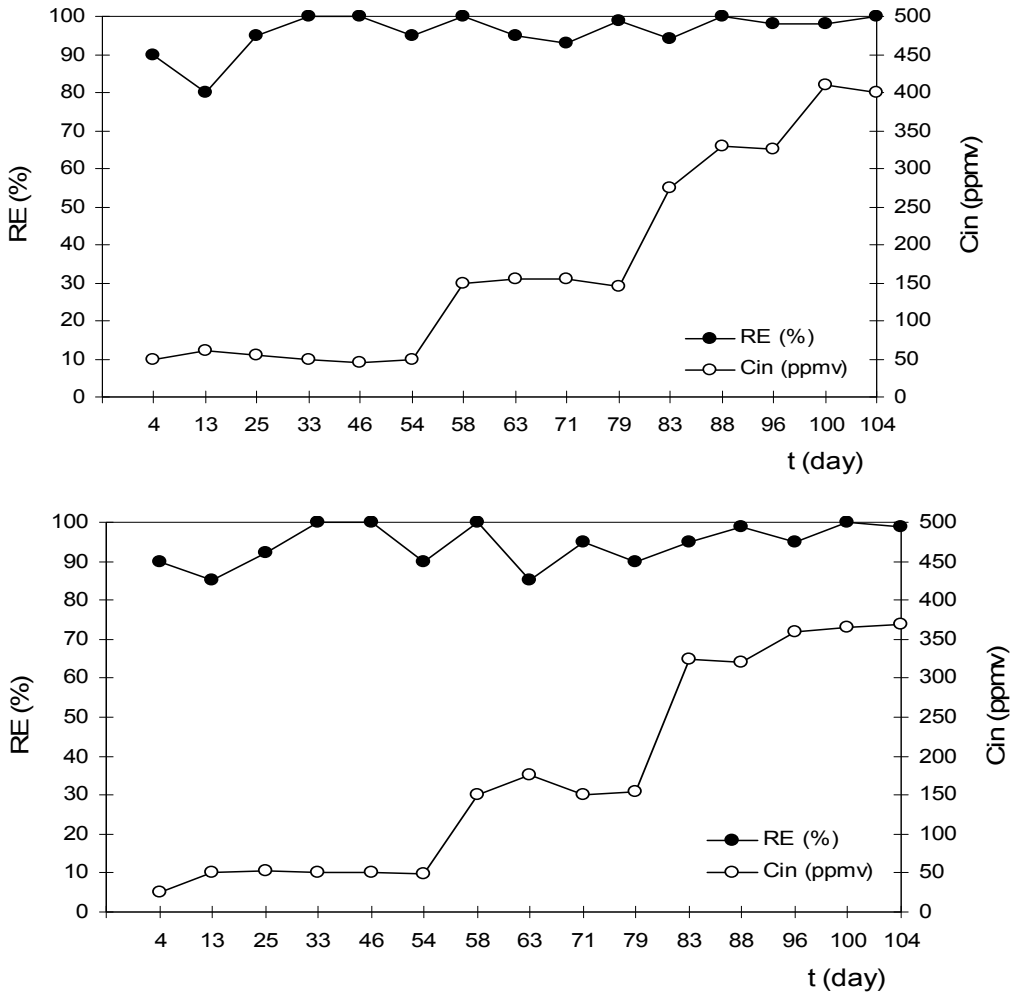

Figure 2.8. Response of (a) biofilter 1 and (b) biofilter 2 during start-up operations.

Bearing in mind that both biofilters were operated for more than 100 days, it was concluded that no significant adsorption took place on the support material, proving that the RE of the bioreactors was attributed to microbe activity.

Figure 2.8, a and b, shows that the start-up period for both biofilters was very short because the RE recorded values always greater than 80 % from the very first moment of operation. No significant differences were found between either biofilter, so they can be considered two replicates differing solely in EBRT (138 s for biofilter 1 and 163 s for biofilter 2). In both cases, stable REs between 80 and 100 % were reached, although inlet pollutant concentration was

increased to 400 ppm_v for biofilter 1 and to 370 ppm_v for biofilter 2. The high EBRT selected for the start-up of the bioreactors also had a positive influence on the high REs. Thus, both inoculated bioreactors reached high RE in a short time compared with the 8-month period for the start-up of a bioreactor without preliminary acclimated inoculation (data not shown).

2.4. Conclusions

Although the ecology of microbial communities in biofilters remains largely unknown, some simple strategies can be carried out for obtaining an active/acclimated inoculum. Certain strategies for successfully acclimating biomass to toluene have been proposed in this work with the objective of obtaining a successfully adapted inoculum for conventional biofilters.

Bearing in mind the inherent problems posed by dealing with growing active biomass and the practical difficulties of collecting representative and homogenous sludge/biomass samples, the bootstrap resampling technique was previously applied to select the best replicates that were the same at a 95 % confidence level. Regarding the selection of the initial sample to be acclimated, no obvious advantage was found in separating the supernatant phase of the sludge before activation/acclimation, and consequently, the use of the whole sample is recommended.

Regarding the decision to previously add an easily degradable carbon source, it was found that the consequence of this addition to a sample with unspecific strains was a quick growth of unspecialized biomass that could damage or inhibit the microorganisms of interest. Regarding the discussion between continuous versus discontinuous acclimation mode, the latter is recommended for rapidly obtaining acclimated sludge samples by operating the system for no more than 1 month. The continuous mode rendered similar degradation rates but required a longer operating time. Nevertheless, the great advantage of the continuous system is that no maintenance is required (apart from regular purges on a weekly basis) and that the inoculum is readily available.

To ascertain the utility of the proposed strategies, two similar biofilters were started using the in-accordance acclimated inoculum. The start-up period in both bioreactors was very short compared with the 8-month period for the start-up of a bioreactor without preliminary acclimated inoculation.

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3. CONTROL PARAMETERS (I)

Evaluating the impact of water supply strategies on *para*-xylene biodegradation performance in an organic media-based biofilter (*J. Hazard. Mater. 185, 1019 – 1026*)

3.1. Introduction

Biofiltration is now one of the most cost-effective and sustainable off-gas treatment technologies due to its minimal energy requirements and low environmental impact (Delhoménie et al., 2005; Revah and Morgan-Sagastume, 2005). Based on its simple operation and high removal efficiencies for both hydrophilic and hydrophobic volatile pollutants, biofiltration is the most popular biotechnologies for the treatment of odours and industrial volatile organic compounds (VOCs) (Kennes, 2001; Kraakman, 2001).

Since their introduction in the 1960s, a considerable amount of research has been conducted to elucidate the mechanisms underlying pollutant removal in biofilters and to enhance their robustness in order to increase their acceptance within the industrial community (Elías et al., 2002; Datta and Allen, 2005). Most studies have focused on the optimization of relevant factors such as the nature of the packing material, moisture content, biomass growth control, pH, nutrient availability, etc. (Gaudin et al., 2008; Ortiz et al., 2008; Jin et al., 2009; Singh et al., 2010). Due to their biological basis, the control of moisture content (water activity) in biofilters is a key operating parameter determining process performance (Datta and Allen, 2005). As a matter of fact, an inefficient control of the moisture content has been reported as the cause of 75 % of biofilter failures (Auria et al., 1998).

Water activity is responsible for the type (bacteria vs. fungi) and level of activity of the microbial community present, and determines the long-term structural stability of the packed bed (compaction, formation of anaerobic zones or preferential pathways, etc) (Corsi and Seed, 1995). However, the control of moisture content in the packing material is compulsory and complex, as a large number of factors need to be considered: the moisture content of the gas stream entering the biofilter, the frequency and flow of external irrigation, the exothermicity of pollutant mineralization, the organic nature of the packing material, the biomass distribution profile within the biofilter and the water retention capacity of each packing material (Kennes, 2001; Sakuma et al., 2009). Water activity in biofiltration systems must therefore be carefully studied on a case by case basis in order to ensure consistent VOC and odour treatment efficiencies, especially when using novel organic packing materials. However, despite the importance of this issue, there are few published studies devoted to understanding and further optimizing the temporal and spatial distribution of moisture content in biofilters (Cox et al., 1996; Auria et al., 1998; Sakuma et al., 2009).

This work focuses the influence of different irrigation and operating strategies on the performance of *para*-xylene abatement using an organic packing material composed of pelletised sawdust and pig manure. Special attention was given to the interdependence between moisture content and pollutant removal efficiency by carefully monitoring the timeline of the profiles of both parameters throughout the biofilter column. *para*-Xylene, was used as a model VOC due to its widespread use in the ever-increasing production of polyethylene terephthalate (METI, 2005).

3.2. Materials and methods

3.2.1. Inoculum preparation

Despite the indigenous microflora present in the packing material used in this work had previously shown a good capacity for degrading H_2S (Barona et al., 2005), it was not capable of mineralizing *para*-xylene. Thus, an aerobic activated sludge collected at the wastewater treatment plant in Muskiz (Bizkaia, Spain) was used as inoculum for *para*-xylene biodegradation. Initially, the activated sludge was continuously exposed for 30 days in a stirred tank bioreactor to a *para*-xylene-laden air at an aeration rate of 0.4 vvm (air volume per unit of liquid volume per minute) and at concentrations ranging from 50 to 100 ppm_v. The measurement of *para*-xylene degradation rate in batch assays confirmed the acclimatization of the microbial population present in the liquid phase. This procedure has already been explained in a previous work (Elías et al., 2010).

3.2.2. Biofilter setup

The biofiltration system consisted of 3 PVC modules (Figure 3.1). The packed bed was divided into the three identical sections with a total volume of 4.5 l. The packing material selected in this work was supplied by SLIR S.L (Specialised Engineering in Recycling Agricultural Residues) and its commercial name was ABONLIR. This material was made up of composted pig manure and sawdust, and the pellets were manufactured by mechanical compression without chemical addition. The compost was stored in sealed plastic bags at room temperature to maintain its original moisture content. Table 3.1 summarizes the main characteristics of the packing material (Barona et al., 2005).

The biofilter was initially irrigated with an activated sludge acclimated according to the procedure published by A. Elías et al. (2010) and it was operated in a downflow configuration at 23 ± 2 °C. The flow of *para*-xylene-contaminated air was added from the top of the biofilter at a flow rate of 1-1.5 l min^{-1} (corresponding to an empty bed residence time ranging from 180 to 270 s) and the contaminated flow was generated by mixing a *para*-xylene-saturated air stream with a humidified *para*-xylene-free air stream in different proportions. The non-humidified fraction of air used for *para*-xylene saturation was a minor fraction of the whole influent air flow and did not significantly impact the moisture of the contaminated air stream entering the biofilter. Indeed, the relative humidity of the contaminated air at the biofilter inlet remained always higher than 98 %. An activated carbon filter was also included in the experimental setup, being fitted to the bioreactor outlet in order to mitigate the environmental impact of the non-degraded contaminant. The biofilter was equipped with several gas sampling valves to monitor the inlet, outlet and inter-module *para*-xylene and CO_2 concentrations. Additionally, it was also provided with several ports located throughout the three PVC modules for measuring the temperature and relative humidity content of the air.

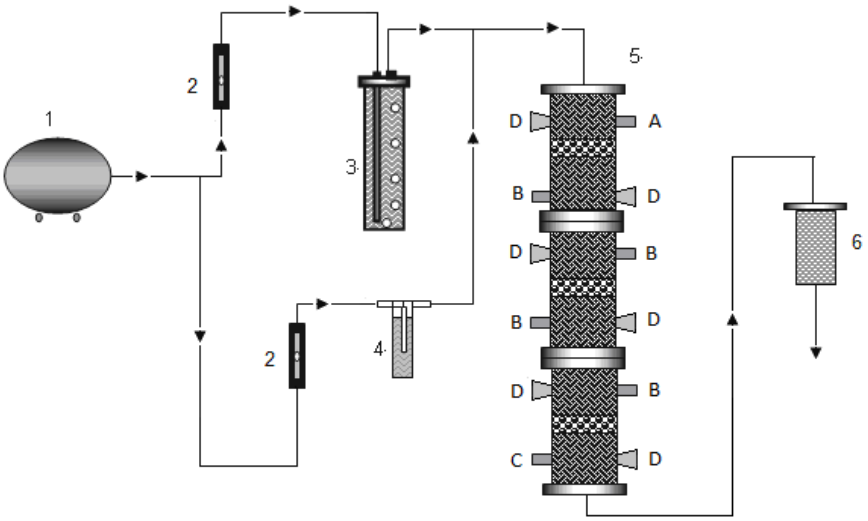

Figure 3.1. Diagram of the biofiltration system: 1. Air-compressor; 2. Flow meter; 3. Humidification chamber; 4. *para*-xylene evaporator; 5. Modular Biofilter; 6. Active carbon chamber; A (inlet gas sampling valve); B (inter-module gas sampling valve); C (outlet gas sampling valve) and D (temperature and air relative humidity measurement ports).

Table 3.1. Physical-chemical properties of the packing material used in the modular biofiltration unit (Barona et al., 2005)

Organic matter (%)[a]	40.0
Total nitrogen (%)	1.4
P_2O_5 (%)	1.1
K_2O (%)	1.5
Total S (%)	3.3
pH	6.5-7.5
Mean length (mm)	10.7
Mean radius (mm)	6.1
Bulk density (g cm^{-3})	1.3
Real density (g cm^{-3})	2.3
B.E.T. (N_2) surface area (m^2 g^{-1})	12.06 ± 0.09
BJH adsorption average pore diameter (Å)	145
Macropore volume in the pellets (%)	89.42
Micropore area (d < 20 Å) (m^2 g^{-1})	0.3
Initial Moisture Content (%)	25.2

Notes: (a) Value provided by the manufacturer.

3.2.3. Influence of water irrigation on the long-term performance of *para*-xylene biodegradation

The system was initially operated for 260 days with no further irrigation, as water was supplied solely via the saturated inlet air stream (relative humidity ≥ 98 %) (dry period). From days 260 to 550 (wet period), each of the 3 modules was individually irrigated every 25 days with 400 ml of mineral salt medium (Barona et al., 2007), in order to avoid drip of water from the top section to the middle and bottom sections. These extended operational periods allowed the development of new microbial characterization techniques for the communities established in the packing material (data not shown in this paper) and the assessment of the structural robustness of the packing material ABONLIR. In this context, the characterization of the dynamics of the predominant microbial species would allow to clarify the influence of adverse environmental conditions (such as lack of irrigation) on process performance. However, due to the size and complexity of these outcomes, this information was not included in the study.

During the dry period, the biofilter was continuously fed with the contaminant at an inlet loading rate (IL) of 8 ± 1 g m^{-3} h^{-1}. On the other hand, *para*-xylene was continuously supplied during the wet period, and the different inlet loading rates during this period were 16 ± 4; 96 ± 10; 30 ± 5; 72 ± 10 and 166 ± 19 g m^{-3} h^{-1}, respectively. Apart from monitoring the overall biofilter biodegradation performance, the elimination capacity and moisture content of each individual module were also periodically recorded.

Samples of the packing material were collected from the 3 modules during the dry and wet periods in order to monitor the dynamics of microbial population by Scanning Electron Microscopy (SEM).

3.2.4. Influence of water irrigation on short-term biofilter and module performance

The transient performance of the overall *para*-xylene removal and the temporal and spatial dynamics of moisture content (MC), temperature and partial elimination capacities in each module were evaluated after each periodic irrigation during the wet period.

3.2.5. Analytical procedure

para-Xylene and CO_2 concentrations were measured using a micro-gas chromatograph CP 4900 (Varian, The Netherlands) equipped with auto-sampling injection, a TCD detector and using He as carrier gas. The Micro GC was equipped with CP-Sil 5 CB (6 m × 0.15 mm × 2 µm) and CP PoraPLOTQ (10 m × 0.25 mm × 8 µm) columns. The oven, injector and TCD detector were maintained at 80 °C, 110 °C and 80 °C, respectively. External standards prepared from a calibration cylinder (Air Liquide, Spain) containing a *para*-xylene in N_2 enabled the quantitative determination of the target VOC.

The temperature of the packing material was determined by using several thermocouple HI-98804 sensors (Hanna Instruments, Italy) located at six different heights in the biofilter column (Figure 3.1). The moisture content within the packing material was determined by a Moisture Analyzer HB43-S (Mettler Toledo) by periodically collecting 2 – 3 g of support material from each module. Air relative humidity and temperature at the inlet and outlet ports of the biofilter column were measured by using a Testo 625 sensor (Testo, Germany).

Biomass samples extracted from the packing material were fixed in 2 % glutaraldehyde in 0.1 M cacodylate buffer (pH = 7.4), washed in iso-osmolar cacodylate/sucrose buffer and postfixed in 1 % Osmium Tetroxide in cacodylate buffer. After repeated washing, samples were dehydrated through an ethanol series and washed in hexamethyldisilazane prior to air drying. Finally, samples were mounted onto stubs and gold coated using a JEOL fine coat ion sputter JFC-1100. Samples were visualized and micrographed using a scanning electron microscope (Hitachi S-4800) at 15 kV accelerating voltage.

3.2.6. Statistical treatment

All results are given as the average value with its corresponding error at 95 %. Results were analyzed using a one-way ANOVA with significance at $P \leq 0.05$. The Excel statistical package (Microsoft Corporation, USA) was used for data processing.

3.3. Results and discussion

3.3.1. Influence of water irrigation on the long-term performance of *para*-xylene biodegradation

During the "dry period" (the first 260 days of operation), process performance was characterised by an average removal efficiency (RE) of 33 ± 7 % (Fig. 4.2a). This poor performance was recorded despite the low inlet loading rate of contaminant (8 ± 1 g m^{-3} h^{-1}) and the long EBRT, which were fairly higher than EBRT used by other authors (Table 3.2). This long period ensured the development and acclimation of an efficient microbial community. The modular biodegradation profile throughout the biofilter column revealed that only the upper module, which received readily available moisture from the inlet stream, was active (data not shown).

Periodic irrigation of the biofilter packing material led to a rapid increase in *para*-xylene removal efficiency, which achieved a sustained RE of 88 ± 15 % from days 259 to 373 (at an inlet loading rate 16 ± 10 g m^{-3} h^{-1}). The irrigated biofilter was also capable of coping with a further increase in IL (days 378 to 428) to 97 ± 18 g m^{-3} h^{-1}, recording a RE of 94 ± 9 %. Similar results (an average RE higher than 93 %) were achieved during operation from days 436 to 505 at an inlet loading rate of 30 ± 9 g m^{-3} h^{-1} and from days 505 to 532 at 72 ± 20 g m^{-3} h^{-1}. However, a sudden increase in pollutant loading rate to 166 ± 24 g m^{-3} h^{-1} resulted in a gradual deterioration of biofilter performance (Figure 3.2a), probably due to a partial inhibition of the microbial community present in the biofilm (Jorio et al., 2000; Gabaldón et al., 2006). These

results therefore confirmed the poor abatement performance reported in uncontrolled biofilters, where the moisture content provided by the humidified air stream might be insufficient to maintain an optimum moisture level for microbial growth (Auria et al., 1998).

Table 3.2. Characteristics of conventional biofilters treating *para*-xylene

Packing material	EC_{MAX} (g m^{-3} h^{-1})	EBRT (s)	Moisture (%)	Reference
Pig manure - sawdust	130 (> 95 % RE)	180 – 270	25 – 35	This study
Conditioned peat	66 IL ≥ 200 g m^{-3} h^{-1}	102	50 – 70	Jorio et al., 1998
Peat balls	67 (max.)	157	-	Jorio et al., 2000
Conditioned peat	236 (66 % RE)	68	16 – 70	Jorio et al., 2002
Compost-wood chip	73 ± 14 (max.)	60	60 – 65	Torkian et al., 2003
Peat + Mineral additive 70:30 (v/v)	61(93 % RE)	150	-	Elmrini et al., 2004
Manure pig compost-forest soil – polyethylene	80 (> 96 % RE)	132	50	Wu et al., 2006
Biosol (glass & cardboard)	160 (> 90 % RE)	47	45	Jeong et al., 2006
Food waste compost + oyster shell + polyurethane foam	21	60	40 – 60	Hwang et al., 2007
Commercial press mud (sugar industry waste)	67	42	50	Saravanan et al., 2009
Wood-chip (fungal inoculum)	77 (53 % RE)	59	-	Jorio et al., 2009
Wood-chip (bacterial inoculum)	58 (49 % RE)	59	-	Jorio et al., 2009
Scoria	450 (66 % RE)	45	-	Kim et al., 2009
Sieved compost + Ceramic balls	101.3 (49 % RE)	48.6	55-60	Rene et al., 2009

Finally, it must be highlighted that despite the low frequency of irrigation (each 25 days), the good stability of the overall reactor performance within these 25 days without irrigation was in agreement with Son and Striebig (2001), who recorded consistent REs greater than 90% in an organic biofilter for 59 days without any nutrient solution or water addition.

The CO_2 production rates recorded also confirmed the higher biofilter performance under periodic irrigation (Figure 3.2b). Hence, while CO_2 production and *para*-xylene removal rates had a poor correlation during the dry period ($R^2 \approx 0.13$), a reasonable correlation was obtained during the wet period ($R^2 \approx 0.80$). The reasons underlying this apparent mismatch are still not

clear, but they confirmed the dysfunctionalities of microbial metabolism at low water activities. An overall carbon balance applied to *para*-xylene mineralization during the wet period revealed that only 32 % of the *para*-xylene removed was converted into CO_2. This unexpectedly low conversion is however similar to that obtained by Garcia-Peña et al. (2008), who also recorded *para*-xylene conversion to CO_2 of 31 %. It is however unlikely that the remaining carbon was totally transformed into biomass, since the production of extracellular metabolites was not analyzed in this work and it might cause a deficit in CO_2 in the gas phase (Jorio et al., 2000). The low values of CO_2 concentration recorded at the biofilter outlet suggest that the organic packing bed was not actively mineralized during the wet period, which agrees with the high stability of the packing material structure even after 550 days of continuous operation. This experimental result agreed well with the carbon distribution determined during toluene mineralization by *Pseudomonas sp*. (Muñoz et al., 2009).

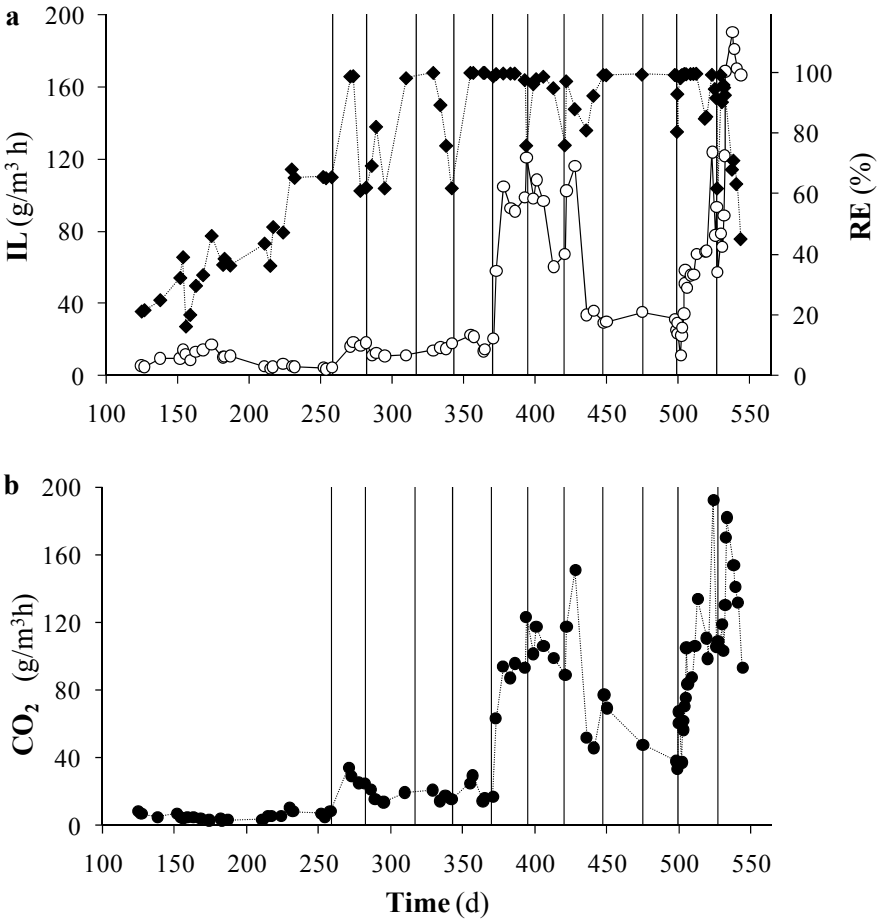

Figure 3.2. Evolution of (a) inlet load and removal efficiency, and (b) CO_2 production during *para*-xylene biofiltration under dry (from days 0 to 260) and wet conditions (from days 260 to 550). Vertical lines represent biofilter irrigation.

It should be noted that pressure drop over the course of the experimental run was negligible (below 3 cm H_2O), which confirmed the stability of this organic packing material. This stability, together with the cost (130 € m^{-3} compared to 475 € m^{-3} for activated carbon or 225 € m^{-3} for

polystyrene foams) makes this packing material an interesting alternative for biofilter scale-up (Prado et al., 2009).

A comparison between the SEM images of the packing material at day 250 (dry period) (Figure 3.3a and 3.3b) and day 427 (wet period) (Figure 3.3c and 3.3d) showed significant differences in the nature and density of the microbial population developed. Hence, while low concentrations of filamentous fungi were present in the filter bed after 8 months of operation in the absence of irrigation, a varied and abundant microbial community composed of both bacteria and fungi was clearly visible after periodic irrigation. These findings are in agreement with previous results on *para*-xylene biofiltration under xerophilic conditions when using the same pelletised carrier material; an abundant fungal biodiversity was observed by culture-independent molecular methods (Prenafeta-Boldú et al., 2008). Similarly, Cox et al. (1996) and Auria et al. (2000) observed a preferential growth of fungi under prolonged periods of low water activity in the biofiltration of styrene and toluene, respectively.

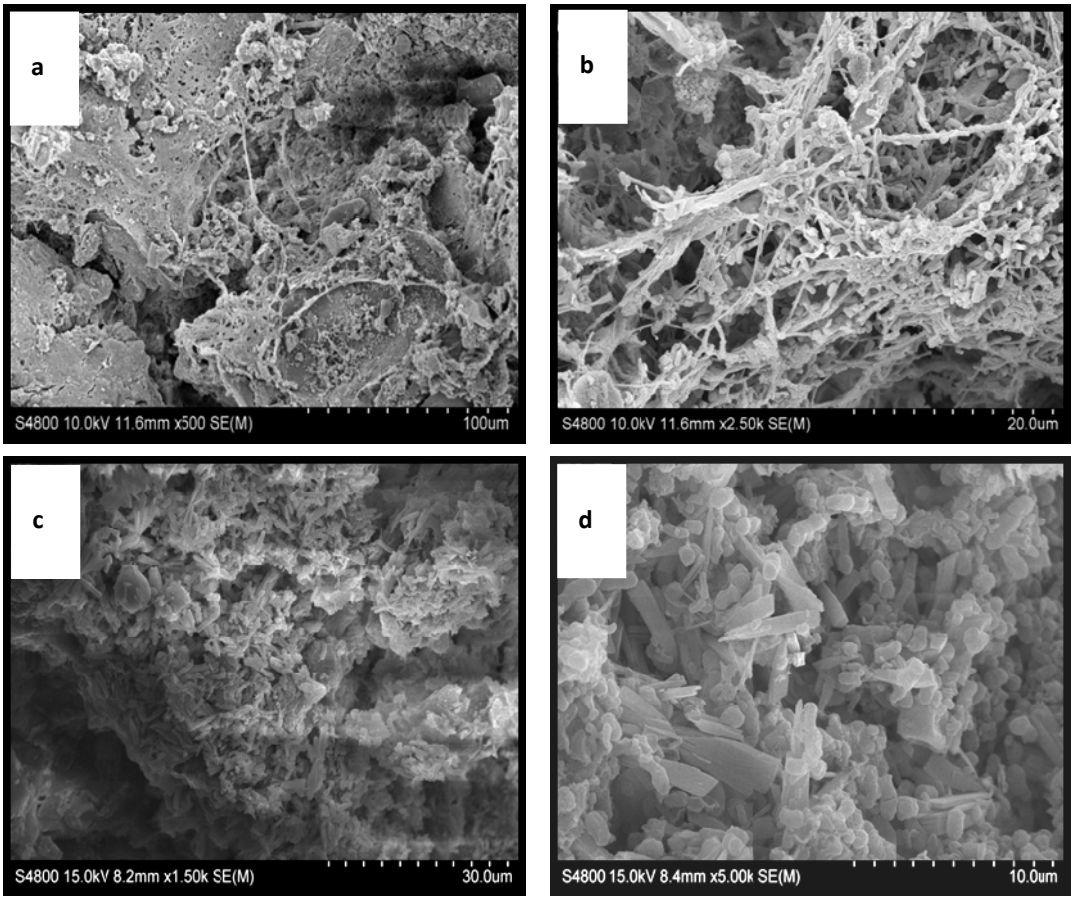

Figure 3.3. SEM photographs of packing media under dry (a, b) and wet (c,d) conditions.

The overall elimination capacity (EC) of the biofilter as a function of the inlet *para*-xylene loading rate during the wet period is shown in Figure 3.4a, while Figure 3.4b, 3.4c and 3.4d show the EC for the upper, middle and lower modules, respectively, as a function of their corresponding IL. Overall *para*-xylene EC increased at increasing loading rates with RE of approximately 100 % up to a critical IL of 120 g m^{-3} h^{-1}, where EC remained constant. A detailed analysis of the data confirmed that *para*-xylene diffusion from the gas phase governed biofilter performance under these particular operating conditions. Thus, when loading rates were lower than 120 g m^{-3} h^{-1}, the *para*-xylene transferred from the gas phase was rapidly consumed in the outer layers of the biofilm, resulting in an insufficient supply of carbon to the internal layers of the biofilm (adjacent to the packing media). However, when the *para*-xylene IL was higher than 120 g m^{-3} h^{-1}, microbial activity, rather than pollutant mass transport, controlled biofilter performance. In addition, it should be noted that the highest EC recorded in this study in most cases exceeded the maximum EC obtained by other researchers under comparable experimental conditions, which can be attributed to the high B.E.T. surface area of the packing material (Table 3.1). Nevertheless, the overall moisture content in our experimental system (25 – 35 %) was slightly lower than those reported in the literature (> 40 – 65 %) (Table 3.2). This high efficiency at lower moisture contents might be explained by the selective pressure placed on microbial enrichment by the initial 8 months of dry period, which rendered a microbial community that was resistant and acclimated to low water activities.

A similar *para*-xylene maximum EC was observed in each of the three modules as shown in the EC vs. IL in Figure 3.4b-d, where IL represents the *para*-xylene loading rate entering each individual module during wet period. Despite this similarity, the middle module recorded the most stable performance (with almost complete VOC depletion up to a loading rate of 65 g m^{-3} h^{-1}), while the upper module surprisingly showed a rather scattered biodegradation pattern. On the other hand, the third module showed a poor process performance at low IL (Figure 3.4d) although comparable performances were recorded above 25 g m^{-3} h^{-1}. A detailed analysis of the correlations between *para*-xylene RE and the moisture content in each individual module revealed only a clear correlation in the upper module (R^2 = 0.82, in the range 7 – 32 %), while no significant variations in RE were recorded at increasing moisture contents in the middle and lower modules (25 – 40 %) (data not shown, R^2 < 0.4). These findings do not rule out a potential correlation between microbial activity and MC, rather than limit the MC range of this correlation. Hence, there was a critical MC (25 – 30 %) below which microbial activity depended on the MC of the packing bed, but above this critical MC, water activity was sufficient to support microbial activity and no correlation between MC and RE was observed.

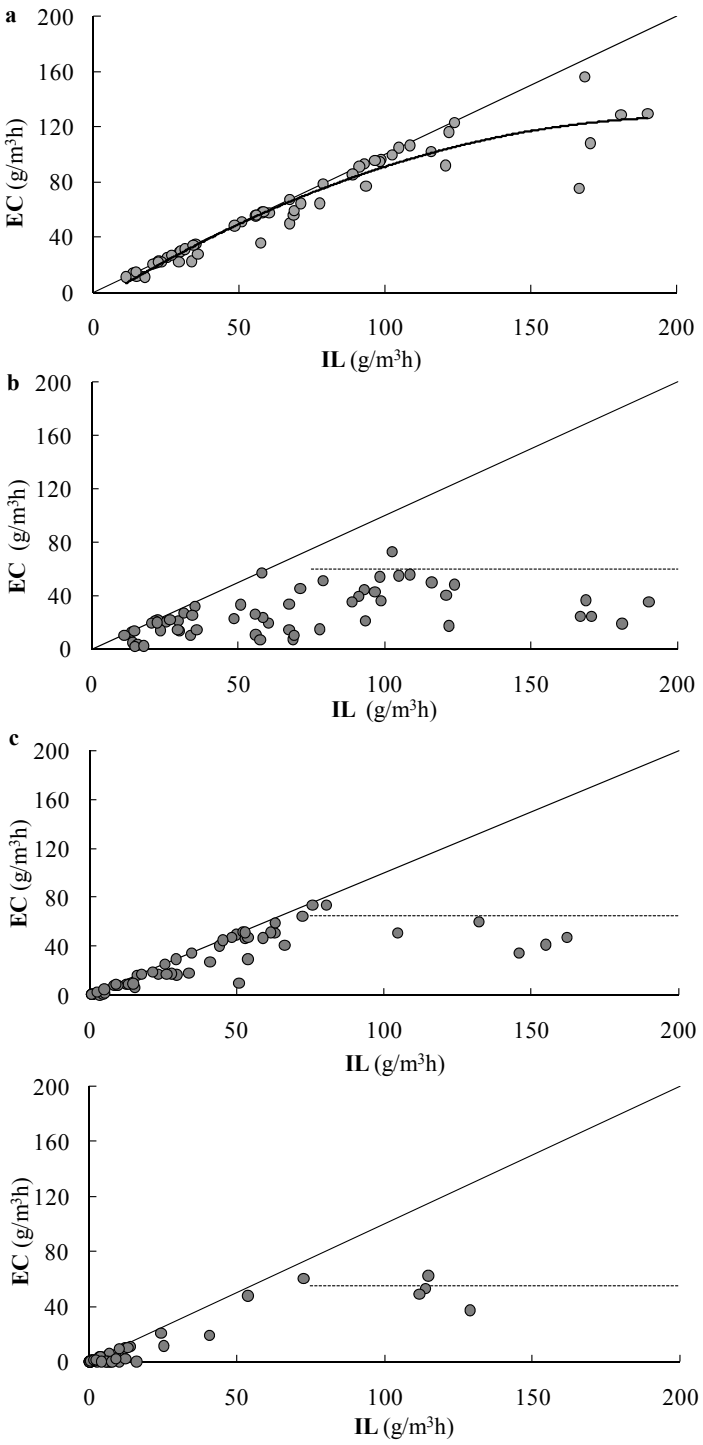

Figure 3.4. Elimination capacity versus inlet load for (a) the overall biofilter, (b) upper module, (c) middle module, and (d) lower module during *para*-xylene biofiltration in the wet period.

3.3.2. Influence of water irrigation on short-term biofilter and module performance

Biofilter performance after one water irrigation and during the subsequent 25 days is shown in Figure 3.5. Steady state performance rapidly recovered a few hours after irrigation. This finding was in agreement with that of Auria et al. (2000), who explained a similar slight RE decrease based on the rapid desorption of the VOC from the packing material. Likewise, an increase in odour concentration in the area surrounding intensive livestock farming facilities is usually found immediately after rainfall due to odorant VOC desorption (flooding-out effect) (Wright et al., 2005). On the other hand, the increase in air velocity (due to pore blocking), together with the increase in the thickness of the water layer surrounding the biofilm, might have resulted in a higher resistance to the mass transfer of hydrophobic pollutants like *para*-xylene (Bagherpour et al., 2005).

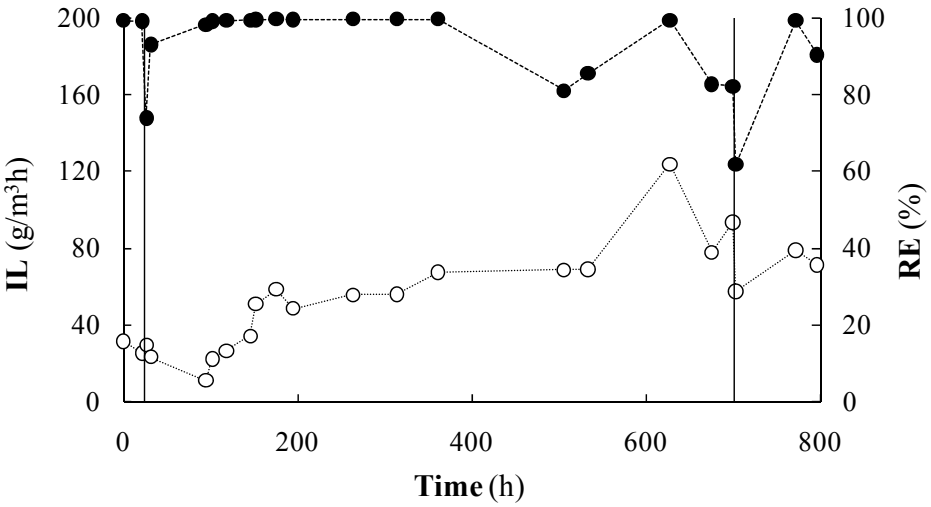

Figure 3.5. Short-term transient effect of irrigation on *para*-xylene removal efficiency (o). Open circles represent the inlet loading rate.

Consistent dynamics for RE and MC were recorded in each module during the wet period, although the temperature patterns slightly differed at the beginning of each irrigation period as shown in Figure 3.6b. Contaminant biodegradation occurred predominantly in the upper zone during the first week following the irrigation of the three modules (Figure 3.6a). Nevertheless, RE in the upper module gradually declined and the RE in the middle module gradually increased within the first 12 days. A steady increase in RE in the lower module from day 12 was observed. The fluctuations in temperature were always correlated with *para*-xylene biodegradation in each module (Figure 3.6b). The temperatures in the middle module increased up to 4 °C by days 13 and 14 after irrigation, while the temperature in the lower

zone gradually increased from the first day and obviously decreased with the next irrigation. This close correlation between biofilter's performance and temperature was also observed by Jorio et al (2009). In accordance with this study, Son et al. (2003) showed that the ethylbenzene was not equally degraded over the depth of a biofilter packed with composting material after water irrigation. Hence, the most dominant stage in ethylbenzene degradation gradually shifted over time from the nearest air-inlet zone to the inner section of the biofiltration units. Finally it must be highlighted that the above mentioned consistency in RE, temperature variations and MC refers to the trend of these parameters rather than to the quantitative extent of these variations, which itself depends on *para*-xylene biodegradation rate. It is clear from Figure 6 than the higher IL on day 528 (compare to day 499) induced higher temperature increases in the modules as a result of a more intense microbial activity. In this context, Son et al. (2003) also observed that higher ethylbenzene degradation rates induced a higher temperature increase in biofiltration packed beds (Son and Striebig, 2003).

On the other hand, the MC in the upper and middle modules progressively declined from 30 to 17 % and 24 %, respectively, while it increased in the lower module from 35 % to 42 % (Figure 3.6c). These dynamics in MC can be explained as a result of water evaporation due to a biodegradation-mediated temperature increase. As a result, the unsaturated polluted air was capable of evaporating water from the packing material. The lower temperatures prevailing in the lower module induced later condensation, which explains the increase in moisture content recorded. These MC profiles agreed with those reported by Abumaizar et al. (1998), who observed higher MC contents in the lower modules of BTEX treating biofilters packed with activated carbon. An optimum MC range of 20 – 40 % was estimated from the correlation of the dynamic profiles of *para*-xylene RE and moisture content. This finding confirms the results of Wang and Govind (1997) who reported that naturally bioactive filter beds (like peat or compost) need a range of suitable water content for high-efficiency operation.

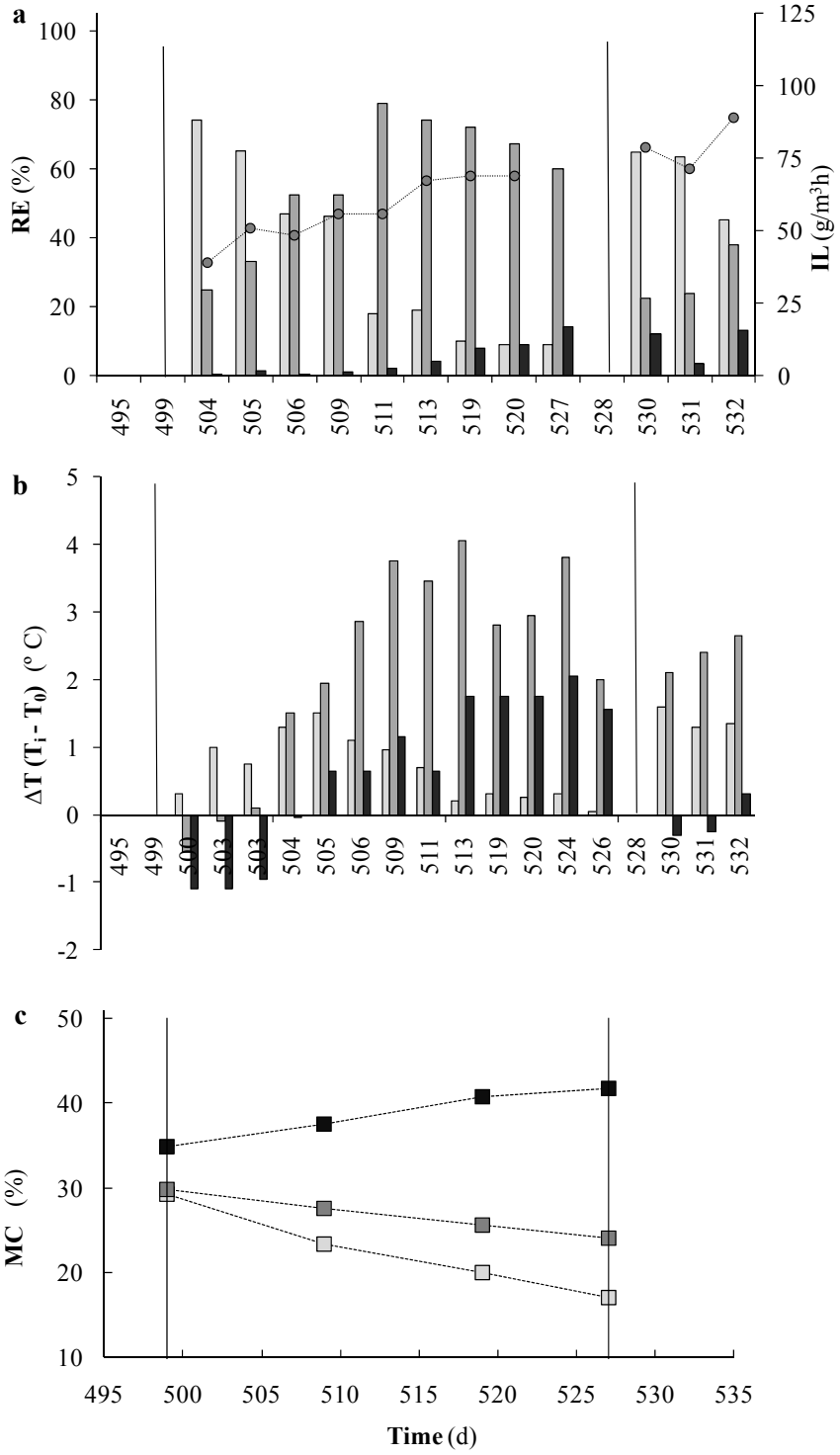

Figure 3.6. Evolution of RE (a), temperature variation (b) and moisture content (b) in the upper (□), middle (▢) and lower (■) modules over time during *para*-xylene biofiltration. Vertical lines represent biofilter irrigation and (●) represents the inlet loading rate.

3.3.3. Water balance modelling

A simple mathematical model was developed for estimating the dynamics of moisture content within the different modules of the biofilter. This modelling approach was based on the separate contribution of water production during *para*-xylene mineralization and water evaporation/condensation as a result of the temporal and spatial dynamics of temperature within the biofilter bed.

Thus, the rate of water evaporation from the biofilter (W_{ev}) can be calculated using the theoretical considerations proposed by Morales et al. (1998):

$$W_{ev} = \frac{(y_{IN}\rho_{IN} - y_{OUT}\rho_{OUT})F_{air}}{V_r} \quad (1)$$

Where ρ is the air density, F_{air} is the volumetric air flow rate, V_r is the volume of the packing bed and is the mass fraction of water contained in the air circulating through the biofilter calculated as follows:

$$y_i = 0.622\frac{Pv_i}{P - Pv_i} \quad (2)$$

The water partial pressure (Pv_i) can be estimated as:

$$Pv_i = RH_i Pvs_i \quad (3)$$

where Pvs_i is the water saturation pressure expressed as:

$$Pvs_i = e^{\left(25.775 - \frac{5281.1}{T_i}\right)} \quad (4)$$

Moreover, air density can be expressed using equation (5):

$$\rho_i = \rho_0\left(\frac{T_0}{T_i}\right)\left(\frac{P}{P_0}\right) \quad (5)$$

Water production from *para*-xylene mineralization can be calculated by experimental stoichiometric considerations. Therefore, assuming a biomass composition of $CH_{1.8}N_{0.2}O_{0.5}$ and based on an experimental CO_2 production yield of 1.24 g CO_2 g *para*-xylene^{-1} degraded, the stoichiometry of *para*-xylene mineralization can be represented by the following equation:

$$C_8H_{10} + 5.49\ O_2 + 1.00\ NH_4^+ \rightarrow 5.01\ CH_{1.8}N_{0.2}O_{0.5} + 2.99\ CO_2 + 2.49\ H_2O$$

The model developed here accurately described the water mass balance within each module. Thus, when applied to the experimental period from days 495 to 435, this model confirmed the very different amounts of evaporated water and biochemically produced water in the upper and middle modules (Figure 3.7a-b). In addition, the model was able to explain the increase in MC recorded in the lower module (Figure 3.7c). Finally, the goodness of estimation of the mathematical approach proposed here was assessed using the abovementioned experimental period. Accordingly, based on Figure 6c, the amount of water loss through evaporation in the upper, middle and lower modules accounted for 238, 112, and -132 g H_2O, respectively and model predictions based on Figures 7 a, b and c accounted for 201, 128, and -60 g H_2O.

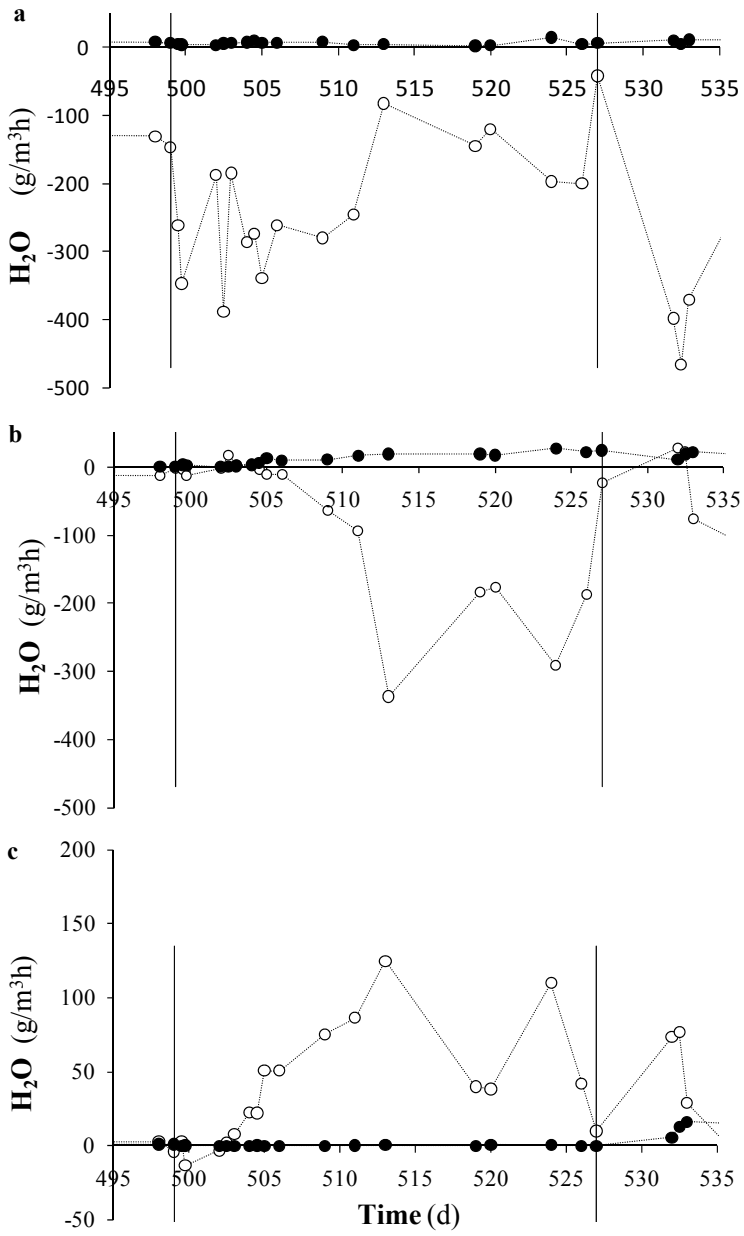

Figure 3.7. Evolution of evaporated water (○) and biochemically produced water (●) in the upper (a), middle (b) and lower (c) modules over time.

3.4. Conclusions

Biofiltration operation in the absence of water irrigation resulted in poor *para*-xylene abatement efficiencies (≈ 33 ± 7 %) even in the presence of filamentous fungi, which are known to improve the absorption of hydrophobic compounds (due to their aerial mycelium) and to maintain their biodegradation activity under low moisturized environment.

On the other hand, a periodic irrigation strategy rendered a considerable increase in the overall RE (about 100 %) for an inlet loading rate lower than 120 g m^{-3} h^{-1} which confirmed previous studies on this topic. These results were confirmed by the poor correlation between CO_2 production and *para*-xylene removal at low water activities, and by the absence of a varied and abundant microbial population during the dry period as shown by the SEM images. A temporary decrease in process performance occurred during the first hours after each irrigation, which was attributed to a water-induced desorption of *para*-xylene from the packing material or a higher water-mediated resistance to mass transfer from the gas phase. Consistent dynamic patterns for RE, T and MC were recorded throughout the biofilter column after irrigation (approx. 300 days). Thus, *para*-xylene biodegradation initially occurred in the upper module, resulting in a temperature increase and a consequent decrease in the moisture content. The decline in moisture content in the upper module gradually led to a higher degradation rate in the second module, while the moisture in the lower module steadily increased as a result of water condensation due to the low removal efficiency. Additionally, mass balance calculations performed using bed temperatures and relatively humidity values were successfully used to account for water balances in the biofilter over time. The negligible pressure drop (< 3 cm H_2O) and the absence of bed compaction after 550 days of continuous operation confirmed the suitability of the pelletized pig manure and sawdust material as packing material in biofiltration processes.

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4. CONTROL PARAMETERS (II)

Comparative response of two organic biofilters treating ethylbenzene and toluene after prolonged exposure (*Process Saf. Environ. Prot. DOI: 10.1016/j.psep.2011.11.006*)

4.1. Introduction

Many studies have confirmed the presence of volatile organic compounds (VOCs) not only in industrial regions (Ras et al., 2009) but also in outdoor environments in US urban areas and in small-medium size European cities (Parra et al., 2009; Roukos et al., 2009). Surprisingly, the concentration of a number of VOCs is higher in indoor air than in outdoor air due to the presence of additional indoor pollution sources such as cooking and the synthetic materials used for decoration and construction (Huang et al., 2011). Unlike discontinuous and highly contaminating industrial emissions, the individual concentration of each contaminant in indoor air is generally low, although a wide range of compounds can be found simultaneously (Guieysse et al., 2008). Nevertheless, alkanes and aromatic hydrocarbons make up half of the one hundred and thirteen VOCs that have been qualitatively identified by Gallego et al. (2009). According to Ohura et al. (2009), the profile of VOCs in living rooms in Japanese homes shows that toluene prevailed, followed by ethylbenzene.

Toluene and ethylbenzene are known to be highly toxic and mutagenic. Chronic exposure even to low doses of toluene could cause damage to immune and neurological functions (Nikiema et al., 2007). Continuous exposure to VOCs could potentially generate a multitude of health effects, such as immune disorders like asthma and allergies, cellular effects such as cancer, as well as cardiovascular, neurological, and sensory effects, and damage to the liver, kidneys, and central nervous system.

Overall, the continental EU air emissions of those two aromatic organic compounds (toluene and ethylbenzene) were 976 kT y^{-1} and 79 kT y^{-1}, respectively (EURAR-T, 2003; EURAR-E, 2007), accounting for 3 % and 0.02 %, respectively, of total non-methane volatile organic compound (NMVOC) emissions. Increasingly restrictive environmental regulations on air emissions and air quality have required the development of effective treatment technologies.

Current technical solutions for improving air quality include combinations of air filtration, ionization, activated carbon adsorption, ozonation and photocatalysis (Guieysse et al., 2008). However, biological methods (conventional biofilters and biotrickling filters) have proven their potential to remove VOCs at low-moderate concentrations of contaminants (lower than 5 – 6 g m^{-3}) and high flows (from 1000 to 10000 Nm^3 h^{-1}) (Weber and Hartmens, 1996; Deshusses et al., 1999; Barona et al., 2004). Moreover, biofiltration is an environmentally friendly and cost-effective alternative because pollutants are finally converted into innocuous compounds such as CO_2, H_2O, and biomass at room temperature (Lebrero et al., 2011).

These methods can be used for the treatment of industrial emissions (Kraakman, 2003; De Visscher and Li, 2008), the deodorization of air emissions (Liu et al., 2009) and the treatment of indoor air (Guieysse et al., 2008).

Many reports have noted the inhibiting effects or enhancing response of biofilters when feeding separate substrates or their mixtures (Qi et al., 2002, Nikolova and Nenov, 2005, García-Peña et al., 2008). García-Peña et al. (2008) found that benzene degradation was negatively affected by both toluene and ethylbenzene and that the toluene degradation rate was hindered by the presence of benzene. Nikolova and Nenov (2005) found that ethylbenzene was easily degraded by different fungal strains in mixtures. Nevertheless, only a handful of researchers have evaluated the performance of biofiltration in the removal of ethylbenzene as a sole contaminant in gas streams (Gabaldón et al., 2006; Álvarez-Hornos et al., 2008).

The goal of this research is to investigate the long-exposure performance of two laboratory-scale biofilters to treat toluene and ethylbenzene as separate substrates. This model of laboratory-scale biofilter used organic wastes for obtaining the packing material and the acclimated inoculum. The support medium (based on wastes like pig manure and sawdust) has already been used by the authors to effectively treat volatile sulphur compounds (Elías et al., 2002).

4.2. Materials and methods

4.2.1. Inoculum preparation and packing material

An aerobic activated sludge, which was collected in the aerobic tank of a sewage treatment plant in Muskiz (Bizkaia, Spain) was used as inoculum. Initially, the activated sludge was continuously exposed to an ethylbenzene and toluene-laden air for 30 days in two different stirred tank bioreactors. The aeration rate was 0.4 vvm (air volume per unit of liquid volume per minute). Contaminant concentration for acclimation purposes ranged from 50 to 100 ppm_v. The effective adaptation of the microbial population to the VOCs in the liquid phase was tested by measuring the contaminant degradation rate after different acclimation periods. This procedure has already been outlined in a previous paper (Elías et al., 2010).

The packing material selected for this research was a commercial soil amendment called ABONLIR, supplied by SLIR S.L. It was made up of composted pig manure and sawdust and was manufactured by mechanical compression with no chemical addition. The pH of this organic material was 8.5 and it showed a good natural buffer capacity for treating H_2S in previous studies (Barona et al., 2004). The main characteristics of the packing material are summarized in a previous paper (Elías et al., 2002).

4.2.2. Biofilter and experimental conditions

Two laboratory-scale bioreactors (biofilters) with an effective volume of 4.5 l were set up to treat an air flow contaminated with ethylbenzene and toluene, respectively. A similar biofilter design with three PVC modules has already been used by the authors in previous research for

treating H_2S and *para*-xylene (Elías et al., 2002; Gallastegui et al., 2011). The schematic of one of the two identical biofilters used in this study is shown in Figure 4.1.

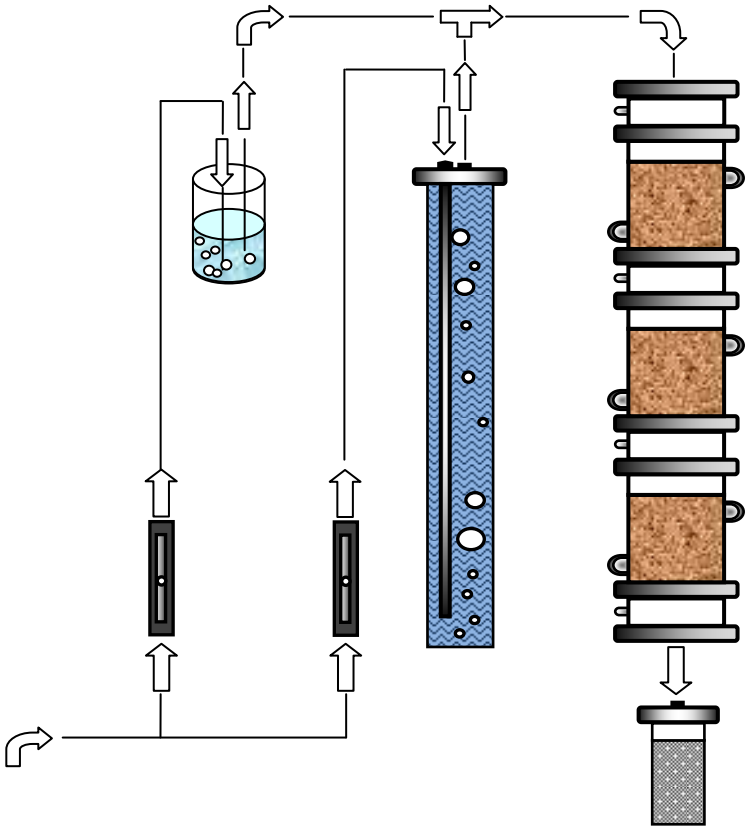

Figure 4.1. Outline of the biofiltration system.

The inlet flow was generated by mixing a dry contaminant-saturated air stream with a humid clean air stream. The non-humidified fraction of air used for contaminant saturation was a minor fraction of the whole influent air flow and did not significantly alter the moisture of the contaminated air stream entering the biofilter. Thus, the relative humidity of the contaminated air at the biofilter inlet remained higher than 98 %. The biofilter was equipped with several gas sampling valves to monitor inlet and outlet contaminant concentration. It was additionally fitted with several ports located along the three PVC modules for measuring room and bed temperatures as well as the relative humidity of the inlet air stream. These ports were also used for sampling the packing material in order to measure the MC (moisture content) in the bed throughout experimentation. The biofilters were operated for more than a year. During the operating period, room temperature oscillated between 18 and 30 °C.

The experimental study of biofilter performance for both contaminants was divided into three stages according to the operating parameters. The range of selected operating parameters for each stage is summarized in Tables 4.1a and 4.1b.

Table 4.1a. Experimental conditions for the biofilter treating ethylbenzene.

Ethylbenzene Biofilter					
Stage	Days	EBRT (s)	Operation Mode	Inlet Load Range (g m^{-3} h^{-1})	Average Removal Efficiency (%)[a]
I	1 – 228	230	Down-flow	10 – 186	90.9 ± 3.3 [(1)]
II	229 – 322	100	Down-flow	26 – 146	72.7 ± 5.6 [(2)]
III	323 – 415	260	Up-flow	9 – 56	75.0 ± 6.4 [(3)]

Notes: (a) At a 95 % confidence level; n (number of data points for calculating average values): [(1)] n = 55; [(2)] n = 40; [(3)] n = 25.

Table 4.1b. Experimental conditions for the biofilter treating toluene.

Toluene Biofilter					
Stage	Days	EBRT (s)	Operation Mode	Inlet Load Range (g m^{-3} h^{-1})	Average Removal Efficiency (%)[a]
I	1 – 227	229	Down-flow	6 – 129	84.5 ± 5.9 [(1)]
II	228 – 342	89	Down-flow	25 – 221	61.0 ± 6.7 [(2)]
III	343 – 472	225	Up-flow	6 – 68	87.5 ± 5.7 [(3)]

Notes: (a) At a 95 % confidence level; n (number of data points for calculating average values): [(1)] n = 55; [(2)] n = 40; [(3)] n = 25.

The frequencies of nutrient solution addition and filter material mixing were established separately for each model to maintain the removal efficiency (RE) of biofilters under 85 % for ethylbenzene and 70 % for toluene in Stage I, and below 50 % during the other stages. The composition of the mineral salt medium added as the nutrient solution has been previously reported by Elías et al. (2010). This nutrient solution includes several salts such as KH_2PO_4 and K_2HPO_4 whose buffer activity promotes neutral or slightly acid conditions within the biofilter. The filter material mixing operation was performed by manually homogenizing the material on a tray, spreading the nutrient solution over the material and, finally, filling back the solid into the module. About 20 – 30 minutes were required to complete the operation.

4.2.3. Analytical procedure

Ethylbenzene and toluene concentrations in the inlet and outlet gas flows were measured using a micro-gas chromatograph CP 4900 (Varian, The Netherlands) equipped with auto-sampling injection, a TCD detector, and using He as carrier gas. The Micro GC was equipped with a CP-Sil 5 CB column (6 m x 0.15 mm x 2 µm) and a CP PoraPLOTQ column (10 m x 0.25 mm x 8 µm). The oven, injector and TCD detector were maintained at 90 °C, 110 °C and 90 °C, respectively. Air relative humidity and temperature at the inlet, middle and outlet sampling ports on the biofilter column were measured using a Testo 625 sensor (Testo, Germany). The temperature of the packing material was recorded with a thermocouple HI-98804 sensor (Hanna Instruments, Italy). The evolution of MC within the packing material throughout the experimentation stages was recorded by a HB43-S Halogen Moisture Analyzer (Mettler Toledo, Ohio, USA) by periodically collecting 2 – 3 g of support material from each module.

Biomass samples were micrographed using a scanning electron microscope (Hitachi S-4800, Japan) at 15 kV accelerating voltage. The samples were extracted from the packing material and fixed in 2 % glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4), washed in an iso-osmolar cacodylate/sucrose buffer and post-fixed in 1 % osmium tetroxide in a cacodylate buffer. After repeated washing, samples were dehydrated through an ethanol series and washed in hexamethyldisilazane prior to air drying. Finally, samples were mounted onto stubs and gold-coated using a JEOL fine coat ion sputter JFC-1100.

The mass units for all the parameters used in this study were the amount of C expressed as g C instead of using the corresponding amount of ethylbenzene or toluene. The rationale for this selection is informed by the need to compare the behaviour of two biofilters fed with different contaminants. The only exception is the amount of CO_2 produced, which has been expressed in g CO_2 m^{-3} h^{-1}.

4.2.4. Start-up

The biofilters were first irrigated with the previously activated sludge. Initially, both biofilters were deliberately operated at low loading rates of ethylbenzene and toluene (< 15 g m^{-3} h^{-1}) corresponding to low inlet concentration and high empty bed residence time (EBRT) (0.5 g m^{-3} and 250 s, respectively) to facilitate the development of an efficient microbial community attached to the solid packing material. The empty bed residence time (EBRT) represents the mean residence time a volatile compound would theoretically spend in an unpacked biofilter (Kennes and Veiga, 2001). Steady-state condition was achieved for both biofilters after 8 – 10 days of operation, which was concluded from the constantly high value of the RE, close to 100 % (data not shown).

The previous adaptation of the inoculum to the contaminants is strongly encouraged to ensure the growth of suitable biomass. Other available and easily degradable carbon sources (such as glucose) were dismissed, as several studies have shown the negative consequence of this

addition, resulting in a quick growth of unspecialized biomass (Elías et al., 2010; Vergara-Fernández et al., 2011).

4.3. Results and discussion

4.3.1. Biofilter performance: Stages I and II

The inlet load of ethylbenzene (IL_e) during Stage I ranged from 10 to 186 g m^{-3} h^{-1} for a constant EBRT of 230 s (Table 4.1a). During this experimental stage, the average RE was 90.9 ± 3.3 % and the highest elimination capacity (EC_{MAX}) recorded was 170 g m^{-3} h^{-1}. In order to improve the performance of the biofilter when the RE was below 85 % (days 103, 130 and 182) or when sudden load increases were fed (days 53 and 210) (Figure 4.2a), the filter material for each module was individually homogenized and the nutrient solution was irrigated from the top module. The frequency of these operations is shown in Figures 4.2a and 4.2b by arrows over the x axis. A sustainable RE close to 100 % was maintained for 50 days when the ethylbenzene inlet concentration (IC_e) was below 2.2 g m^{-3} (3^{rd} homogenization on day 130).

During Stage II, the gas flow rate was increased so that the EBRT was 100 s. The IL_e varied from 26 to 146 g m^{-3} h^{-1}. An average RE of 72.7 ± 5.6 % was recorded, with an EC_{MAX} of 116 g m^{-3} h^{-1}. These results were consistent with those reported by other authors who obtained a similar EC_{MAX} of 120 g m^{-3} h^{-1} when using a packing material made of fibrous peat at an EBRT of 127 s (Gabaldón et al., 2006; Álvarez-Hornos et al., 2008).

Bearing in mind that the overly frequent application of homogenization and nutrient addition strategies could involve unnecessary water addition, changes in bed porosity, erosion of packing material and, finally, high pressure drop, they were applied only when RE was under 50 % (on days 235, 251, 276 and 291). Nevertheless, the improvement in biofilter performance did not last long, which is consistent with other previous studies showing that enhanced removal after homogenization was efficiently maintained for only 2 – 4 days (Ortiz et al., 2003; Znad et al., 2007).

Contrary to expectations from the visual appearance of biomass growth, pressure drop across the biofilter never exceeded 40 mm H_2O, which did not significantly hinder the overall performance. Bibeau et al. (1997) reported a ceiling value of 60 mm H_2O per meter of bioreactor after which compaction problems occurred in a biofilter packed with peat. Periodical bed unpacking or homogenization had a positive effect in decreasing pressure drop and in avoiding the accumulation of inactive biomass within the biofilm phase, as also reported by other authors (Song and Kinney, 2005; Maestre et al., 2007; Znad et al., 2007). Thus, although water saturated zones and the partial disintegration of the organic material were observed at the bottom of the columns, homogenisation and nutrient addition were applied to counteract their negative consequences.

Although homogenization has proven to be an interesting strategy (Delhoménie et al., 2003), it is not usually performed in full-scale biofilters due to the large volume of material to be mixed.

As far as the toluene biofilter is concerned, the inlet load of toluene (IL_t) during Stage I ranged from 6 to 129 g m^{-3} h^{-1} for a constant EBRT of 229 s (Table 4.1b). During this experimental stage, the average RE was 84.5 ± 5.9 % and the EC_{MAX} recorded was 99 g m^{-3} h^{-1}. Homogenization and nutrient addition strategies were applied four times after the RE fell below 70 % (arrows over the x axis in Figure 4.2b).

The EBRT during Stage II was considerably lower (89 s), which rendered a sharp decrease in the average RE (61.0 ± 6.7 %) for an inlet load ranging from 25 to 221 g m^{-3} h^{-1}. Nevertheless, a punctual EC_{MAX} of 138 g m^{-3} h^{-1} was recorded. In spite of the high frequency of homogenization and nutrient addition (7 times in 114 days) to improve biofilter performance, the RE of toluene always kept below 70 %.

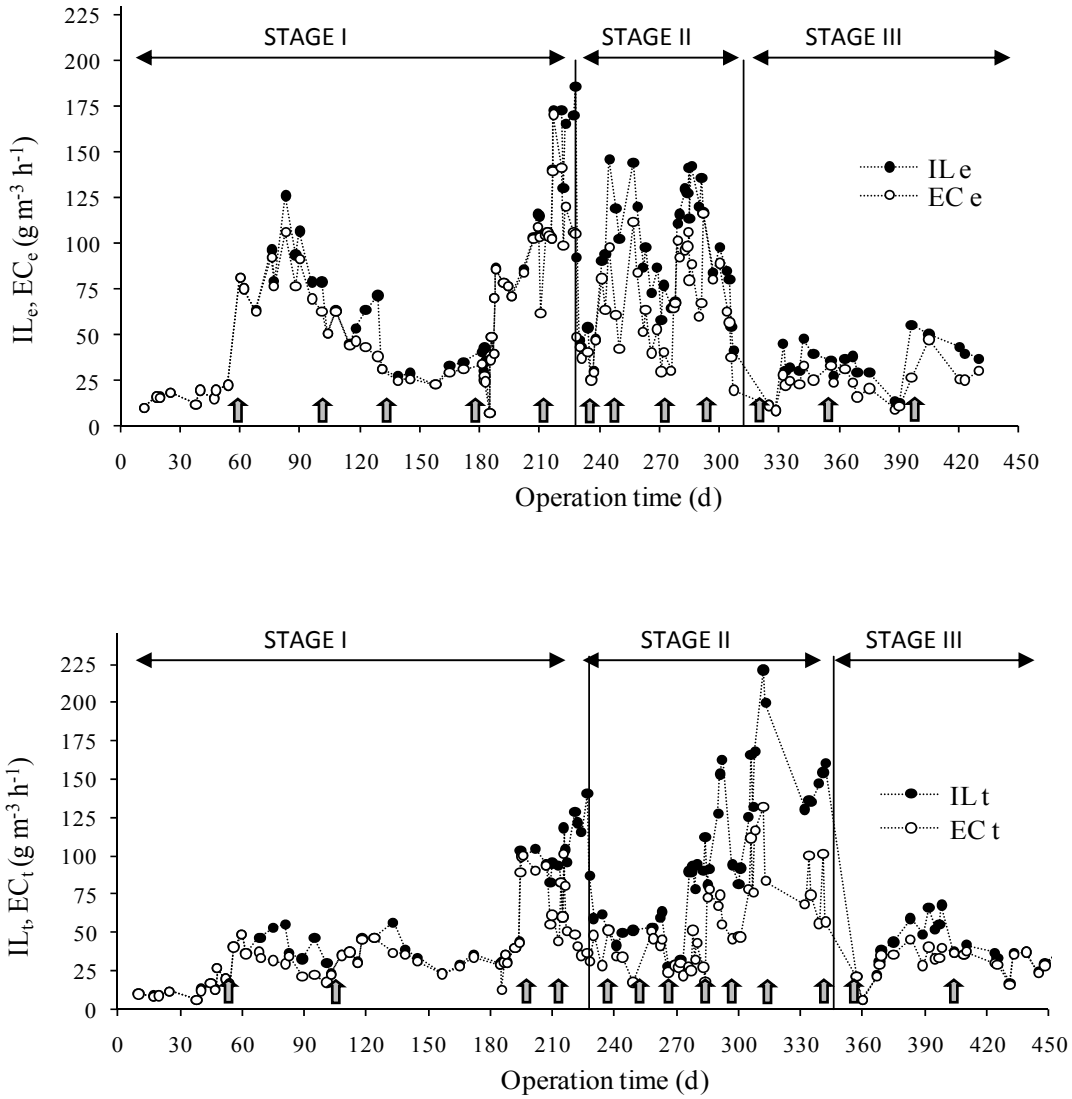

Figure 4.2. Evolution of the inlet load (IL) and elimination capacity (EC) with operation time. Arrows over the x axis represent nutrient solution addition and packing material homogenisation. (a) Top: ethylbenzene degrading biofilter; (b) Bottom: toluene degrading biofilter.

4.3.2. Influence of flow rate: Stages I and II

Air flow rate is a major limiting parameter in the biodegradation process. The elimination efficiency of ethylbenzene and toluene (expressed as the amount of C removed per m^3 of treated air, $C_{REMOVED}$) was plotted against IL_e and IL_t, respectively (Figures 4.3a and 4.3b). The experimental data were obtained from Stages I and II, and two EBRT were tested for each contaminant (Tables 4.1a and 4.1b).

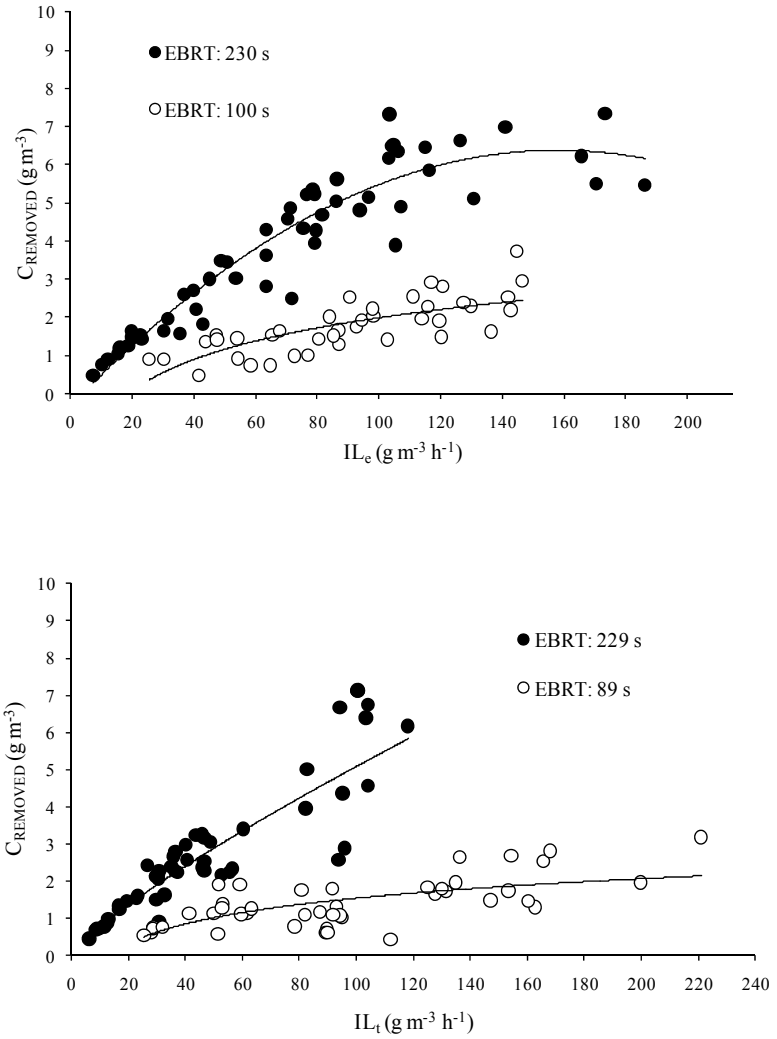

Figure 4.3. Concentration of removed carbon as inlet load is increased (Stages I and II) for (a- top) ethylbenzene degrading biofilter; (b- bottom) toluene degrading biofilter.

The higher EBRT in both cases (230 s for ethylbenzene and 229 s for toluene) rendered a linear response between the IL and $C_{REMOVED}$. The highest value of $C_{REMOVED}$ ranged from 7.5 to 7.1 g m^{-3} when the IL for both compounds was in the 100 to 120 g m^{-3} h^{-1} range.

Nevertheless, when the EBRT for the ethylbenzene biofilter was reduced to 100 s during Stage II, the slope of the linear adjustment was twofold lower than the value for the higher EBRT. It was therefore concluded that the contact time between the contaminant and the bed was too low for the biomass to fully degrade the substrate.

Regarding the toluene biofilter, when the EBRT was 89 s (Stage II) and the IL_t was higher than 140 g m^{-3} h^{-1}, the $C_{REMOVED}$ remained nearly constant. For IL_t values lower than 140 g m^{-3} h^{-1}, the EBRT was too low to ensure the degradation of toluene with a high RE. Therefore, the EBRT should exceed the time required for diffusion processes, as is the case for low operating flow rates. If flow rate is too high, the contact time between microorganisms and gas is too short, and consequently, the biodegradation reaction cannot be completed.

4.3.3. Influence of flow mode: Stage III

The behaviour of both bioreactors after a change in the flow mode (from down-flow to up-flow) was studied during Stage III (Tables 4.1a and 4.1b). Although the IL for both compounds was low after the flow mode change, a gradual increase in contaminant concentration, up to 56 g m^{-3} h^{-1} for ethylbenzene and up to 68 g m^{-3} h^{-1} for toluene, led to a gradual deterioration in the response of both biofilters, recording a RE below 65 % for the highest IL values (Figures 4.2a and 4.2b).

For a brief period of time after flow mode change, biofilter performance was not influenced by the flow reversal. Thus, a RE of 100 % was achieved for ethylbenzene and toluene when IL was lower than 12 g m^{-3} h^{-1} and 21 g m^{-3} h^{-1}, respectively, for the first 5 – 9 days after the flow mode change. However, gradual increases in contaminant concentration up to 48 g m^{-3} h^{-1} for ethylbenzene and 68 g m^{-3} h^{-1} for toluene were detrimental to both biofilters, reaching a final RE of under 65 % after 25 – 30 days of operation.

In the case of ethylbenzene, consecutive homogenizations were applied on days 323, 356 and 397. An immediate and momentary improvement in the EC was measured within 12 – 24 h after homogenization, but it did not last long and, consequently, a new critical EC [$EC_{CRITICAL}$] (defined as the loading rate where the elimination capacity is exceeded and the RE will consequently be lower than 100 %) at 25 – 30 g m^{-3} h^{-1} was established.

In the case of toluene, the packing material was remixed on days 357 and 402. The second homogenization allowed a sustained $EC_{CRITICAL}$ of around 35 g m^{-3} h^{-1} and a RE higher than 80 %, which were maintained for 60 days. However, the biofilter could not handle the higher IL as in Stage I, where an $EC_{CRITICAL}$ of around 70 – 80 g m^{-3} h^{-1} was recorded.

According to the literature, the prolonged exposure of biomass to contaminants may reduce the active fraction of a biofilm and lead to a gradual and selective loss of catabolic functions (Arcangeli and Arvin, 1992; Leddy et al., 1995). Omri et al. (2011) found a sharp drop in species diversity in the inlet gas zone in a peat biofilter treating H_2S in downflow mode, and Bayle et al. (2009) reported that the low diversity of the main degrading bacterial groups led to a low effective performance in terms of elimination efficiency.

The first module was the zone supporting the higher mass load during downflow operation. Thus, a simplification of the genetic structure of the total microbial community with the appearance of a dominant microflora in this region could be expected (Omri et al., 2011).

The lower the diversity of the microbial community, the more resistant the system is to recovering from disturbances. Specifically, Cabrol and Malhautier (2011) stated that the resilience capacity of a biofilter (engineering resilience is the rate at which a system returns to its original state after being disturbed) is high when the microbial community is flexible and can induce metabolic and structural changes. In consequence, after long exposure periods (228+93 and 227+114 days for ethylbenzene and toluene, respectively), a genetically simplified (or "non-diverse") microbial community is expected to hinder the recovery of a high and stable removal performance after severe system disturbances, such as flow reversal.

Based on these results, switching the flow mode is not advisable if the biofilter has been fed for a long time under the same flow mode because this change cannot promote the growth of stressed biomass (or at least not in a short time).

4.3.4. Degradation profile along the bed

In order to understand the dynamics of the removal of both contaminants, the profile of the normalized concentration (the ratio between the outlet and inlet concentrations, C_{out}/C_{in}) during Stage I was plotted along each biofilter height (Figure 4.4a and 4.4b).

When the inlet concentration of ethylbenzene is low (2.02 ± 0.42 g m^{-3}), the distribution curve of the ethylbenzene concentration along the filter bed showed that the upper module of the filter bed made a significant contribution to the overall RE (Figure 4.4a). This could be explained by the presence of a higher amount of available carbon source (ethylbenzene) in the upper zone, which favoured the transfer of contaminant mass to the biofilm, rendering a higher metabolic reaction and biodegradation rate. The bottom section of the biofilter hardly presented any microbial activity at all (< 5 %).

As inlet concentration increased to 5.39 ± 0.43 g m^{-3}, the overall RE was maintained at high values (92 %), but both the mass transfer capacity and the reaction rate of the upper section of the bed were exceeded, and contaminant moved into the downstream section. Thus, spatial RE in the biofilter became more uniform between the first two sections (44 % and 38 %, respectively). However, microbial population and reaction capacity remained low in the lowest part of the biofilter (RE of 10%). This evidence is consistent with other authors, who concluded

that for low xylene IL_x (12.8 g m^{-3} h^{-1}), the contribution of the different sections of the biofilter was not evenly balanced (Elmrini et al., 2004).

In the latter case, a high ethylbenzene concentration (8.72 ± 0.78 g m^{-3}) exceeding typical VOC values from 0 to 5 g m^{-3} in biofilters was tested (Delhoménie and Heitz, 2005). Each section eliminated 27 %, 17 % and 31 %, respectively, although ethylbenzene was not completely removed from the biofilter.

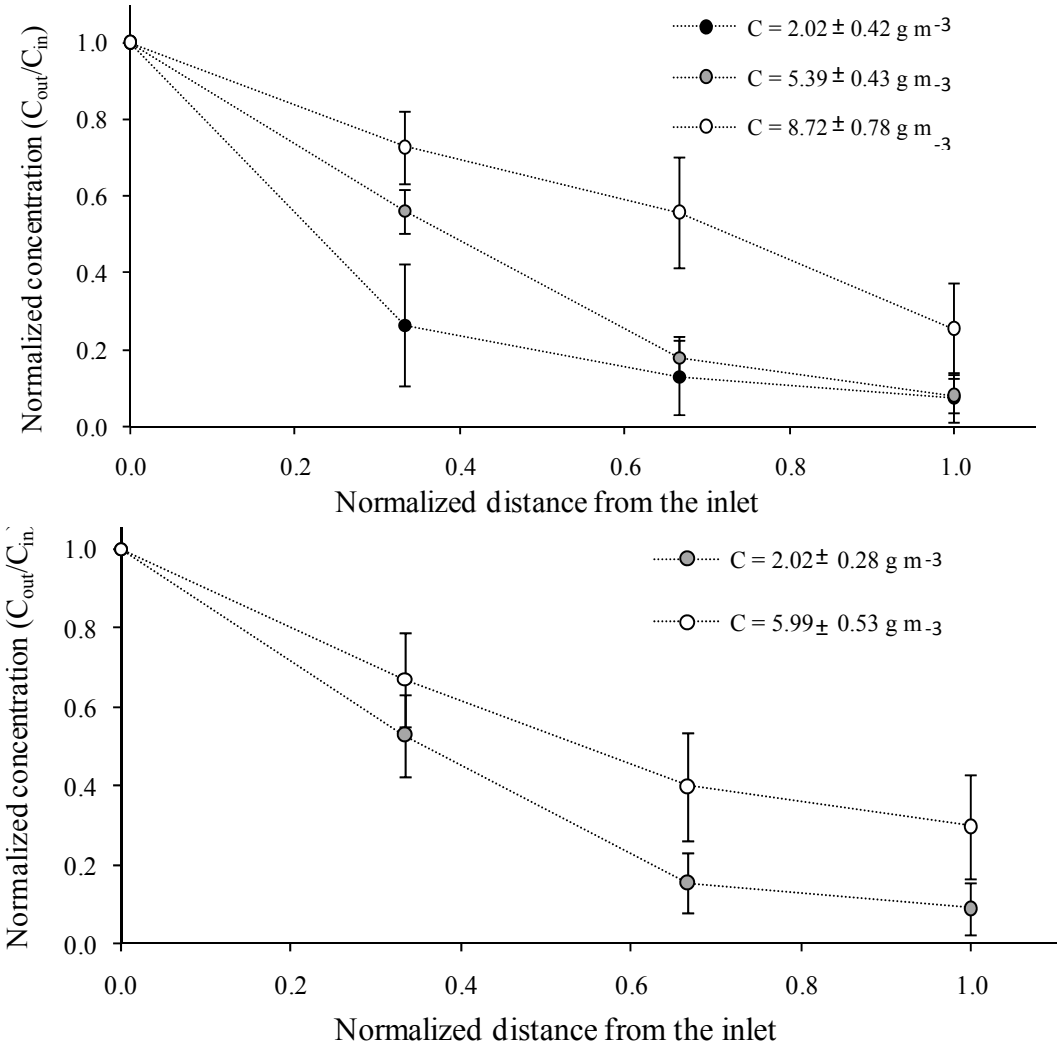

Figure 4.4. Contribution of each module to (a- top) ethylbenzene degradation; (b- bottom) toluene degradation (during Stage I).

As far as the toluene biofilter is concerned, a similar behaviour was found for two concentrations (Figure 4.4b). As shown in this figure, the two first sections accounted for 60 % and 82 % of the total RE for the high and low concentrations, respectively. The last section in

both cases (for a normalized distance from the inlet of 0.68 onwards) has a similar slope, although the biofilter with an inlet concentration of 2.02 g m^{-3} had a higher overall RE (about 90 %) compared to 67 % for the higher inlet concentration.

4.3.5. Evolution of MC

The presence of water is an important parameter in biofiltration studies because microorganisms require a minimum water content for optimal growth and activity. The influence of bed MC on the amount of removed C per m^3 of air and litre of biofilter ($C_{REMOVED}$) is shown in Figure 4.5a and Figure 4.5b for both contaminants. Figures 4.5a and 4.5b were constructed by plotting the experimental points obtained when the biofilters were governed by microbial activity and had an inlet concentration higher than 1 g m^{-3} and a RE lower than 75 %.

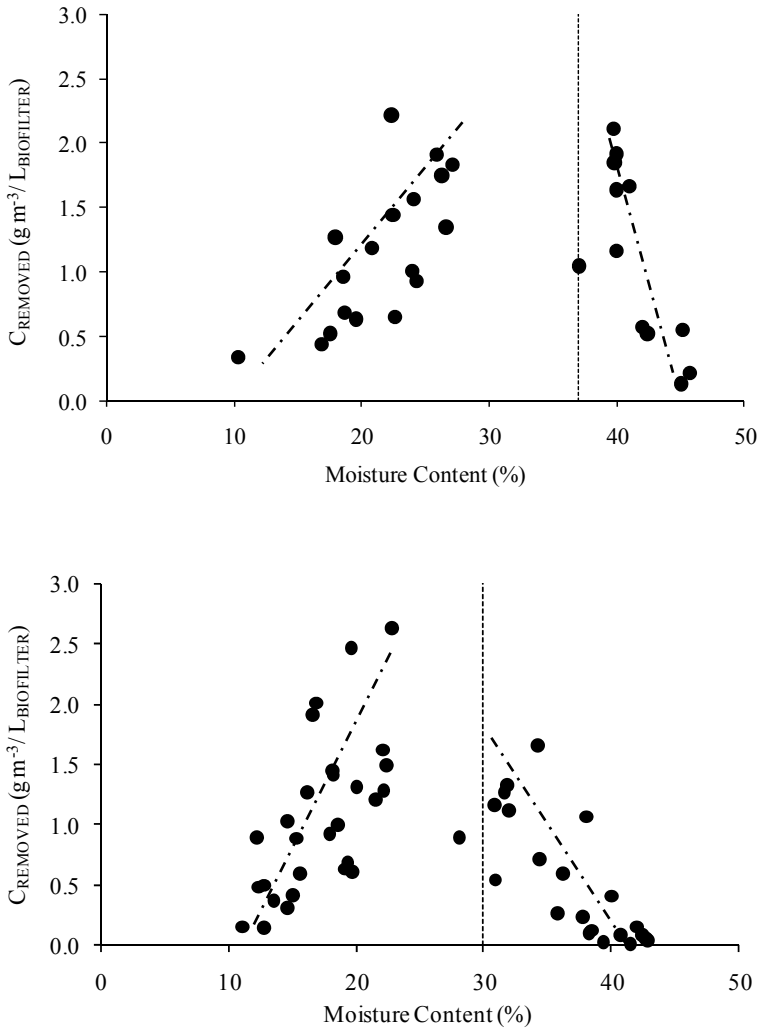

Figure 4.5. Relationship between MC in the packing material and the concentration of removed carbon per volume of biofilter treating (a- top) ethylbenzene; (b- bottom) toluene.

A sudden decrease in the performance of both biofilters occurred when the MC value was higher than 37 % for ethylbenzene and 30 % for toluene. Water saturated zones and partial disintegration of the organic material, which are considered to be intrinsic characteristics of natural organic materials, were visually observed at the bottom of the columns when those limit values were exceeded. This phenomenon may be explained by the formation of anaerobic zones and the increasing mass transfer resistance at the gas-biofilm interface. MC in the range between 30 % and 60 % wt/wt has been recommended by Nikiema et al. (2007) for optimum biodegradation activity in organic filter media. Nevertheless, the recommended MC for the organic packing material in this study ranged from 15 % to 30 %.

Morales et al. (2003) used a dynamic drying study to estimate the critical water content for a peat bioreactor treating toluene. They found that with a MC below 58 % there was insufficient flux of water to maintain optimum EC. Shareefdeen et al. (2010) likewise observed that a peat biofilter performed efficiently when the water content was around 50 %.

The explanation for the low MC optimal range could be related to the fungal invasion within the bioreactors. The development of fungi over bacteria in biofilters allows maintaining a reduced MC, as they are known to maintain their activity under these conditions (Prenafeta-Boldú et al., 2008). Aizpuru et al. (2005) measured higher EC (275 g m^{-3} h^{-1}) in a fungal biofilter treating toluene when MC was 19 %, while an EC of around 185 g m^{-3} h^{-1} was obtained when MC was 47 %.

4.3.6. Temperature

The relationship between biofilter performance and filter bed temperature is shown in Figures 4.6a and 4.6b. The experimental data points were taken from the three modules during stage I. The biodegradation of organic contaminants is known to be an exothermic process; it thus generates heat that is released into the biofilter, increasing the temperature of the system (packing material + outlet gas stream). Regarding the influence of MC on bed temperature, a modified temperature parameter was proposed (ΔT* = ΔT/MC). Hence, the effect of removed contaminant concentration (expressed as grams of removed C per m^{-3} of air and per litre of biofilter) on this pseudo-temperature increase (ΔT*) is illustrated for the biofilters treating ethylbenzene (Figure 4.6a) and toluene (Figure 4.7b) during Stage I.

As far as the ethylbenzene bioreactor is concerned, pseudo-temperature increases (ΔT*) of between 0.01 – 0.04 °C were recorded when the $C_{REMOVED}$ values were lower than 1.0 g m^{-3} $l_{Biofilter}^{-1}$. When the $C_{REMOVED}$ values were higher than 1.0 g m^{-3}, an exponential increase in the pseudo-temperature was reported, achieving a maximum value of 0.20 °C for an ethylbenzene elimination concentration of 1.95 g $m^{-3}l_{Biofilter}^{-1}$. A similar performance was recorded with the biofilter degrading toluene. Low $C_{REMOVED}$ values (≤ 1.0 g $m^{-3}l_{Biofilter}^{-1}$) rendered a ΔT* lower than 0.03 °C. In this case, a maximum value of 0.23 °C was observed for a $C_{REMOVED}$ of more than 2.50 g m^{-3} $l_{Biofilter}^{-1}$.

Different patterns are found when comparing these results with previously published studies. Son and Striebig (2001) observed that for every 1 kg of ethylbenzene degraded per cubic meter

of biofilter medium (a compost mixing hardwood saw dust and municipal wastewater sludge), a temperature increase of 0.41 °C was measured. Delhoménie et al. (2005) found that temperature increased 3.0 °C per g m^{-3} of toluene removed for a biofilter filled with a compost and an organic binder (90:10, v:v) and with a constant MC around 55 – 60 %. Singh et al. (2010) found a difference of 1.5 – 2.5 °C between bed temperature and ambient temperature due to exothermic bio-oxidation taking place in the wood charcoal media degrading toluene.

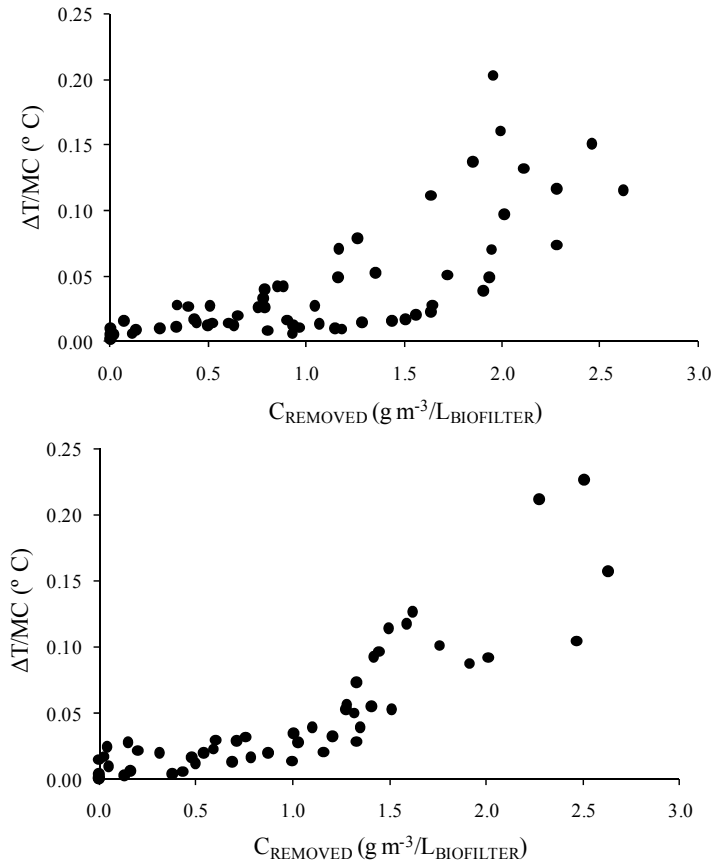

Figure 4.6. Relationship between the pseudo-temperature increase and the concentration of removed carbon per volume of biofilter treating (a-top) ethylbenzene; (b- bottom) toluene.

4.3.7. Evolution of CO_2

Monitoring the CO_2 concentration in the gas phase provided information on biomass activity and the contaminant's mineralization level. The CO_2 production rate (P_{CO2}) during down-flow mode (Stages I and II) as a function of the EC for ethylbenzene and toluene is shown in Figures 4.7a and 4.7b, respectively. Based on these figures, the production of P_{CO2} (expressed as g CO_2 m^{-3} h^{-1}) was concluded to linearly increase along with the EC for both EBRT tested in Stages I and II.

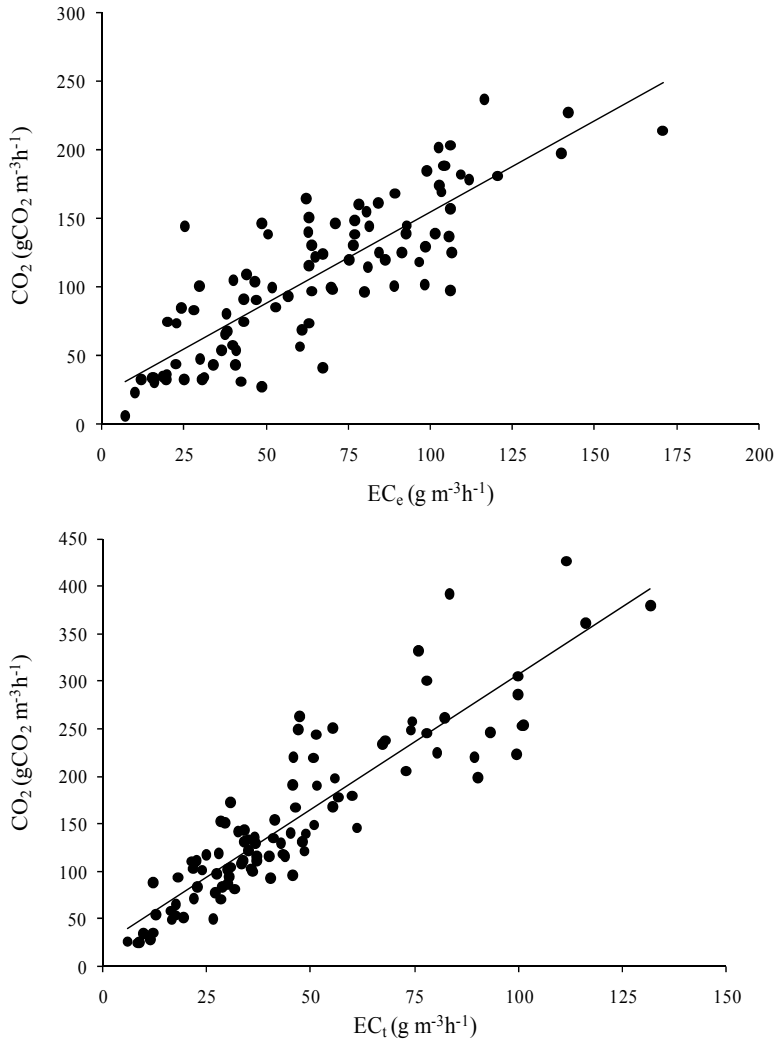

Figure 4.7. Carbon dioxide production rate during down-flow mode (Stages I and II) as a function of the EC for (a- top) ethylbenzene; (b- bottom) toluene.

A linear regression, calculated according to the least-squares method, provided the following equations for ethylbenzene and toluene degradation:

Ethylbenzene biofilter: $P_{CO2} = 1.36EC + 21.8$ $R^2 = 0.71$ (1)

Toluene biofilter: $P_{CO2} = 2.84EC + 23.1$ $R^2 = 0.80$ (2)

where P_{CO2} is expressed in g CO_2 m^{-3} h^{-1} and EC in g C m^{-3} h^{-1}.

According to equations (1) and (2), the biodegradation of toluene rendered an amount of CO_2 twofold higher than that for ethylbenzene, assuming a similar elimination capacity. The significant disparity between the fractions recovered as CO_2 for both contaminants might be attributed to the dominant degrading species within the biofilter. Specifically, Six et al. (2006) stated that the production of microbial biomass and by-products is greater in solid supports (soils) where the microbial community is composed predominantly of fungi, as fungi have

higher "microbial growth efficiency" than bacteria. Fungal-dominated communities will, therefore, retain more C in biomass per unit substrate consumed and release less as CO_2.

Regarding the hypothesis on the production of volatile intermediates to explain CO_2 emission, an increase in CO_2 production and an increase in O_2 consumption (together with a constant TOC) are related to the straight biodegradation of toluene without intermediates (Muñoz et al., 2008). Other authors (Yu et al., 2001) have concluded that when oxygen is available to *P. putida* F1, toluene is transformed by a dioxygenase reaction that produces a catechol intermediate, but does not support biomass growth. Therefore, the reaction rate from toluene to the intermediates and the degradation rate of the intermediates may be related to CO_2 accumulation.

According to Six et al. (2006), low quality substrates (high C/N) favour fungi and high quality (low C/N) substrates favour bacteria. The C/N ratio of the organic packing material used in our study was 10.1 and, thus, a high fungi/bacteria biomass ratio is expected to be found in both biofilters.

Several SEM photographs of the packing material taken after 110 days of operation in the toluene bioreactor showed that fungi had colonized the biofilter (Figure 4.8a).

The SEM photographs for the ethylbenzene bioreactor were similar and have not been included. As shown in Figure 4.8b, bacteria seem to be attached onto the fungal hyphae surface, which is in agreement with Kohlmeier et al. (2005), who reported that fungal hyphae acted as vectors for bacterial transport, suggesting stimulation on the mobilization of pollutant-degrading bacteria, and improved the biodegradation rate. Synergism between fungi and bacteria for the mineralization of aromatic hydrocarbons has been previously posited (Stapleton et al., 1998; Li et al., 2008).

Nevertheless, SEM photographs show only an extremely reduced area of the whole packing material surface and, obviously, no assertion can be made about the dominant species in each biofilter. Thus, the individual contributions of fungi and bacteria to CO_2 production in the two biofilters could not be established. Therefore, the only measure of CO_2 was not concluded to be a determining index for explaining the EC recorded in the two biofilters. Further detailed microbiological studies are required to explain the biofilter response on the basis of CO_2 production.

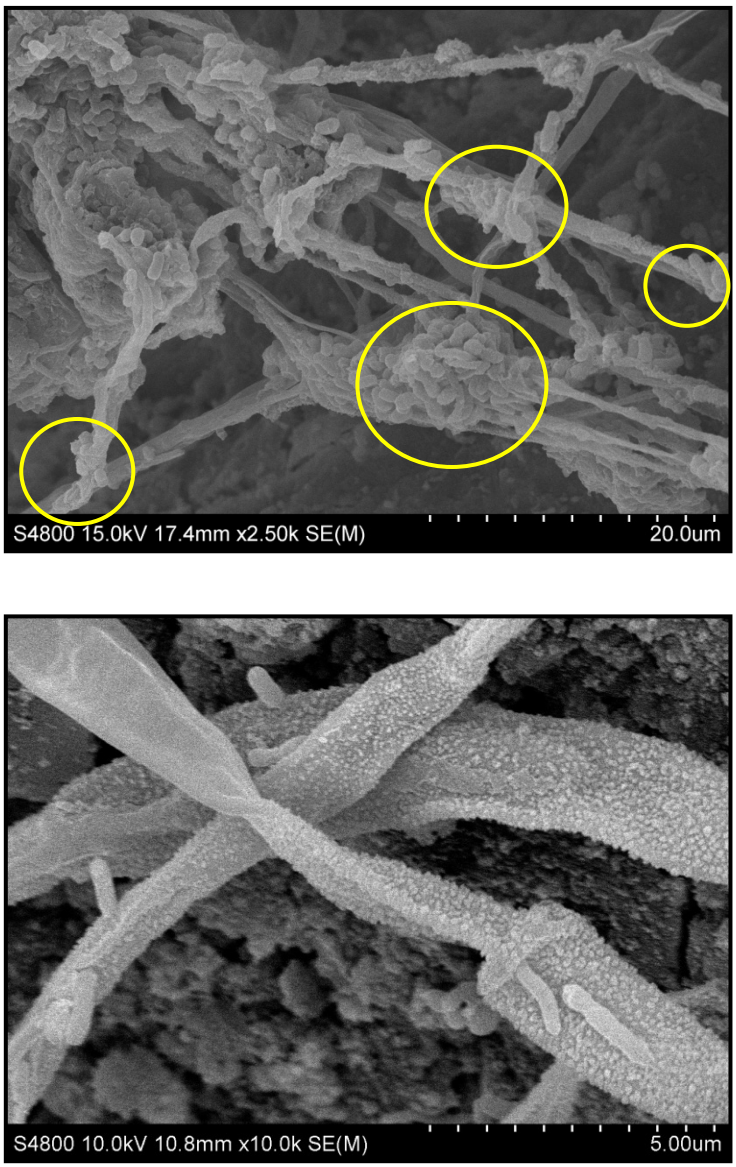

Figure 4.8. SEM photographs of the packing material taken after 110 days of operation in the toluene bioreactor.

4.4. Conclusions

Two biofilters filled with an organic waste material for treating ethylbenzene and toluene were operated under similar conditions for 415 and 472 days, respectively, and the influence of several relevant operating parameters was reported over time. As expected, the higher EBRT in both cases (230 s for ethylbenzene and 229 s for toluene) rendered a linear response between the IL and the concentration of removed carbon ($C_{REMOVED}$). However, when EBRT was decreased to 100 s for ethylbenzene and 89 s for toluene, the average RE was as low as 72.7 % for the former and 61 % for the latter. As far as the degradation profile along the biofilter is

concerned, as inlet concentration was increased, the mass transfer capacity and the reaction rate of the upper section of the bed were exceeded and the contaminants moved into the downstream section. The MC of the packing material proved to be a crucial factor for bioreactor efficiency. A sudden decrease in the performance of both biofilters occurred when the MC value was higher than 37 % for ethylbenzene and 30 % for toluene. Thus, the recommended MC for this organic material ranged between 15 % and 30 %, which is a very low value in comparison with other materials. The bioreactors' high RE when operated at low MC was attributed to the synergism effect between a prevailing fungi colony and bacteria attached to the fungal hyphae. Nevertheless, the individual contributions of fungi and bacteria to CO_2 production (and consequently to EC) in the two biofilters could not be established.

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5. BIOFILTRATION OF MIXTURES

Performance and macrokinetic analysis of biofiltration of toluene and p-xylene mixtures in a conventional biofilter packed with inert material (*Bioresour. Technol. 102 (17), 7657 – 7665*)

5.1. Introduction

Toluene and *para*-xylene, together with benzene and ethyl benzene, constitute the volatile organic compound (VOC) group BTEX (benzene, toluene, ethylbenzene and xylene). This group accounts for up to 59 % (w/w) of gasoline pollutants (Barona et al., 2007) and they are likewise included as regulated hazardous air pollutants in the Clean Air Act Amendments proposed in 1990 in the US (EPA, 1990). Benzene is a potent carcinogen while toluene, ethyl benzene and *para*-xylene are highly toxic and mutagenic. In Europe, these contaminants are integrated in the European Pollutant Release and Transfer Register (E-PRTR) (European Union, 2006). For operating facilities required to report their release in 2009, toluene was emitted to the atmosphere at a rate of 12.2 kt yr^{-1} in the USA, and 3.9 kt yr^{-1} in Canada. In addition, 5.7 kt yr^{-1} of xylene (all isomers) were emitted to the atmosphere in the USA, while 5.1 kt yr^{-1} were emitted in Canada (NPRI, 2010; TRI Program, 2010).

Recently, biological processes have frequently been applied to control waste gas emissions polluted with relatively low concentration of VOCs (Iranpour et al., 2005). Among all the biological technologies, biofiltration is one of the most successful techniques. Biofiltration has shown proven ability to remove a wide range of VOCs achieving high pollutant removal capacities (> 90 %). In Europe, more than 600 chemical processing industries use biofilters for odour and VOC treatment (Mudliar et al., 2010).

Although toluene and *para*-xylene are commonly released together, many studies have focused on the biofiltration of each pollutant individually, and less effort has been made in order to understand interactions between these two monoaromatic compounds during in situ biofiltration. Jorio et al. (1998) indicated that the removal efficiency for toluene decreased due to the presence of a mixture of xylene isomers in the airstream while the presence of toluene had negligible effect on the xylenes removal efficiency. Correspondingly, Strauss et al. (2004) observed that toluene removal efficiency was inhibited by the presence of *para*-xylene for toluene-acclimatized biofilter consortium under mesophilic conditions. On the contrary, toluene had an enhancing effect on the removal efficiency of the *para*-xylene compared to removal efficiencies for individual compounds.

On the other hand, several studies have been made using monocultures and microbial associations in homogeneous batch reactors containing suspended cell cultures to investigate toluene and *para*-xylene substrate interactions: Deeb and Álvarez-Cohen (1998) reported that using a 35 °C-enriched consortium, *para*-xylene was inhibited by the presence of toluene, while no effect was observed on toluene degradation. On the other hand, Kim et al. (2009) stated that toluene degradation was inhibited by the presence of xylene, but xylene degradation was stimulated when toluene was present in a mixture. Oh et al. (1997) (with pure and mixed cultures) and Chang et al. (1993) (with pure cultures) found that *para*-xylene was degraded through a cometabolic process in the presence of toluene. Finally, Prenafeta-Boldú

et al. (2002) (with the soil fungus *Cladophialophora sp.* strain T1) observed that *para*-xylene partial depletion occurred when this compound was combined with toluene, but only after the toluene was exhausted.

A number of theoretical and empirical models have been reported for describing the kinetics of biodegradation of VOCs using a macrokinetic approach. In general, the biodegradation rate of a pollutant in a biofilter by the microbial flora has been described by the Michaelis-Menten relationship (Hirai et al., 1990).

Nevertheless, in the case of biodegradation of two pollutants, the metabolic activity may involve the mechanism of induction, inhibition or cometabolism. To our knowledge, details of the macrokinetics of such biodegradation processes in biofilters are rare in the relevant literature (Sologar et al., 2003).

As cosubstrate interactions are crucial for understanding and predicting the performance of a biofilter, the aim of this work is to study macrokinetics of three lab scale biofilters treating toluene, *para*-xylene and mixtures of both VOCs. The present research has also the objective to determine the interactions of toluene and *para*-xylene in a biofilter packed with inert material and their effect on the biofilter performance.

5.2. Materials and methods

5.2.1. Biofilter setup

Three identical biofilters were constructed from Plexiglas cylinders having an internal diameter of 0.15 m and a total bed height of 1 m divided into three sections. Figure 5.1 shows the experimental setup of one of these units. The inert material (small stones) employed in the bioreactors had the following characteristics: equivalent diameter 7.28 ± 0.19 mm, specific surface area 470 m^2 m^{-3}, and void space volume 0.43 ± 0.01. The nature of the packing material cannot be revealed for confidential reasons.

The biofilters were operated in an up-flow configuration at an average room temperature of 23.0 ± 0.2 °C. The pollutant was continuously supplied to the bottom of the biofilter at a constant flowrate of 0.354 m^3 h^{-1}, for an empty bed residence time (EBRT) of 180 s. The synthetic polluted air was generated by mixing an airstream saturated with vapours of toluene (99.9 % Fisher Scientific, US) and/or *para*-xylene (99.9 %, Fisher Scientific, US) with an uncontaminant and humidified airstream. The concentration of pollutants was fixed by means of massflow meters and air flow meters (all from Brooks Instrument, US).

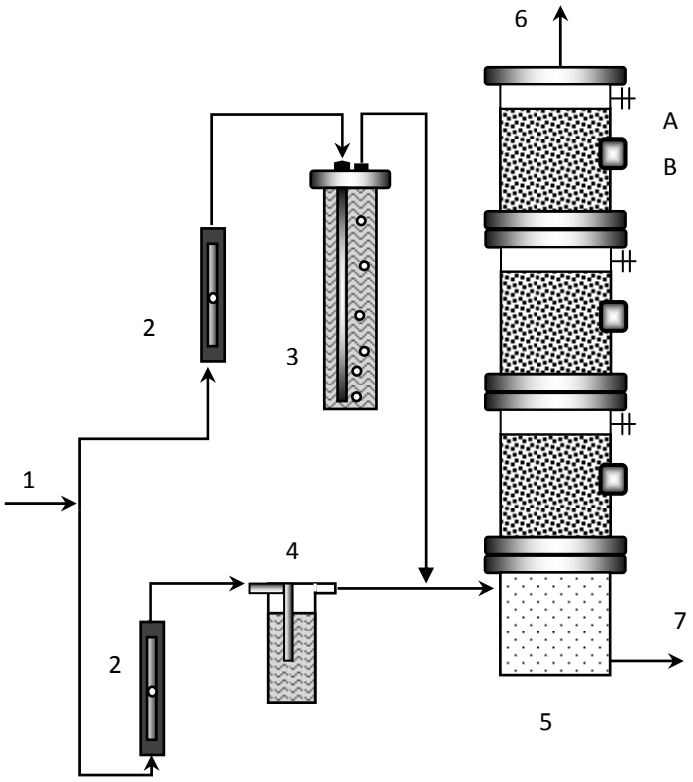

Figure 5.1. Diagram of the biofiltration system : 1 Dry influent air; 2 Flow meter; 3 Humidification column; 4 Pollutant evaporator; 5 Packed bed reactor; 6 Effluent air; 7 Leachate; A (Gas sampling valve); B (Packing material sampling port).

The different ILs during this study for the biofilter degrading *para*-xylene were 20.0 ± 0.7; 43.2 ± 2.4; 57.9 ± 1.2 and 70.4 ± 2.0 g C m^{-3} h^{-1}, while the ILs for the biofilter degrading toluene were 21.0 ± 0.7; 32.9 ± 1.0; 53.4 ± 1.6; 63.8 ± 1.1 and 89.3 ± 1.9 g C m^{-3} h^{-1}. The chronology of events and different contaminant combinations carried out in the mixed biofilter are summarized in Table 5.1. During each stage, the inlet load of *para*-xylene ($IL_{p\text{-}Xyl}$) was kept constant, while three increasing toluene loads (IL_{Tol}) steps were tested. All combinations were carried out until the biofilter operated under steady state for at least one week. The average values of performance parameters were obtained by daily measurements on the biofilter. The performance of biofilters is expressed in terms of elimination capacity (EC), removal efficiency (RE), CO_2 production rate (PCO_2) and biomass production rate (PB). All performance parameters are defined in Table 5.2. Each biofilter was irrigated every day with 1 l of mineral salt medium (MSM) as reported by Barona et al. (2007).

Table 5.1. Chronology of different contaminant combinations for the mixed biofilter.

Operating time (d)	Stage Nº	IL p-Xylene (g C m^{-3} h^{-1})	$[IL\ Toluene]_1$ (g C m^{-3} h^{-1})	$[IL\ Toluene]_2$ (g C m^{-3} h^{-1})	$[IL\ Toluene]_3$ (g C m^{-3} h^{-1})
54	I	18.89 ± 1.27	8.52 ± 0.44	21.47 ± 0.39	38.24 ± 1.21
47	II	38.64 ± 1.22	4.25 ± 0.24	19.62 ± 0.75	32.38 ± 1.47
38	III	8.84 ± 0.29	6.52 ± 0.37	19.84 ± 1.02	35.91 ± 1.55
39	IV	61.86 ± 1.85	7.55 ± 0.49	24.05 ± 0.63	38.74 ± 0.81

Table 5.2. Definition of biofilter performance parameters.

Parameter	Definition	Units
Inlet load	$IL = \frac{Q \cdot C}{V}$	g C m^{-3} h^{-1}
Elimination capacity	$EC = \frac{Q \cdot (C_I - C_0)}{V}$	g C m^{-3} h^{-1}
Global Removal efficiency	$RE = \frac{(C_I - C_0)}{C_I}$	Dimensionless
Biomass production rate	$PB = \frac{C_B}{\Theta V}$	g B m^{-3} h^{-1}
Carbon dioxide production rate	$PCO_2 = \frac{Q \cdot (CO_{2_O} - CO_{2_I})}{V}$	g CO_2 m^{-3} h^{-1}
Biomass yield coefficient	$YB = \frac{PB}{EC}$	g Biomass produced g^{-1} of pollutant consumed
Carbon dioxide yield coefficient	$YCO_2 = \frac{PCO_2}{EC}$	g CO_2 produced g^{-1} of pollutant consumed

Where Q is the total air flowrate (m^3 h^{-1}); V is the empty bed volume (m^3); Θ is the data acquisition period (h); C_B is the dry biomass concentration in the biofilm (g B m^{-3}); C is the pollutant concentration and CO_2 is the carbon dioxide concentration; all concentrations in (g m^{-3}). The subscript "I" indicates that the compound is in an inlet stream and subscript "O" indicates that the compound is in an outlet stream.

5.2.2. MSM selection

Originally, $(NH_4)_2SO_4$ was supplied as the main nitrogen source (pH original MSM: 6.0 ± 0.2) (operational period not taken into account and not shown in the rest of the sections). However, it was changed to $(NH_4)HCO_3$ (pH: 8.0 ± 0.0) due to a progressive acidification of the filter media with $(NH_4)_2SO_4$. This implied a severe RE decrease in the biofilters treating *para*-xylene ($pH_{leachate}$ = 5.8) and the mixture of both pollutants ($pH_{leachate}$ = 4.9), while a slight worsening was noticed in the biofilter treating toluene ($pH_{leachate}$ = 5.7) (Table 5.3). This finding is in agreement with previous results reported by Lu et al. (2002). These authors observed that bacteria responsible for BTEX removal preferred a weak basic environment (pH =7.5 – 8.0), with a continuous microbial activity drop as the pH of the nutrient feed decreased from 8.0 (RE_{BTEX} ≥ 80 %) to 5.0 (RE_{BTEX} ≈ 65 %). Singh et al. (2010) also observed a very favourable growth of the microbial strain *P. putida* (MTCC 102) when pH variation of the leachate stabilized around 7.0 in a wood charcoal biofilter treating toluene.

Table 5.3. Inlet load, removal efficiency and pH values of the MSM after the addition of all the salts and the leachate of each biofilter.

Biofilter	$(NH_4)_2SO_4$				$(NH_4)HCO_3$			
	pH MSM	pH Leachate	IL ($g\ C\ m^{-3}\ h^{-1}$)	RE (%)	pH MSM	pH Leachate	IL ($g\ C\ m^{-3}\ h^{-1}$)	RE (%)
p-Xylene	6.0 ± 0.2	5.8 ± 0.2	22.0 ± 0.5	19.2 ± 3.1	8.0 ± 0.0	7.4 ± 0.0	20.0 ± 0.1	54.1 ± 1.4
Toluene	6.0 ± 0.2	5.7 ± 0.4	21.1 ± 0.7	77.4 ± 3.6	8.0 ± 0.0	7.2 ± 0.0	21.0 ± 0.7	79.8 ± 2.6
Mixture (Stage I)	6.0 ± 0.2	4.9 ± 0.2	38.6 ± 1.5	16.1 ± 3.3	8.0 ± 0.0	7.5 ± 0.0	42.9 ± 0.9	71.7 ± 2.1

5.2.3. Analytical procedure

Toluene and *para*-xylene concentrations in the mixed biofilter were measured using a gas chromatograph (GC) Varian GC CP-3800 (Varian, USA) equipped with a FID detector and using He as a carrier gas. The GC was equipped with a FactorFour Capillary Column VF-5ms 30 m x 0.25 mm x 0.39 mm (Varian, USA). The oven, injector and FID detector were maintained at 110 °C, 250 °C and 250 °C, respectively.

In the two biofilters treating toluene and *para*-xylene individually, the concentrations of VOCs and CO_2 in the gas phase were measured by means of a total hydrocarbon analyzer FIA-510 (Horiba, USA) and Ultramat 22P (Siemens AG, Germany), respectively.

The pH at 25 °C of the nutrient solution and the lixiviate produced were measured daily using a Fisher Accumet Model 25 pH/ Ion Meter (Fisher Scientific, USA).

The determination of biomass and water content in the packed bed was realized twice per week as already described elsewhere (Ávalos Ramirez et al., 2008a), while total carbon content and inorganic carbon content in leachate and nutrient solution were measured using a Total Organic Carbon analyzer – TOC-V Series (Shimadzu, Japan).

5.2.4. Macrokinetic models for VOC biodegradation

In a biofilter, the EC commonly follows a behaviour that can be adequately described by a Michaelis-Menten type model (Hirai et al., 1990):

$$EC = \frac{EC_{max} C_{ln}}{K_S + C_{ln}} \qquad (1)$$

where EC_{max} (g m^{-3} h^{-1}) is the maximal EC, C_{ln} (g m^{-3}) is the logarithmic average of the inlet and outlet concentrations of pollutants in the gas phase and K_S (g m^{-3}) is the saturation constant. When EC presents inhibition, a Haldane type model including a substrate inhibition term could be fitted to experimental EC (Ávalos Ramirez et al., 2008b):

$$EC = \frac{EC^* C_{ln}}{K'_S + C_{ln} + \left(C_{ln}^2 / K_I \right)} \qquad (2)$$

where EC* (g m^{-3} h^{-1}) is the maximal EC in the absence of inhibition, K'_S (g m^{-3}) is the saturation constant and K_I (g m^{-3}) is the inhibition constant for the Haldane type model.

In this case, EC_{max} is obtained following the mathematical analysis reported by Sologar et al. (2003) for the maximal specific growth rate (μ_{max}). The equation to calculate EC_{max} is as follows:

$$EC_{max} = \frac{EC^*}{1 + 2 \cdot \left(K'_S / K_I \right)^{0.5}} \qquad (3)$$

Taking account of the definition of the saturation constant (K_S) and Eq. (2), the value of K_S for the inhibition case can be calculated as follows:

$$K_S = \frac{\left(1 + 4 \cdot \left(K'_S / K_I \right)^{0.5} \right) \pm \sqrt{\left(1 + 4 \cdot \left(K'_S / K_I \right)^{0.5} \right)^2 - 4K'_S \left(1 / K_I \right)}}{2 \cdot \left(1 / K_I \right)} \qquad (4)$$

The specific growth rate (μ) of the microorganisms present in the biofilters could be calculated using the exponential growth equation integrated as follows:

$$\ln(B) = \ln(B_0) + \mu \cdot t \qquad (5)$$

where B_0 (g) is the biomass content in the packed bed at t = 0 and B (g) is the biomass content at the elapsed time (t).

5.2.5. Carbon balance

A carbon balance to predict the outlet carbon rate was herein developed in Equation (6):

$$C_{OUT-CALC} = C_{IN-EXP} - C_{ACC-EXP} \quad (6)$$

where C_{IN-EXP} (g C m^{-3} h^{-1}) and $C_{ACC-EXP}$ (g C m^{-3} h^{-1}) are the total inlet and accumulation carbon rates obtained by means of experimental measures and $C_{OUT-CALC}$ (g C m^{-3} h^{-1}) is defined as the hypothetical outlet carbon rate. They represent the total mass of carbon that entered in (C_{IN-EXP}), left from ($C_{OUT-CALC}$) and accumulated in ($C_{ACC-EXP}$) the empty bed volume.

The compounds considered for C_{IN-EXP} were the VOC of the inlet gas stream and the carbon content of the nutrient solution. $C_{ACC-EXP}$ was obtained by measuring twice per week the total mass of the biofilter. Samples of biofilm were taken from each biofilter section in order to determine the average solid content in the packed bed at each stage. A weighted arithmetic average of dry biomass accumulation rate for whole biofilter was calculated assuming that dry biomass had the empirical formula $CH_{1.98}O_{0.44}N_{0.18}$ reported by Ávalos Ramirez et al. (2008b) for a biofilter treating toluene. With this average composition the dry biomass accumulation rate was converted to the carbon accumulation rate ($C_{ACC-EXP}$).

The theoretical $C_{OUT-CALC}$ was compared with the experimental $C_{OUT-EXP}$ in order to determine the error of the calculated value. $C_{OUT-EXP}$ was determined with the addition to the airstream of the CO_2 produced by the microorganisms (PCO_2), the unconverted VOC present in the outlet airstream and the total carbon content of the leachate.

5.3. Results and discussion

5.3.1. Influence of VOC inlet concentration and interactions of VOCs

Figure 5.2a and Figure 5.2b illustrate the overall EC of *para*-xylene and toluene biofilters as a function of the IL. In the biofilter treating *para*-xylene (Figure 5.2a), the IL was successively increased from 20.0 ± 0.7 to 70.4 ± 2.0 g C m^{-3} h^{-1}. The EC increased with the IL up to EC_{max} of 26.5 g C m^{-3} h^{-1}, which occurred at an IL of 65.6 g C m^{-3} h^{-1} (RE = 40.3 %).

In the biofilter treating toluene (Figure 5.2b), the inlet load was successively increased from 21.0 ± 0.7 to 89.3 ± 1.9 g C m^{-3} h^{-1}. In this case, the IL tested was higher than for *para*-xylene biofilter as the toluene biofilter seemed to be close to an inhibition zone at IL ≈ 65 – 70 g C m^{-3} h^{-1}. At the IL of 94.5 g C m^{-3} h^{-1} the toluene biofilter performance had deteriorated, probably

due to a partial inhibition of the microbial community present in the biofilm (Jorio et al., 2000). Hence, an EC_{max} of 40.3 g C m^{-3} h^{-1} was observed at an IL of 57.8 g C m^{-3} h^{-1} (RE = 69.6 %).

A comparison of Figure 5.2a and Figure 5.2b confirms that toluene is more amenable to biotreatment than *para*-xylene for inlet loads up to 70 g C m^{-3} h^{-1}, with an EC_{max} for toluene being 52 % higher compared to that of *para*-xylene. This evidence concurs with results of other authors (Jorio et al., 1998; Kim et al., 2009; Strauss et al., 2004).

Figure 5.2c illustrates the overall EC of the mixed biofilter as a function of the overall IL for the 4 stages analyzed in this study (previously defined in Table 5.1). For similar ILs, very different ECs are reached as a result of the different contribution of each contaminant to the overall IL. Thus, better EC were obtained in those mixtures with a higher proportion of toluene. For instance, for an overall IL ≈ 50 g C m^{-3} h^{-1}, a EC ≈ 15 g C m^{-3} h^{-1} was obtained when the percentages of *para*-xylene/toluene in the mixture were 53/47 % (w/w), while a EC ≈ 35 – 40 g C m^{-3} h^{-1} was achieved with a *para*-xylene/toluene mixture of 19/81 % (w/w).

In order to better understand the dynamics of *para*-xylene and toluene removal, the RE of the three sections of both biofilters is shown in Figure 5.3a and Figure 5.3b. In the case of *para*-xylene, experimental data revealed that the contributions of the different sections of the biofilter changed depending on the tested ILs. As IL increased, the RE of the lower part descended from 48 % to 32 %, while the upper part improved from 26 % to 42 %. On the contrary, the middle section showed a similar performance in all cases (RE 18 – 25 %). For the biofilter treating toluene, except for the smallest IL (21.0 ± 0.7 g C m^{-3} h^{-1}), the results indicate that the RE was always more efficient in the upper part of the filter, where 47 – 60 % of the toluene was removed.

Previously published studies reached comparable patterns to those obtained in the *para*-xylene biofilter. Rene et al. (2005) reported that the removal is more efficient in the lower part than in the upper part of a compost-based biofilter treating toluene for a similar up-flow configuration and an IL of 3 – 12 g m^{-3} h^{-1}. Elmrini et al. (2004) observed that for relatively small ILs (12 – 34 g m^{-3} h^{-1}), most of the xylene was eliminated in the first lower/inlet section of a peat-based biofilter. However, for relatively high ILs of the pollutant (87 g m^{-3} h^{-1}), the performance in the different sections was relatively uniform. Differences between these results and the values attained in the biofilter treating toluene might be attributed to the dissimilar nature of the packing material. Organic filter beds like compost or peat naturally hold a large quantity of indigenous microflora and contain the necessary nutrients for bacteria to develop. Moreover, the degree of compaction during operation is extremely different, which could contribute with the different pattern of the removal efficiencies.

In the same way, Figure 5.3c and Figure 5.3d illustrate the overall and the modular REs achieved for each pollutant when the airstream is polluted by the mixture of *para*-xylene and toluene. For example, in Figure 5.3c, for a fixed $IL_{p\text{-}Xyl}$ nearly constant at 40 g C m^{-3} h^{-1}, the $RE_{p\text{-}Xyl}$ decreased from 39 % to 18 % due to the increase of toluene in the airstream from 0 to 35.9 g C m^{-3} h^{-1}. Moreover, the EC_{max} for *para*-xylene in the mixed biofilter was 18.1 g C m^{-3} h^{-1} ($IL_{p\text{-}Xyl}$ = 67.0 g C m^{-3} h^{-1}; IL_{Tol} = 8.5 g C m^{-3} h^{-1}) (data not shown), which is lower than the EC_{max} obtained as a single pollutant (26.5 g C m^{-3} h^{-1}). This information suggests that inhibition of

para-xylene degradation took place because of the presence of toluene in the air contaminated by the mixture.

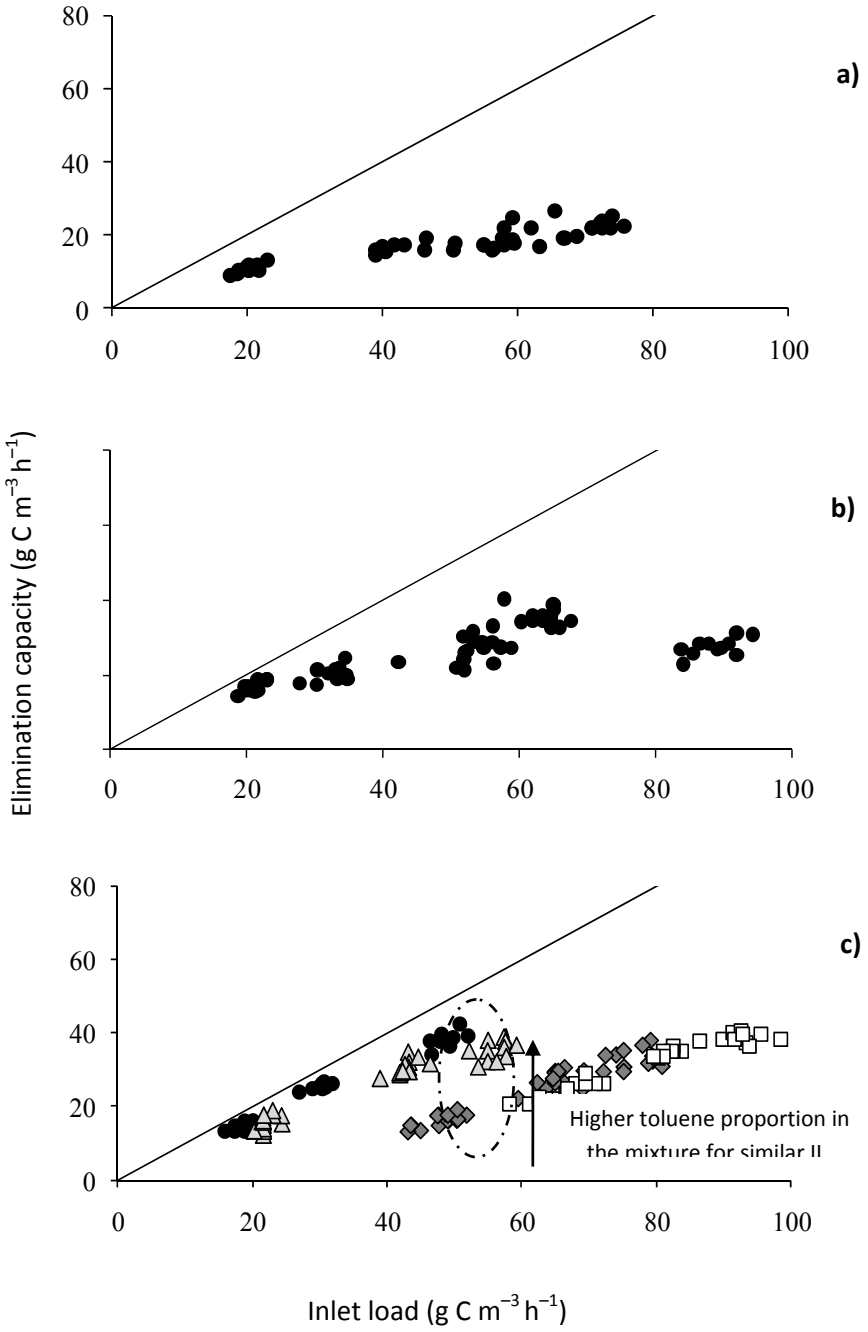

Figure 5.2. Elimination capacity as a function of inlet load for biofilters treating (a) *para*-xylene, (b) toluene, and (c) mixed biofilter for Stage I (△), Stage II (◆), Stage III (●) and Stage IV (□).

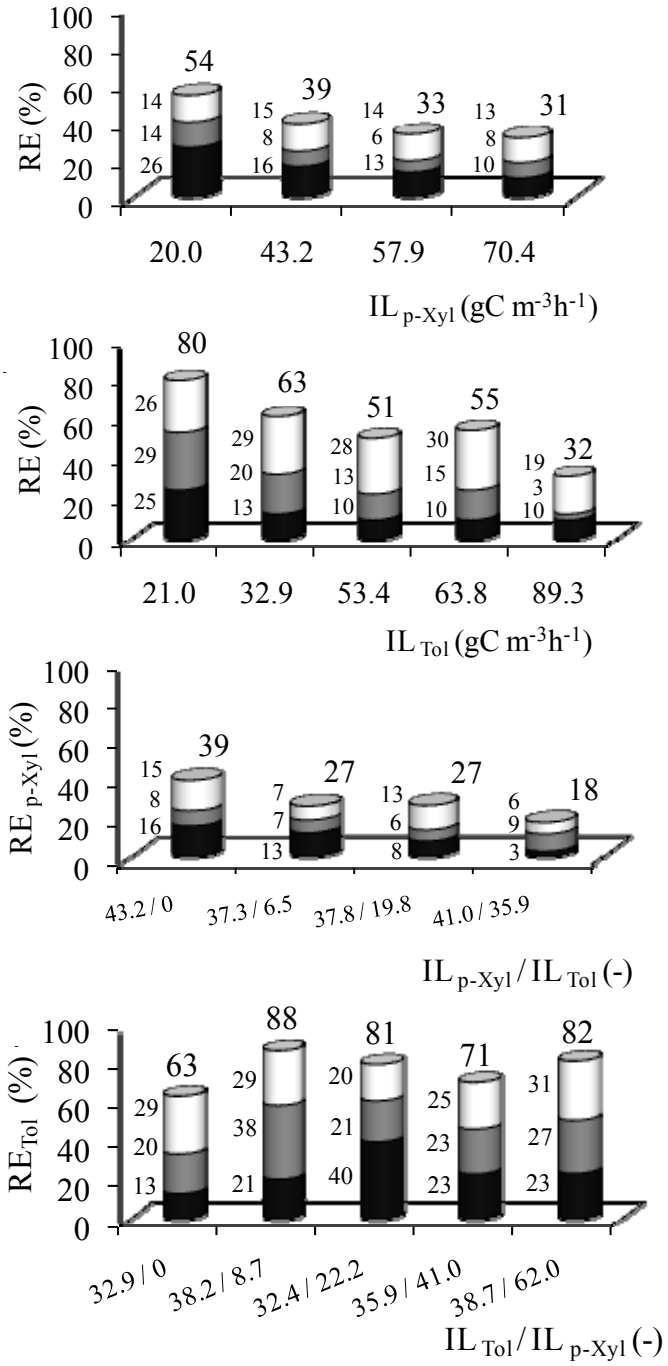

Figure 5.3. Overall and modular removal efficiency (RE) for biofilters treating (a) *para*-xylene; (b) toluene; (c) *para*-xylene removal efficiency ($RE_{p\text{-}Xyl}$) for mixed biofilter when *para*-xylene inlet load ($IL_{p\text{-}Xyl}$) was nearly constant at 40 g C m^{-3} h^{-1}; and (d) Toluene removal efficiency (RE_{Tol}) when toluene inlet load (IL_{Tol}) was nearly constant at 35 g C m^{-3} h^{-1}. (■) Removal efficiency for lower-inlet module, (▩) Removal efficiency for middle module and (□) Removal efficiency for upper-outlet module.

The competitive effect might be explained by the similarities in the catabolic pathways and enzymatic systems of both pollutants. In both cases, aerobic biodegradation could be initiated

by progressive oxidation of the alkyl side chain of the aromatic ring (methyl-group hydroxylation via catechol and subsequent meta-cleavage pathway) (Smith, 1990). Nonetheless, the greater *para*-xylene molecular weight and the steric hindrance caused by the second methyl group in the *para*-xylene molecule might contribute to the lowering of the biodegradation rate of *para*-xylene in the presence of toluene (Jorio et al., 1998).

Additionally, *para*-xylene degradation in the mixture biofilter followed essentially the same pattern developed in the biofilter treating *para*-xylene as sole contaminant, where no priority of any of the modules to biodegrade the contaminant was observed.

Figure 5.3d shows that the opposite occurred for toluene case. For an IL_{Tol} nearly constant at 35 g C m^{-3} h^{-1} the RE_{Tol} increased when *para*-xylene was involved. In all mixture cases, RE_{Tol} ranged from 71 to 88 %, while a RE of 63 % was achieved when toluene was fed as sole carbon source into the biofilter.

A comparison between EC_{max} for toluene in mixed biofilter and in toluene biofilter cannot be done as EC_{max} for toluene in the mixed biofilter was not reached because the low IL_{Tol} evaluated (Maximum IL_{Tol} around 90 and 38 g C m^{-3} h^{-1} were tested for toluene biofilter and mixed biofilter, respectively). However, results reported in all stages suggested the possibility of higher EC_{max} in toluene biotreatment mixed with *para*-xylene. Hence, the presence of *para*-xylene had an enhancing effect on toluene removal efficiency.

5.3.2. CO_2 production rate

In the biofiltration process, *para*-xylene (C_8H_{10}) and toluene (C_7H_8) are converted under aerobic conditions to CO_2, water and biomass due to global biochemical reactions catalyzed by microorganisms as follows:

$$aC_7H_8 + bO_2 + c(NH_4)HCO_3 \xrightarrow{\text{Microorganisms}} CH_{1.98}O_{0.44}N_{0.18} + dH_2O + eCO_2 \quad (7)$$

$$fC_8H_{10} + gO_2 + h(NH_4)HCO_3 \xrightarrow{\text{Microorganisms}} CH_{1.98}O_{0.44}N_{0.18} + iH_2O + jCO_2 \quad (8)$$

Monitoring the CO_2 concentration in the gas phase provides valuable information on the operation of the biofilter and the mineralization level of the pollutant. The CO_2 production rate (PCO_2) as a function of EC for *para*-xylene, toluene and the mixture of both is plotted in Figures 5.4a, 5.4b and 5.4c.

Experimental data reveal that the variation of PCO_2 versus EC was essentially linear in all cases. The values of YCO_2 obtained were 2.47 g CO_2 g C^{-1} for *para*-xylene, 2.41 g CO_2 g C^{-1} for toluene and 2.60 g CO_2 g C^{-1} for the mixture. These experimental values were lower than 3.32 g CO_2 g C^{-1} and 3.34 g CO_2 g C^{-1}, the stoichiometric ratio in the case of complete chemical oxidation of

para-xylene and toluene respectively. However, a great portion of the removed pollutant was effectively mineralized to CO_2 when they were individually treated (*para*-xylene 74 % and toluene 72 %), which indicates that pollutants were really eliminated by biodegradation.

Figure 5.4c shows a general tendency for the whole of the experimental data obtained. The average YCO_2 was 2.60 g CO_2 g C^{-1}, which was higher than YCO_2 of single pollutants. This finding agrees with García-Peña et al. (2008), who obtained higher values of carbon recovered as CO_2 for BTEX binary mixtures in comparison with single pollutants assimilation.

The values of YCO_2 are slightly lower than most of the results reported in other studies, which used organic packing media in all cases (Table 5.4). The lower levels of CO_2 released might be partly explained by the inorganic nature of the filter media used, which was limited in moisture, nutrient content and indigenous microflora.

Table 5.4. Carbon dioxide yield coefficient, maximum elimination capacity and packing material employed in conventional biofilters treating *para*-xylene and toluene.

Contaminant	Carbon dioxide yield coefficient (g CO_2 g C^{-1})	EC_{MAX} (g m^{-3} h^{-1})	Packing material	Author
para-Xylene	2.47	26.5	Inert material (small stones)	This study
Mixture of xylene isomers (19 % m-/65 % p-/16 % o-)	2.5	67 (max.)	Peat balls	(Jorio et al.. 2000)
Xylene	2.72	61 (93 % RE)	Peat + Mineral additive with good binding capacity 70:30 (v/v)	(Elmirini et al.. 2004)
Toluene	2.41	40.3	Inert material (small stones)	This study
Toluene	2.8	90	Pellet of compost-organic binder (90/10 v/v) with 10 mm size.	(Delhoménie et al.. 2002)
Toluene	2.48	360 (48 % RE)	Peat with an organic content of 95 wt %	(Álvarez-Hornos et al.. 2008)
p-Xylene/Toluene mixture	2.60	-	Inert material (small stones)	This study

In this case, the CO_2 emitted came largely from VOC biodegradation and to a lesser extent from $((NH_4)HCO_3$ contribution (calculated as the difference between the inorganic carbon content in the MSM and the leachate), which was the 24.3 ± 2.0 % and 18.8 ± 1.4 % of total CO_2 emissions for *para*-xylene and toluene biofilters, respectively. This assumption could not be made when organic packing materials were employed: no information about degradation of organic packing materials and their possible contribution to CO_2 production was generally provided. This can be the difference between the YCO_2 obtained in the present study and the values reported in literature shown in Table 5.4.

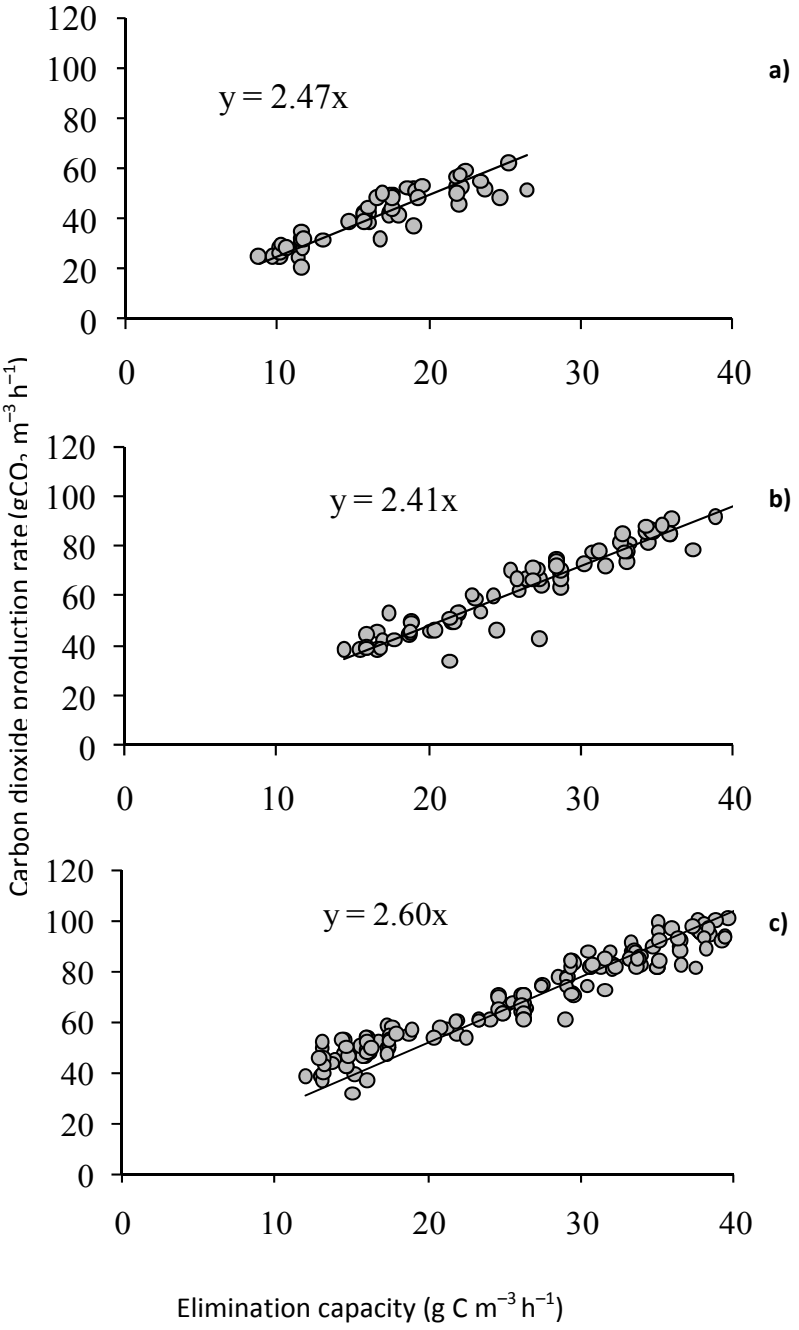

Figure 5.4. Effect of elimination capacity on carbon dioxide production rate for (a) *para*-xylene biofilter, (b) toluene biofilter, and (c) mixed biofilter for stages I to IV.

5.3.3. Biomass production rate

PB against EC for *para*-xylene and toluene is plotted in Figure 5.5a and Figure 5.5b. For both cases PB increased with EC.

A linear regression of the data, using the least squares method, provided biomass yield coefficients for *para*-xylene (YB – p-XYL) and toluene (YB – TOL) of 0.37 g B g C^{-1} and 0.34 g B g C^{-1}, respectively.

Biomass yield is an important parameter for the numerical modelling of biofilter and related technologies. Grove et al. (2009) suggested that a value in the range of 0.17 – 0.43 g B g $_{Compound}^{-1}$ would be appropriate for the degradation of most substrates. Few data is available in the literature concerning biomass yield in real biofilters. For batch culture experiments, where microorganisms were grown on a mineral salt medium in shaken watertight flasks, Oh and Bartha (1997) calculated a value of 0.52 g B g C^{-1} for *para*-xylene and 0.61 g B g C^{-1} for toluene. Likewise, Song et al. (2000) calculated a value of 0.65 g B g C^{-1} for toluene in a vapour-phase bioreactor packed with porous silicate pellets. The difference with the present study could be based on the fact that Song et al. (2000) used an acclimated inoculum, which was recirculated throughout the bioreactor for several hours. Additionally, operating parameters such as contaminant concentration were constant throughout the experimental period.

Figure 5.5c shows PB as a function of EC for the mixture biofilter. Previously, in Figure 5.4c, all data obtained in the different stages presented the same pattern for YCO_2. For YB, a common trend was not observed and experimental points could not be aligned. In this case, each stage was indicated with a different symbol as it is described in Figure 5.5c caption. Linear regression of experimental data gave YB_{stage} II = 0.17 g B g C^{-1} and YB_{stage} IV = 0.31 g B g C^{-1}. In comparison, the stages with lower $IL_{p\text{-}Xyl}$ (stage I and stage III), the biomass yield was higher with YB_{stage} I = 0.38 g B g C^{-1} and YB_{stage} III = 0.62 g B g C^{-1}. This shows that biofilters with lower amounts of *para*-xylene in the mixture exhibit fewer difficulties to produce biomass, as microorganisms require less energy to biodegrade toluene.

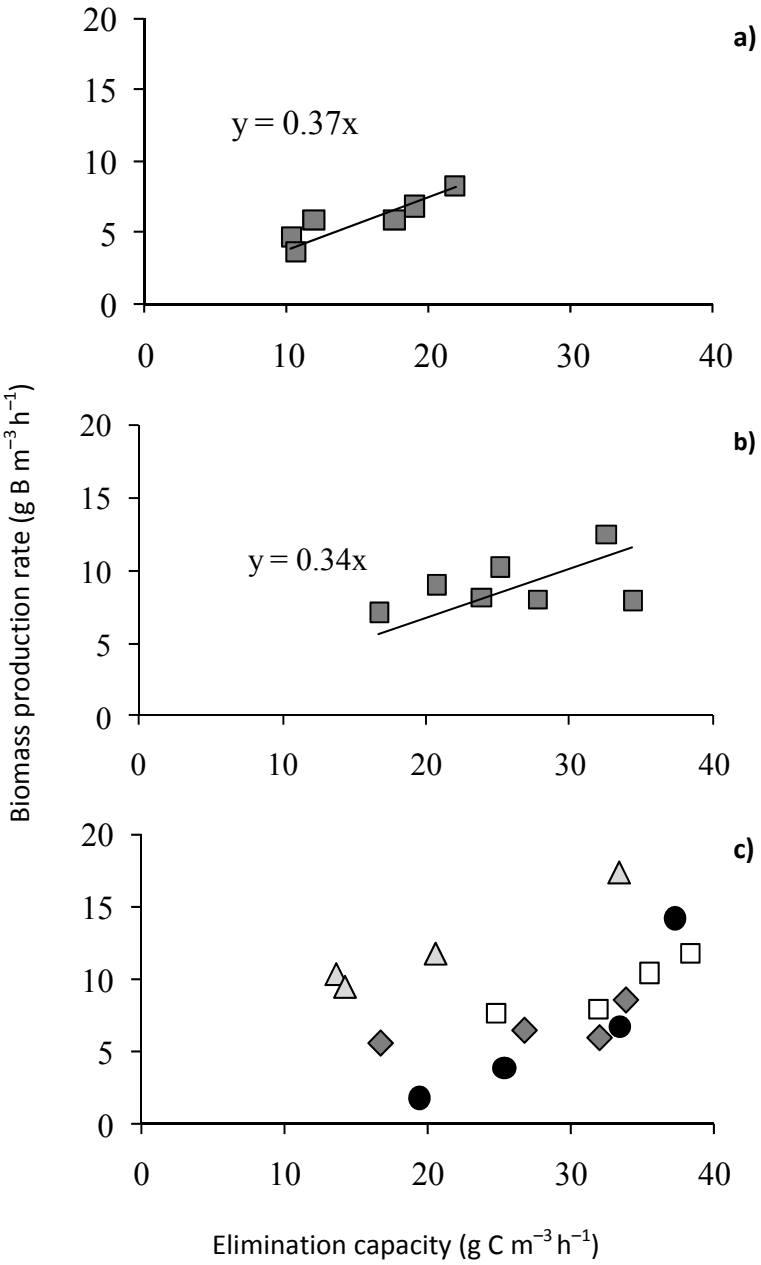

Figure 5.5. Effect of elimination capacity on biomass production rate for (a) *para*-xylene biofilter, (b) toluene biofilter, and (c) Stage I (△), Stage II (◆), Stage III (●) and Stage IV (□) of mixed biofilter.

5.3.4. Carbon balance

Tables 5.5a, 5.5b and 5.5c show the carbon balance for biofilters treating *para*-xylene, toluene and mixture of both pollutants in stage nº II ($IL_{p\text{-}Xyl}$ 38.64 ± 1.22 g C m^{-3} h^{-1} and IL_{Tol} from 4.25 ± 0.24 g C m^{-3} h^{-1} to 32.38 ± 1.47 g C m^{-3} h^{-1}). The carbon balance for stage nº II is shown as it can be related to Figure 5.3c; however, the behaviour was analogous for the four stages. For

the biofilter treating *para*-xylene, the calculated $C_{OUT-CALC}$ presented a range of error from -2.2 % to 6.0 %. Except for one of the inlet loads studied (41.2 ± 1.5 g C m^{-3} h^{-1}), "$C_{OUT-CALC}$" is slightly higher than "$C_{OUT-EXP}$". This means in fact, more carbon was accumulated inside the biofilter than the measures realized. The small difference between the experimental value and the calculated value, beyond the experimental error, might be due to the packing material loss during experimental manipulation of the biofilters. The water content of the biofilm (90.6 ± 0.9 %) and the moisture of the packed bed could present variations due to condensation/evaporation processes caused by microbial activity and temperature changes in the biofilter and the laboratory.

Table 5.5a. Mass carbon balances for biofilter treating *para*-xylene.

Inlet load (g C m^{-3} h^{-1})	20.0 ± 0.7	41.2 ± 1.5	56.1 ± 2.2	70.4 ± 2.1
C_{IN-EXP} (g C m^{-3} h^{-1})	20.2 ± 0.7	41.4 ± 1.5	56.3 ± 2.2	70.6 ± 2.1
$C_{ACC-EXP}$ (g C m^{-3} h^{-1})	2.7 ± 0.9	6.0 ± 0.9	3.3 ± 0.9	3.3 ± 0.9
$C_{OUT-EXP}$ (g C m^{-3} h^{-1})	17.1 ± 0.6	36.3 ± 1.5	50.4 ± 2.6	63.5 ± 2.1
$C_{OUT-CALC}$ (g C m^{-3} h^{-1})	17.5 ± 0.7	35.5 ± 1.5	53.0 ± 2.2	67.3 ± 2.1
Error of $C_{OUT-CALC}$ (%)	+1.9	-2.2	+5.1	+6.0

Table 5.5b. Mass carbon balances for biofilter treating toluene.

Inlet load (g C m^{-3} h^{-1})	21.0 ± 0.7	32.9 ± 1.0	53.4 ± 1.6	63.8 ± 1.2	89.3 ± 1.9
C_{IN-EXP} (g C m^{-3} h^{-1})	21.2 ± 0.7	33.1 ± 1.0	53.6 ± 1.6	64.0 ± 1.2	89.5 ± 1.9
$C_{ACC-EXP}$ (g C m^{-3} h^{-1})	3.9 ± 0.9	5.1 ± 0.9	3.5 ± 0.9	4.1 ± 0.9	6.8 ± 0.9
$C_{OUT-EXP}$ (g C m^{-3} h^{-1})	15.9 ± 0.7	25.3 ± 1.7	43.9 ± 1.5	51.9 ± 1.7	81.0 ± 1.7
$C_{OUT-CALC}$ (g C m^{-3} h^{-1})	17.3 ± 0.7	28.1 ± 1.0	50.1 ± 1.6	59.9 ± 1.2	82.6 ± 1.9
Error of $C_{OUT-CALC}$ (%)	+8.8	+11.0	+14.1	+15.4	+2.0

Table 5.5c. Mass carbon balances for mixture biofilter.

Total inlet load (g C m^{-3} h^{-1})	47.9 ± 1.5	65.1 ± 1.3	77.1 ± 1.7
C_{IN-EXP} (g C m^{-3} h^{-1})	48.1 ± 1.5	65.3 ± 1.3	77.3 ± 1.7
$C_{ACC-EXP}$ (g C m^{-3} h^{-1})	2.5 ± 0.9	3.1 ± 0.9	4.6 ± 0.9
$C_{OUT-EXP}$ (g C m^{-3} h^{-1})	45.6 ± 1.2	57.4 ± 1.2	67.8 ± 1.7
$C_{OUT-CALC}$ (g C m^{-3} h^{-1})	45.6 ± 1.5	62.2 ± 1.3	72.8 ± 1.7
Error of $C_{OUT-CALC}$ (%)	-0.1	+8.4	+7.3

For the biofilter treating toluene, the calculated COUT-CALC presented a range of error from 2.0 % to 15.4 %. In this case, differences around 10% between "$C_{OUT-CALC}$" and "$C_{OUT-EXP}$" in all the stages except the last one (89.3 ± 1.9 g C m^{-3} h^{-1}) were recorded. The error obtained in the research is acceptable and the carbon balance accurately describes the outlet carbon rate

within each biofilter. In the mixed biofilter, the calculated $C_{OUT-CALC}$ presented a range of error from -0.1 % to 8.5 %.

Since the biomass contains around 50 % carbon (Ávalos Ramirez et al., 2008a) the experimental determination of biomass in the packing bed is essential for carbon balances. On the other hand, it was found that the daily total carbon flux disposed through leachate was much less than the values of carbon fluxes due to CO_2 evolved from contaminant biodegradation and undegraded contaminant. The total carbon rates in the leachate were only 1.1 ± 0.2 % and 0.6 ± 0.1 % of the total outlet carbon rate (sum of the CO_2 produced by the microorganisms (PCO_2), the unconverted VOC present in the outlet airstream and the total carbon content of the leachate) for *para*-xylene and toluene, respectively. Therefore, this carbon sink is commonly neglected for closing carbon balances (Song et al., 2000).

5.3.5. Macrokinetic analysis

Figure 5.6a and Figure 5.6b show the experimental EC for the biofilters treating *para*-xylene at a C_{In} from 0.6 to 3.1 g C m^{-3}, and toluene at a C_{In} from 0.5 to 3.9 g C m^{-3}. The EC estimated based on Michaelis-Menten type model fitted experimental data for *para*-xylene. The experimental EC_{max} for *para*-xylene was 26.5 g C m^{-3} h^{-1}, while the EC_{max} estimated with the Michaelis-Menten type model was 30.4 g C m^{-3} h^{-1}. A K_S of 1.32 g C m^{-3} was obtained.

In contrast, the Haldane type model fitted the data better for the toluene biofilter, as the EC decayed due to substrate inhibition for C_{In} higher than 3.0 g C m^{-3}. This inferior performance was not predicted by Michaelis-Menten type model. In the case of toluene, the experimental EC_{max} was 40.3 g C m^{-3} h^{-1}, while the values of EC_{max} and K_S estimated with the Haldane type model (Equations 3 and 4) were 31.1 g C m^{-3} h^{-1} and 0.57 g C m^{-3}, respectively. Zamir et al. (2011) calculated values of 50 g m^{-3} h^{-1} and 3.50 g m^{-3} for EC_{max} and K_S in a compost fungal-biofilter treating toluene. They observed there was no inhibition effect of the substrate on the microbial population under the studied experimental conditions (inlet concentration in the range of 52.6 – 173.1 mg m^{-3} and EBRT in the range of 66 – 264 s). The higher results obtained might be explained because of the low inlet load tested (≤ 10 g m^{-3} h^{-1}). Besides, studies published earlier showed that fungal-biofilters have attained higher hydrophobic compounds elimination rates compared to bacterial systems due to the direct contact between the fungal mycelia and the gaseous pollutant (Estévez et al., 2005).

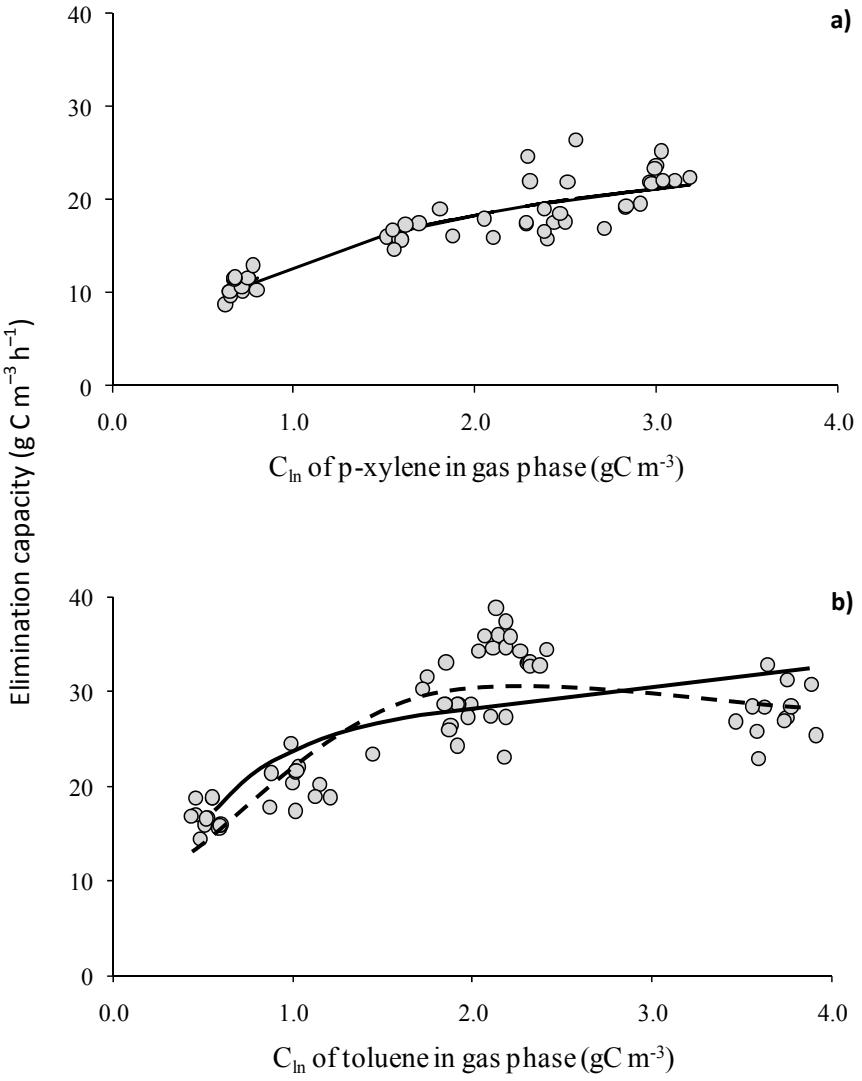

Figure 5.6. Elimination capacity as a function of the logarithmic average of inlet and outlet concentrations in gas phase of biofilters (C_{ln}) for *para*-xylene (a) and toluene (b). Experimental EC (○), estimated EC with Michaelis-Menten type model (—) and with Haldane type model (- -).

Table 5.6 (a and b) shows the μ values in biofilters packed with inert material for *para*-xylene, toluene and the mixed biofilter. For the case of the biofilter treating *para*-xylene as sole contaminant, Table 5.6a shows μ as a function of the logarithmic average of *para*-xylene concentration in the biofilm ($C_{biofilm}$) (calculated employing air/water distribution coefficients) over a range of 2.3 to 9.7 g C m^{-3}. The microorganisms were inhibited at concentrations in the biofilm at greater levels than 8 g C m^{-3}. μ_{max} for *para*-xylene case was 0.0048 h^{-1}.

Table 5.6a. Specific growth rate (μ) as a function of the pollutant logarithmic average concentration in biofilm ($C_{biofilm}$) for *para*-xylene and toluene biofilters

para-Xylene biofilter		Toluene biofilter	
$C_{biofilm}$ (g C m^{-3})	μ (h^{-1})	$C_{biofilm}$ (g C m^{-3})	μ (h^{-1})
2.28	0.0034	1.98	0.0034
5.33	0.0045	3.90	0.0037
7.85	0.0048	7.26	0.0041
9.65	0.0043	8.34	0.0081
		14.06	0.0046

Table 5.6b. Specific growth rate (μ) as a function of the pollutant logarithmic average concentration in biofilm ($C_{biofilm}$) for mixture biofilter

Stage - I		Stage - II		Stage - III	
$C_{biofilm}$ (g C m^{-3})	μ (h^{-1})	$C_{biofilm}$ (g C m^{-3})	μ (h^{-1})	$C_{biofilm}$ (g C m^{-3})	μ (h^{-1})
1.45	0.0030	5.73	0.0021	1.41	0.0064
3.70	0.0036	7.09	0.0019	2.16	0.0055
5.89	0.0054	10.02	0.0035	4.08	0.0068

In the case of the biofilter treating toluene, μ is calculated as a function of $C_{biofilm}$ of toluene over a range of 2.0 to 14.1 g C m^{-3}. The microorganisms were inhibited at concentration in the biofilm at greater levels than 9 – 10 g C m^{-3}. The μ_{max} for toluene case was 0.0081 h^{-1}.

The value of μ_{max} for *para*-xylene determined in the present study was smaller than those reported in the literature when batch tests with specific strains were performed. Accordingly, Chang et al. (1993) reported a value of 0.54 h^{-1} using a *Pseudomonas* species designated as X1. Kim et al. (2009) determined a value of 0.034 h^{-1} using a *Bacillus* sp. strain. However, when the same methodology as described here to calculate μ_{max} was followed, similar results were obtained. Kermanshahi et al. (2006) obtained a value of 0.0047 h^{-1} using data from a continuous operation of a bioreactor with immobilized soil. Identical conclusion is valid for toluene. For microorganisms degrading toluene in batch essays, μ_{max} has been observed within the range of 0.046 to 0.54 h^{-1} (Chang et al., 1993; Kim et al., 2009). Ávalos Ramirez et al. (2008b) obtained a comparable value to the present study of 0.007 h^{-1} from a biofilter packed with clay spheres.

Prenafeta-Boldú et al. (2008) stated that biodegradation kinetic parameters obtained through microorganisms growth in suspended cultures may be considerably different from those found

in real biofilters. Such differences might be attributed to rate limiting diffusion or to biomass physiological changes that are embedded in the observed kinetic parameters.

In the mixed biofilter (Table 5.6b), μ values from 0.019 to 0.068 h^{-1} were calculated, where the highest rate obtained (0.068 h^{-1}) was equal to the pollutant mixture with the lowest *para*-xylene level ($IL_{p\text{-}Xyl}$ 8.84 ± 0.29 g C m^{-3} h^{-1}). Stage nº IV has not been introduced in Table 5.6b due to equipment failures during the experimentation.

No inhibition processes in any of the studied stages were noticed. In accordance with the conclusion pointed out in Figure 5.2c, higher μ were obtained in those mixtures with similar total contaminant concentration in the biofilm but comparatively greater toluene proportions. For instance, for a C_{In} ≈ 6 g C m^{-3}, a μ of 0.0054 h^{-1} was recorded when *para*-xylene/toluene proportion was approximately 48/52 % (w/w) (stage nº I), but this value decreased to a value of 0.0021 h^{-1} when the proportion varied to 92/8 % (w/w) (stage nº II).

5.4. Conclusions

para-Xylene, toluene and a mixture of both vapours were treated with three up-flow lab scale biofilters filled with inert packing media. Optimum behaviour was achieved under weak basic environment. *para*-Xylene was inhibited by the presence of toluene, but toluene degradation was stimulated when *para*-xylene was present in the mixture. Michaelis-Menten and Haldane type models were fitted to experimental EC for *para*-xylene and toluene biofilters, respectively.

Biomass yield coefficient (YB) and specific growth rate (μ) strongly depended on $IL_{p\text{-}Xyl}$ in the mixture biofilter, with YB_{max} (0.62 g B g C^{-1}) and μ_{max} (0.068 h^{-1}) were obtained for the lowest *para*-xylene/toluene proportion (19/81%) (w/w).

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6. PACKING MATERIAL

Fabrication of yttria-stabilized zirconia-based honeycomb biofilters *(Int. J. Appl. Ceram. Technol. 8 (6), 1305 – 1311)*

6.1. Introduction

The worldwide power production is currently based on the combustion of fossil fuels, which aggravates all the problems related to the quality of the air and global-warming effects. A competitive alternative to traditional combustion processes (incineration and catalytic oxidation) or chemical oxidation technologies for air pollution control are the use of biological technologies whose efficiency relies on the ability of microorganisms to biodegrade a large variety of contaminants. The most popular and oldest bioreactor configuration is the conventional biofilter (or fixed-bed bioreactor), which has now been used for several decades (Kennes et al., 2009). It has proven to be both economical (€10000/1000 m^3 h^{-1} when the air flows range from 10^4 to 10^5 m^3 h^{-1}) and environmentally viable (Malhautier et al., 2005). The lack of a liquid phase in its configuration makes it especially suitable for the treatment of hydrophobic and poorly water-soluble compounds.

The nature of the packing material is a crucial factor for a successful industrial application of biofilters, affecting both the removal performance related to bacterial activity and the bioreactor long-term operational stability. Additionally, the correct choice of support media has important implications in the economic assessment, not only because of the high volumes usually required for biofilter construction and medium replacement due to limited durability, but also because of electrical costs correlated to pressure drop (Prado et al., 2009).

Consequently, an ideal packing material should exhibit the following properties (Dumont et al., 2008): suitable particle size, void fraction and specific surface area, high nutritive capacity, high moisture retention capacity, high buffering capacity avoiding large pH fluctuations and mechanically resistant, chemically inert and stable. Dorado et al. (2010) demonstrated that the election of the most appropriate material to pack a biofilter depends on its physical and chemical properties according to its role upon operation. They proposed a function defined by the degree of suitability values of four parameters: a main parameter that has larger impact in the case study (45 %), a secondary parameter, which may play an important role in the case study (25 %) (i.e. water-holding capacity and water retentivity would be the main and secondary parameters, respectively, to be considered in the case of a biofilter operating with an inlet gas stream with low-relative humidity), pressure drop (15 %), and annual material cost (15 %).

The most commonly used organic biofilter media cited in literature are soil, compost, peat, wood bark, and coconut fiber, as they satisfy most of the required criteria, and they are widely available at low cost. However, they tend to be mechanically fragile, which implies a relatively low durability. Alternatively, several synthetic or inert carriers such as activated carbon, perlite, glass beds, ceramic rings, or polyurethane foam have been studied, though their high price and the requirement of a periodical nutrient supply may hinder their use on an industrial scale. Consequently, combinations of organic and inorganic materials (Hernández et al., 2010), formulation of new packing materials focusing in the simplification of the biofiltration process

(Gaudin et al., 2008) and development of new reactor systems have been tested (Fang et al., 2007).

A common inorganic material is zirconia, which is typically used in the fabrication of ceramic-based membranes for microfiltration (MF) and removal of toxic elements (Hestekin et al., 1998). Zirconia-based membranes are more stable than those based on TiO_2 and □-Al_2O_3 (Bhave, 1991) and they can be cleaned with steam and a basic solution (Wu and Cheng, 2000), performing steadily under high concentration of hydrocarbons for the treatment of wastewater of oil refineries.

Zirconia exhibits three crystallographic phases: cubic, tetragonal, and monoclinic. The cubic polymorph is typically used in MF processes. The use of a doping element (e.g. 8 – 12 % yttrium oxide) is a typical strategy to stabilize the cubic phase at room temperature, which helps avoiding the formation of cracks in the ceramic support due to phase transitions during heating/cooling processes. This cubic yttria-doped zirconia is commonly referred as YSZ and is also the *state-of-the-art* electrolyte material for solid oxide fuel cells (Minh and Takahashi, 1995).

The aim of this work was to investigate the possibility of employing YSZ as support matrix for biomass nesting and growth in biofilters applied to waste gas treatment. In order to achieve this, a simple procedure for replicating organic-based ceramics has been developed and will be described herein. Toluene was selected as representative of hydrophobic monoaromatic hydrocarbon compounds as it is moderately biodegradable.

6.2. Materials and methods

6.2.1. Slurry preparation

A slurry of the ceramic target material were prepared by mixing 10 g of YSZ (PI-KEM, Staffordshire, U.K.) in a zirconia-jar with the following organic components: 8 g of a solvent mixture of methyl–ethyl–ketone and ethanol (3:2, w/w); 0.5 g of triton-Q (dispersant); 1 g of polyethyleneglycol PEG400 (plasticizer); 1 g of dibutyl phthalate (plasticizer); and 1 g of butvar polyvinyl butyral (binder). All of them were purchased from Sigma-Aldrich (Madrid, Spain). These components were ball-milled (Pulverisette, Fritsch, Germany) for 1 h at 150 rpm using zirconia balls. After that, the slurries were sonicated for 15 min to allow degasification. The slurries were preserved in glass bottles, under continuous movement in a roller mixer to avoid agglomeration of the solid particles with time.

Once the slurries have been prepared they can be used to coat any type of organic-based structure (e.g. polyester and NOMEX™ (DUPONT™, Goodfellow, Huntingdon, U.K.) meshes, carbon-based microfibers, cotton, acrylic fibers, polyurethane foams, etc.) (Ruiz-Morales et al., 2010a). The organic structure is impregnated by dip-coating at room temperature and then left to dry overnight. After solvent evaporation, the coated material is fired at temperatures between 1000 °C and 1400 °C for 2 – 4 h, which results in a ceramic body that replicates the structure of the organic-based material used as mould.

6.2.2. Biofilter setup

The biofiltration system consisted of three PVC modules (Figure 6.1). The packed bed was divided into three identical sections with a total volume of 4.5 l. The packing material selected in this work was commercial ABONLIR^{TM} supplied by SLIR S.L (Specialised Engineering in Recycling Agricultural Residues, Carcastillo, Navarra, Spain). This material was made up of composted pig manure and sawdust and the pellets were manufactured by mechanical compression without chemical addition. The compost was stored in sealed plastic bags at room temperature to maintain its original moisture content. Table 6.1 summarises the main characteristics of the packing material (Barona et al., 2005).

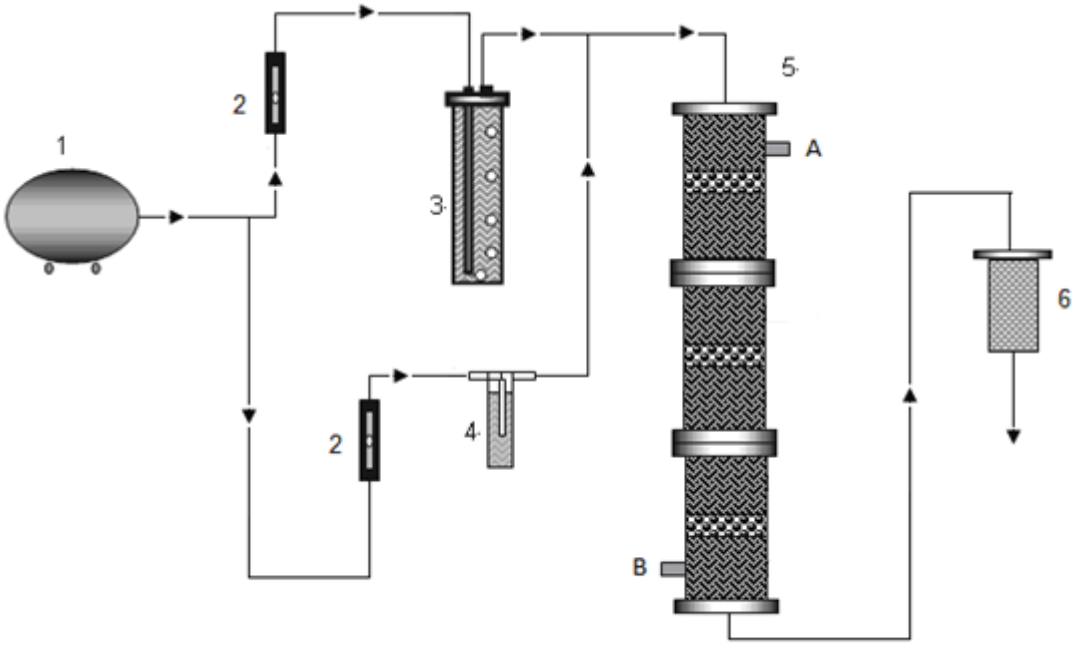

Figure 6.1. Diagram of the biofiltration system: 1 Air-compressor; 2 Flow meter; 3 Humidification chamber; 4 Toluene evaporator; 5 Modular Biofilter; 6 Active carbon chamber; A (inlet gas sampling valve); B (outlet gas sampling valve).

The biofilter was initially irrigated with an activated sludge acclimatized according to the procedure published by Elías et al. (2010) and was operated in a downflow configuration at room temperature. The flow of toluene-contaminated air was added from the top of the biofilter at a flow rate of 1.5 l min^{-1} (corresponding to an empty bed residence time of 180 s) and the contaminated flow was generated by mixing a toluene (99.5 %, Panreac, Castellar del Vallès, Barcelona, Spain) saturated air stream with a humidified toluene-free air stream in different proportions. The relative humidity of the contaminated air at the biofilter inlet remained always higher than 98 %. An activated carbon filter was also included in the experimental setup, being fitted to the bioreactor outlet to mitigate the environmental impact of the nondegraded contaminant. The biofilter was equipped with gas sampling valves to monitor the inlet and outlet toluene concentrations.

Table 6.1. Physical-chemical properties of the packing material used in the modular biofiltration unit (Barona et al., 2005)

Property	Value
Organic matter (%)[a]	40.0
Total nitrogen (%)	1.4
P_2O_5 (%)	1.1
K_2O (%)	1.5
Total S (%)	3.3
pH	6.5 – 7.5
Mean length (mm)	10.7
Mean radius (mm)	6.1
Bulk density (g cm^{-3})	1.3
Real density (g cm^{-3})	2.3
B.E.T. (N_2) surface area (m^2g^{-1})	12.06 ± 0.09
BJH adsorption average pore diameter (Å)	145
Macropore volume in the pellets (%)	89.42
Micropore area (d < 20 Å) (m^2 g^{-1})	0.3
Initial Moisture Content (%)	25.2

Notes: (a) Value provided by the manufacturer

Three samples of 2 – 3 g of honeycomb–YSZ support were introduced in each module following an adapted composting test based on standard UNE-EN ISO 846. The samples were immersed in the bioreactor for up to 180 days once the biofilter operated in steady-state. This condition successfully degraded the contaminant. During the studied period, the biofilter was continuously fed with toluene at an inlet loading (IL) rate ranging from 7 to 114 g m^{-3} h^{-1}.

6.2.3. Analytical procedure

The toluene concentration was measured using a microgas chromatograph CP 4900 (Varian, Houten, The Netherlands) with auto-sampling injection, a TCD detector and using He as carrier gas. The Micro GC was equipped with CP-Sil 5 CB (6 m * 0.15 mm * 2 µm) and CP PoraPLOTQ (10 m * 0.25 mm * 8 µm) columns. The oven, injector, and TCD detector were maintained at 80 °C, 110 °C, and 80 °C, respectively. External standards prepared from a calibration cylinder (Air Liquide, Madrid, Spain) containing a toluene in nitrogen enabled the quantitative determination of the target volatile organic compounds (VOC).

6.2.4. Microstructural characterization

Optical images (Leica Zoom 2000, Leica Microsistemas, Barcelona, Spain) and scanning electron microscopy micrographs (JEOL JSM-6300, Tokyo, Japan) were used to obtain information about the microstructure of the ceramic-based material after the sintering process and, specially, to verify the colonization of the inorganic support and also to check any evidence of degradation due to the microorganisms on the surface of the mentioned material.

6.3. Results and discussion

6.3.1. Slurry coating

Ceramic materials are typically used in several technological areas such as fuel cells, materials for exhaust catalyst, hydrogen storage, high-temperature electrolysers, photocatalyst, ceramics membranes, etc. Typically, a moulding process is applied to the ceramic powders to produce materials with an adequate porosity, mechanical properties, thermal stability, and so forth (Scheffler and Colombo, 2005).

A typical method for the fabrication of ceramic thin layers is the tape-casting procedure (Mistler and Twiname, 2000). As explained in the experimental section, the method consists on mixing ceramic powders with some organic compounds: solvents allow controlling the viscosity of the slurry and the rate of evaporation; plasticizers help to introduce flexibility on the material in the green state; dispersing agents are used to avoid the agglomeration of the solid particles and the binder is used to bind together ceramic particles during the sintering process. After the mixing procedure the slurry can be deposited in a suitable substrate, for example a thin layer of Mylar, and the so-called doctor blade allows the deposition onto the substrate regulating the thickness of the deposited layers. After solvent evaporation the material deposited in the green state remains as a flexible layer than can be easily peeled-off from the Mylar substrate and a subsequent thermal treatment allows obtaining a dense thin layer of the moulded ceramic material.

In the proposed approach the 2D Mylar substrate was replaced by a three-dimensional (3D) organic-based material, which acted as a mould. The idea was to produce a 3D replication of the organic mould in an inorganic material. The composition of the slurries helped to produce a dense material when firing at high temperature, thus avoiding the replicated structures to collapse even after working at 1500 °C.

As common organic materials, polyester meshes (Figure 6.2a), carbon microfibers meshes (Figure 6.2c), cotton fabrics (Figure 6.2e), acrylic fibers (Figure 6.2g), and foams (Figure 6.2i), have been used in this approach (Ruiz-Morales et al., 2010a). After dip-coating in the YSZ-based slurry, the fresh-coated material was left to dry for 0.5 – 2 h. Then the sample was fired up to 1400 °C for 2 h, thus rendering perfectly replicated structures, as shown in Figures 7.2b, d, f, h and j.

The same methodology was applied to fabricate a zirconia-based support due to the aforementioned good physico-chemical properties of this material. The morphology of the support was fabricated as a honeycomb arrangement due to two factors. First, it is well-known that the honeycomb arrangement provides mechanical strength to any supported structure due to an effective distribution of the load (Foo et al., 2007). For the use of biological reactors, at mass scale, the weight of the supporting material may be critical and hence, a material and structure with high-mechanical strength is highly desirable. Secondly, the size of the honeycomb cells can be totally controlled, which may help to avoid occlusion problems during the filtration process.

A commercial NOMEX^{TM} mesh was used as a moulding agent (Figure 6.2k). The NOMEX^{TM} mesh was dip-coated in the YSZ slurry and left to dry several times. This allowed the production of a honeycomb arrangement with thinner walls after three to five cycles. The sample was finally fired up to 1400 °C for 2 h (Figure 6.2l), rendering the final ceramic product. The coated NOMEX^{TM}, in the green state, can be easily cut as circular, squares, etc., pieces to be adapted to the specific design of the bioreactor. These pieces can be stacked for the final dip-coating cycle, and hence, the whole assembly can be coated and fired as single unit (Figure 6.2l). Mechanical tests performed in these structures indicated that the critical stress to break the structure is 37.2 MPa and furthermore, it was verified that the fabrication process did not affect the properties of YSZ (Ruiz-Morales et al., 2010b).

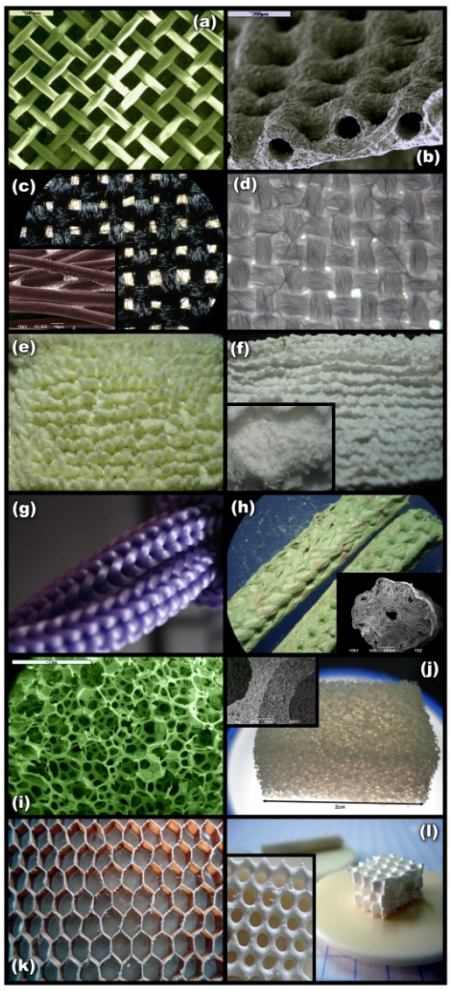

Figure 6.2. (a) Polyester mesh, (c) carbon-microfibers mesh, (e) cotton, (g) acrylic fibers, (i) polyurethane foams, and (k) NOMEX^{TM} mesh can be covered with an inorganic-based material by slurry coating and after firing at high temperature (1000 – 1400 °C, 2 – 4 h) a perfect replicated structure will be produced as shown in: (b), (d), (f) fabricated with YSZ, (h) composite NiO–YSZ, inset shows crosssection of the moulded material, (j) YSZ foam, inset shows an alumina-based foam, (l) 3D-honeycomb arrangements fabricated in YSZ, inset shows a single honeycomb YSZ layer.

6.3.2. Application of YSZ-based honeycombs in biofiltration

A biofilm was generated progressively (visual observation) over the surface of the YSZ-based honeycomb structures after the samples were introduced in the bioreactor together with the organic packing material. The defects and porosity of the YSZ-supporting layer (Figure 6.3a) might act as attachment points for microorganisms growth (Figure 6.3b and c).

These biofilms are typically made of bacteria, which excrete extracellular polymeric substances or sticky polymers showing the structure of a spider-web (Mittelman, 1985). The polymeric material known as glycocalyx favors the microbial immobilization and retains VOC like toluene, which could favur the contact between the biomass and the pollutant (Figure 6.3d). This

adsorptive capacity may be interesting to reduce the inhibitory effect for high contamination applications.

A mature biofilm can be developed in several hours or several weeks, depending on the system (Mittelman, 1985). In any case, the main element in a mature biofilm is the glycocalyx matrix (75 – 95 %), being the bacterial cells the minor element (5 – 25 %) (Geesey et al., 1994). The common ‘‘pioneer’’ bacteria is the Pseudomonas aeruginosa as it seems to be confirmed from our biofilms (Figure 6.3e), although other types of bacterias, such as *Pseudomonas* sp., when feeding toluene (Daugulis, 2001) can be expected.

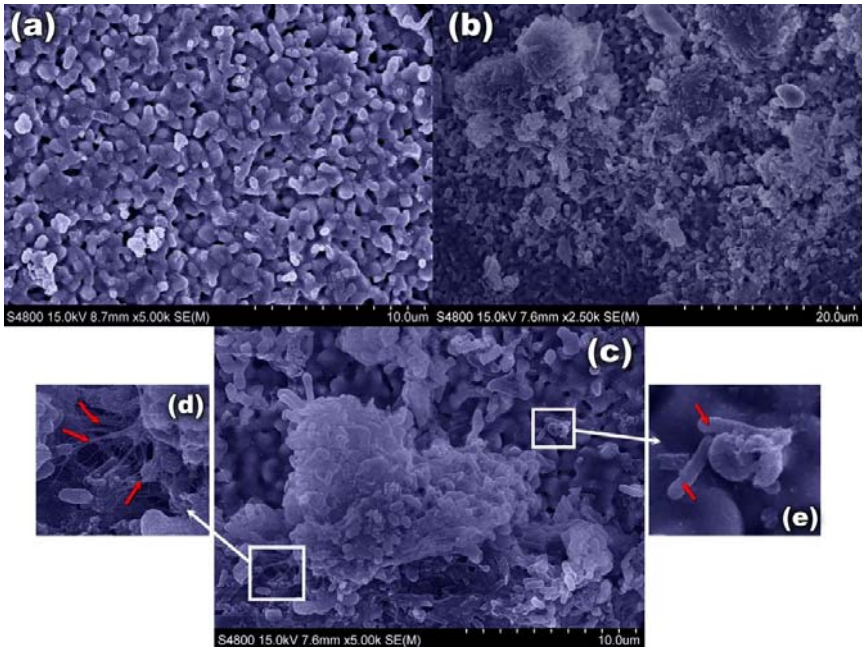

Figure 6.3. SEM micrographs of the (a) surface of a yttrium-stabilized zirconia biofilter media. (b, c) Details of the attached biofilms after 45 days of experimentation. (d) Magnification of the sticky spider-web glycocalyx. (e) Magnification of some ‘‘pioneer’’ bacteria observed in the biofilms.

The removal efficiency (RE) of the contaminant was 88.1 ± 5.2 % during experimentation (EC_{MAX} = 109 g m^{-3} h^{-1} (Figure 6.4), which involved the growth and development of specific biomass capable of toluene degradation. This confirms the good behavior of the proposed system for the successful removal of VOC. Additionally, less compaction of the organic packing material was observed around the synthetic samples. This result enhanced the idea of employing higher quantities of this material mixed with the organic packing material (50:50 vol:vol) in order to avoid problems like clogging, channelling, and excessive head loss within the biofilter bed, which involves bed compaction and deterioration of the overall performance.

SEM micrographs recorded after 6 months of operation did not show any evidence of degradation in the YSZ-supporting honeycomb structure as no roughening of the surface, holes/cracks, defragmentation or changes in color were observed.

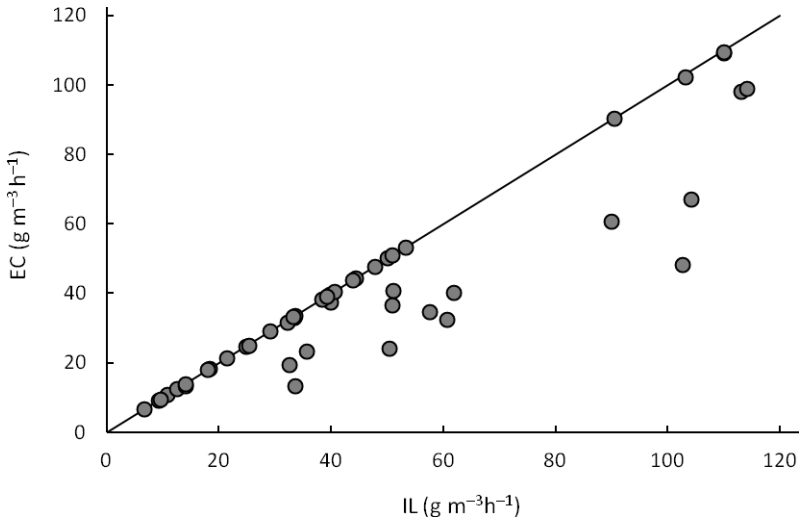

Figure 6.4. Toluene inlet load (IL) versus elimination capacity (EC) during the 180 day experiment.

6.3.3. Economical assessment

The purchase cost of the packing material has a significant impact on the overall cost (Prado et al., 2009). The price of YSZ-based honeycomb could be estimated in €160 m^{-3}, where the fabrication/man work was the 30 % of the final cost. Once RE decreases due to biomass accumulation and pore clogging, the synthetic filter could be taken away and burnt at 600 °C in order to be reutilized. With this assumption, an average durability of 10 years was supposed, which implies an annual material cost of €16 $(m^3\ year)^{-1}$. The price and the characteristics provided by this material make it a reasonable alternative to typical biofiltration packing materials like compost (€2 – 5 $(m^3 year)^{-1}$) (Dorado et al., 2010) or fibrous peat (€30 – 50 $(m^3\ year)^{-1}$) with an average durability of 3 – 5 years, as estimated by these authors (Álvarez-Hornos et al., 2008; Dumont et al., 2008).

Another factor that can drastically reduce the production costs with this approach is the possibility of recover YSZ from solid oxide fuel cells that have reached the end of its lifetime. This would allow using residual YSZ, from aged SOFC devices, for the fabrication of new biofilter supports.

6.4. Conclusions

A simple and cost-effective approach were used to replicate any type of organic-based structures. The same slurry coating process were used to fabricate YSZ structures in honeycomb arrangements. These structures exhibit high thermal and mechanical resistance and they can withstand harsh media conditions, being ideal to be used as biofilm support. YSZ-based biofilters were fabricated and tested with a typical hydrophobic VOC such as toluene.

Preliminary tests show that the YSZ structures are stable; allowing the attachment of biofilms and the performance of such systems for removing contaminants reaching a very promising RE > 85 %. Further research must be done utilizing this synthetic filter alone as the body of the bioreactor or mixed with other organic media in order to analyze the removal rates obtained and the stability for long-term operation.

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7. MICROBIOLOGICAL CHARACTERIZATION

Fungal/bacterial interactions during the biodegradation of TEX hydrocarbons (toluene, ethylbenzene and p-xylene) in gas biofilters operated under xerophilic conditions (*Under construction*)

7.1. Introduction

An air biofilter is the simplest configuration of a vaporphase bioreactor used for the treatment of air polluted with volatile organic compounds. The contaminated gas stream is forced through a column packed with a porous support material, where the volatile contaminants are ad/absorbed and metabolized by a microbial biofilm thatdevelops on the packing. A conventional biofilter is characterized by the absence of a free liquid phase, and nutrients needed for microbial growth are supplied either by the packing itself, when organic materials such as compost or peat are used, or are supplemented through discontinuous irrigation of the bed in case of inert or synthetic materials. Humidification of the biofilter bed is therefore a critical control parameter to keep the availability of water for microbial growth at an acceptable level (Bohn and Bohn, 1999). Yet, excess of water has been reported as detrimental for the elimination of relatively insoluble substrates because of the mass transfer limitation phenomena and packing deterioration (Kennes and Veiga, 2004).

Air biofiltration was initially developed for the removal of odorous compounds, but its use has progressively been extended to the biodegradation of hazardous volatile pollutants such as benzene, toluene, ethylbenzene, and xylene (Kennes et al., 2009) – monoaromatic hydrocarbons known collectively as BTEX. Significant atmospheric BTEX pollution arises from petrochemical and chemical industries, particularly where containment and/or gas treatment is inadequate (Swoboda-Colberg, 1995). The biological treatment of gas streams containing relatively low concentrations of BTEX has been found to be more economical and environmentally friendly than other physicochemical alternatives such as adsorption, condensation, and incineration, which require more input (i.e. materials, reagents, and energy) and produce toxic wastes (van Groenestijn and Hesselink, 1993). As reviewed recently (Kennes and Veiga, 2004), an increasing number of studies have pointed to the fact that the presence of fungi in the biofiltration of specific pollutants offers some advantages with respect to the stability and biodegradation activity. Fungi are generally better suited than bacteria to grow on a solid support, to deal with water and nutrient scarcity and to tolerate low pH conditions. Hence, fungi have primarily been used in combination with inert packing media (e.g. perlite, polyurethane foam, vermiculite, etc.) for an extended biofilter operation, and sustained elimination capacity (EC) values ranging from 20 to 100 g m^{-3} h^{-1} have generally been obtained (Cox et al., 1996; García-Peña et al., 2001; Kennes and Veiga, 2004; Prenafeta-Boldú et al., 2008).

Research on the microbial ecology of biofilters related to the biofiltration of BTEX-polluted gases has fundamentally been focused on population dynamics and composition of heterotrophic bacteria, particularly when biofilters were operated under optimal or suboptimal conditions (Cabrol et al., 2012). Extensive reviews have also been published on the aerobic metabolism of aromatic hydrocarbons by bacteria (Gibson and Harwood, 2002;

O'Leary et al., 2002), but studies on the fungal counterparts are comparatively scarce (Prenafeta-Boldú et al., 2006). Interactions between fungi and bacteria in gas biofilters, and the consequences on the overall bioreactor performance, remain a controversial issue. Fungal dominance has been claimed both as beneficial and detrimental to the feasibility of biofiltration. Besides the already mentioned advantages of fungi to withstand growth-limiting conditions, the development of fungal biomass in biofilters has also been related to the presence of potential pathogens (Prenafeta-Boldú et al., 2006), the frequent incidence of clogging due to the filamentous biomass (Aizpuru et al., 2005), and the generally lower metabolic rates when compared to most of aerobic bacteria (Prenafeta-Boldú et al., 2001). While the presence of an adapted microbial community is essential for the stable operation of a biofilter, relatively little research has been carried out in characterizing the structure and dynamics of the underlying microbial populations. A biofilter is an ecosystem in which diverse organisms are subjected to several environmental stresses, including changes in water content, pH, temperature, and exposure to toxic chemicals. Moreover, the system is likely to be heterogeneous, and important environmental gradients might occur inside the bioreactor in time and/or space. Altogether, biofilter operation and performance may be correlated directly with the microbial community dynamics. A biofilter is analogous to a soil bed, and molecular techniques aimed to microbial community analysis in soil would seem directly applicable to biofiltration research.

In this study, the microbial communities of bacteria and fungi in samples taken from different laboratory-scale biofilters were studied by culture-independent molecular methods to gain new insights into the relationship between microbial community structure and biofilter operational parameters. The biofilters were used for the treatment of gas streams containing toluene, ethylbenzene, and *para*-xylene. Operation was performed at relatively low water content to study the effect of bed drying out on biofilter performance and on the microbial populations from the bioreactor bed. Microbial community shifts were characterized by comparing the dominance and composition of fungal and bacterial genotypes enriched in the biofilters, in relation to those initially present in the original inoculum and organic packing.

7.2. Materials and methods

7.2.1. Biofilter set-up and operation

Gas biofiltration experiments were performed in three identical columns, fed respectively with toluene, ethylbenzene, and *para*-xylene. Each biofilter consisted of two interchangeable cylindrical modules mounted on top of each other, named M1 and M2, respectively, accordingly to their initial vertical position. Each module was made of PVC (10 cm diameter * 33 cm length) and had a hollowed bottom plate to sustain the packing. Biofilter modules were packed with a pelletized mixture of animal manure and sawdust, commercialized as a plant fertilizer under the name of Abonlir ^{TM} (SLIR S. L., Carcastillo, Spain). Pellets were previously sieved to homogenize the particle size and characterized in physicochemical terms (Table 7.1). The packing was then soaked with a toluene degrading liquid enrichment culture prepared as

described elsewhere (Elías et al., 2010), and about 1.6 dm^3 of the material was loaded into each biofilter module. Subsequent gas feeding in biofiltration experiments was applied in a down-flow mode, and the polluted air was generated by mixing two compressed air streams that were regulated with rotameter assemblies.

Table 7.1. Physicochemical characteristics of the bed packing material used in biofiltration experiments.

Property	Type/Value[a]
Physical parameter:	
Particle shape	Cylindrical
Bed apparent density (g m^{-1})	0.98 ± 0.07
Pellet apparent density (g ml^{-1})	1.29 ± 0.08
Material real density (g ml^{-1})	2.72 ± 0.21
BET superficial area (m^2 g^{-1})	12.06 ± 0.09
Langmuir superficial area (m^2 g^{-1})	17.41 ± 0.74
Chemical composition:	
Total Organic matter (%)[b]	72
Labile Organic matter (%)	40
Total Carbon content (%)	32
Total Nitrogen content (%)	2
Total Hidrogen content (%)	3
Total Phosphorous content (%)	0.2
Total Sulphur content (%)	3
Elemental sulphur content (%)	< 0.1
Sulphate content	< 0.1
Water content (%)	23.2
pH	6.5 – 7.5

Notes: (a) Average and standard deviation values; (b) Value provided by the manufacturer.

The main stream consisted of air bubbled through a distilled water column for humidification, while a secondary air stream was saturated with the selected volatile substrate in a gas washing bottle. The relative humidity of the contaminated air at the biofilter inlet remained always higher than 98 %. Biofiltration experiments lasted for 185 days during which step changes were applied to the volumetric organic loading rate (OLR), either by increasing the substrate concentrations in the influent air, the fed gas flow rate, or by removing one of the biofilter modules. The effect of drought was studied by applying sporadic irrigations and interchanging the position of the two biofilter modules. The water content in each packed

module was monitored gravimetrically upon drying a sample of support material (2 – 3 g) at 105 °C. An equivalent mass of the withdrawn filter media was replaced with new packing material. All biofiltration experiments were performed at room temperature (approximately 25 °C).

7.2.2. Analytical and microscopy methods

In relation to the physicochemical characterization of the packing material, the pellets were previously sieved so that only those with a diameter between 6.3 and 8.0 mm were used as packing material. ASTM sieves were used for the particle size distribution. The BET surface area and external surface area were estimated by means of the nitrogen adsorption technique with an ASAP 2010 V5.02H analyzer (Micromeritics, Georgia). The original moisture content was determined in a Leco TGA 500 thermobalance (LECO Corporation, MI). The pH value was measured in a 1 : 9 dry material weight to water volume ratio. Total C, H, and N contents were measured in an EAI Exeter Analytical analyzer (Exeter Analytical Inc., MA), and the S total content was determined in a Leco SC 132 analyzer (LECO Corporation). The content of elemental sulfur was determined by dissolving the elemental sulfur component of the sample into carbon sulfide.

The EC and efficiency in each biofilter were determined by measuring inlet and outlet concentrations of the applied volatile organic compounds in a gas chromatograph (microGC CP 4900) equipped with a CP-Sil 5 CB (6 m × 0.15 mm × 2 µm) column and a TCD detector. The column and injector temperatures were set at 80 °C and 100 °C, respectively. For scanning electron microscopy (SEM) imaging, samples of the packing were fixed in 2 % glutaraldehyde in 0.1 M cacodylate buffer (pH = 7.4), washed in iso-osmolar cacodylate/sucrose buffer, and postfixed in 1 % osmium tetroxide in cacodylate buffer. Samples were then dehydrated through an ethanol series and washed in hexamethyldisilazane prior to air-drying. Finally, samples were mounted onto stubs and gold-coated using a JEOL fine-coat ion sputter JFC-1100. Samples were visualized and micrographed using a scanning electron microscope (Hitachi S-4800) at 15 kV accelerating voltage.

7.2.3. Denaturing gradient gel electrophoresis (DGGE) molecular profiling

Bed samples were withdrawn at the end of experiments from each biofilter bottom module (M2). Samples were also taken from the carrier material. Total DNA was extracted from approximately 0.25 g of each sample with the PowerSoil ™ DNA Isolation kit (MoBio Laboratories, Inc., Carlsbad, CA), according to the instructions of the manufacturer. Two primer sets were used to selectively amplify bacterial and fungal rDNA fragments. Universal eubacterial forward F341GC and reverse R907 primers were used to amplify the hypervariable V3-V5 region from the 16S rRNA gene, as previously reported (Yu and Morrison, 2004). The

fungal first internal transcriber spacer (ITS1) from the ribosomal DNA was amplified with the primer pair ITS5 and ITS2 (White et al., 1990). The forward primer ITS5 and F341 contained the GC clamp 5'- CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGG-3'. All PCRs were performed with a Mastercycler (Eppendorf, Hamburg, Germany), and each reaction mix (25 µL mix/reaction) contained 1.25 U of ExTaq DNA polymerase (Takara Bio, Otsu, Shiga, Japan), 12.5 mM dNTPs, 0.25 µM of each primer and 100 ng of DNA.

The obtained PCR amplicons were loaded in two 8 % (w/v) polyacrylamide gels with a chemical denaturing gradient ranging from 30 % to 70 % [100 % denaturant contained 7 M urea and 40 % formamide (w/v)] and electrophoretically resolved in a DGGE-4001 equipment (CBS Scientific Company, Del Mar, CA). Electrophoresis was carried out at 60 °C and at 100 V for 16 h in a 19 TAE buffer solution (40 mM Tris, 20 mM sodium acetate, 1 mM EDTA, pH 7.4). The DGGE gels were stained for 45 min in 19 TAE buffer solution containing Sybr-Gold™ (Molecular Probes, Inc., Eugene, OR) and then scanned under blue light by means of a blue converter plate (UV Products Ltd, Cambridge, UK). Predominant DGGE bands were excised with a sterile filter tip, resuspended in 50 µL of sterilized Milli-Q water, and stored at 4 °C overnight. A 1 : 50 dilution of the supernatants was subsequently reamplified by PCR as described previously and sequenced using R907 and ITS2 primers, for eubacterial and fungal sequences, respectively.

Sequencing was accomplished using the ABI Prism Big Dye Terminator Cycle-Sequencing Reaction kit v. 3.1 and an ABI 3700 DNA sequencer (both Perkin–Elmer Applied Biosystems, Waltham, MA), according to the manufacturer's instructions. Sequences were edited using the BIOEDIT software package v. 7.0.9 (Ibis Biosciences, Carlsbad, CA) and aligned with the NCBI genomic database using the BLAST search alignment tool. The bacterial 16S rRNA and fungal ITS1 rRNA gene nucleotide sequences determined in this study were deposited in the GenBank database under accession numbers JN982532–JN982549 and JN982550–JN982558, respectively.

7.2.4. Quantitative PCR assay

Gene copy numbers of eubacterial 16S rRNA and fungal ITS1 rRNA fragments were quantified with the quantitative real-time PCR (qPCR). Each sample was analyzed in triplicate by means of three independent DNA extracts. The analysis was carried out using Brilliant II SYBR® Green qPCR Master Mix (Stratagene, La Jolla, CA) in a Real Time PCR System MX3000-P (Stratagene) operated with the following protocol: 10 min at 95 °C, followed by 40 cycles of denaturation at 95 °C for 30 s, annealing for 30 s at 50 and 55 °C (for 16S rRNA and ITS1 rRNA, respectively), extension at 72 °C for 45 s, and fluorescence measurement at 80 °C. The specificity of PCR amplification was determined by observations on a melting curve and gel electrophoresis profile. Melting curve analysis to detect the presence of primer dimers was performed after the final extension by increasing the temperature from 55 to 95 °C with a heating rate of 0.05 °C per cycle. Each reaction was performed in a 25-µL volume containing 2 µL of DNA template (approximately 100 ng of DNA), 200 nM of each primer, 12.5 µL of the ready reaction mix, and 30 nM of ROX reference dye. The primer set for eubacterial population was 519F and 907R

(Lane, 1992; Muyzer et al. 1995) and for fungal population was ITS5 and ITS2 (both couple of primers were purified by HPLC). The standard curves were performed with the following reference genes: 16S rRNA gene from *Desulfovibrio vulgaris* ssp. vulgaris ATCC 29579, inserted in a TOPO TA vector (Invitrogen, Belgium); and an ITS1 gene fragment obtained from a single DGGE band (Gen- Bank accession no. JN982550) cloned onto the PGEM plasmid vector using PGEM-T Easy Vector System I (Promega, Madison, WI). All reference genes were quantified by Quant-iT^{TM} PicoGreen® dsDNA Reagent using MX3000P (Stratagene) as a detector system. Tenfold serial dilutions of known copy numbers of the plasmid DNA in the range from 101 to 108 copies were subjected to aqPCR assay in duplicate to generate the standard curves. The qPCR efficiencies of amplification were greater than 96 %; the Pearson correlation coefficients (R^2) of the standard curves were between 0.997 and 0.994; and the slopes were between –3.353 and–3.416 for 16S rRNA and ITS rRNA, respectively. All the results were processed by means of MxPro^{TM} QPCR Software.

7.3. Results

7.3.1. Operation of biofilters

High removal efficiencies (RE > 90 %) were immediately achieved in the start-up operation at low OLRs [OLR < 10 g m^{-3} h^{-1}, empty bed residence time (EBRT) > 2 min], conditions that were maintained constant for about 30 days (Figure 7.1). Thereafter, the OLR was progressively increased by applying higher substrate concentrations in the influent air (C_i), until a significant drop in the RE was observed. An adaptation period of variable length, depending on the substrate, was generally required prior to the recovery of the previously recorded RE values. The maximum EC that was achieved during this first period was 40 g m^{-3} h^{-1} for toluene (RE = 97 %), 34 g m^{-3} h^{-1} for ethylbenzene (RE = 97 %), and 29 g m^{-3} h^{-1} for *para*-xylene (RE = 92 %). Neither irrigation nor nutrient amendments were applied during this initial stage, and the water content remained close to that of the original packing material (23 %) in all modules that composed the biofilters treating toluene and ethylbenzene. Conversely, the *para*-xylene biofilter displayed a marked humidity gradient (Figure 7.2); while water content in the top module (M1) stabilized at about 20 %, it remained close to 40 % in the bottom module (M2). To check whether such behavior was caused by heterogeneous packing, the position of the two modules was reversed after 85 days of operation in all biofilters prior to bed watering, so that the bottom module (M2) was replaced at the top position and vice versa. In just a few days of operation after module reversal (as measured in day 116), the water content of modules M1 now displaced to the bottom position raised in all biofilters up to 27 – 36 %, while it remained fairly constant in the now top modules M2 in relation to the previously measured values.

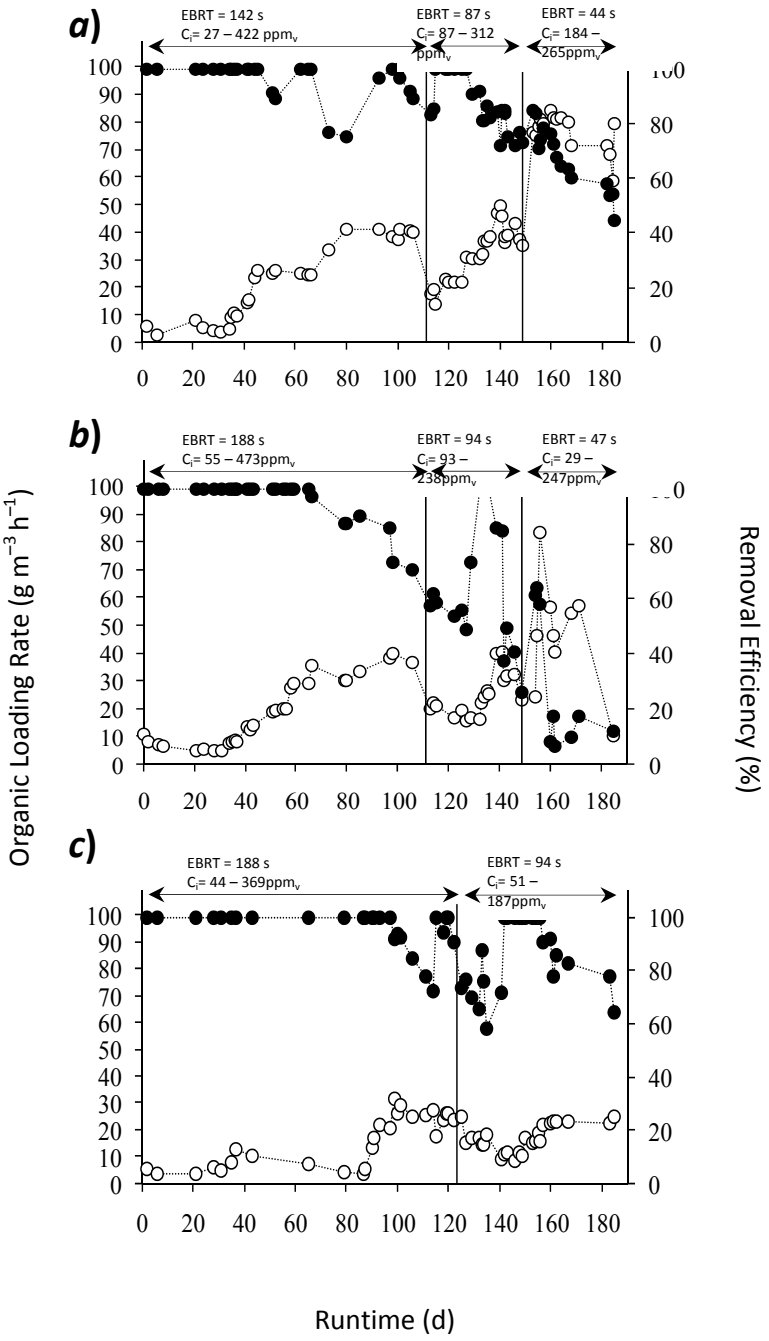

Figure 7.1. Organic loading rate (o) and substrate removal efficiency (●) of three identical biofilters operated in parallel and fed respectively with toluene (a), ethylbenzene (b) and p-xylene (c). Different operation phases in relation to the empty bed residence time (EBRT) and influent substrate concentration (C_i) are separated by vertical lines.

In a second operational stage, the EBRT was reduced to about 90 s by increasing the gas flow after 111 days of operation of the biofilters with toluene and ethylbenzene, and after 122 days for the *para*-xylene biofilter. Yet, despite packing moistening and the application of a lower OLR at the beginning of this second period, the performance of the biofilters tended to

deteriorate and the previously recorded EC values could not be maintained for a long time, particularly for the biofilter with ethylbenzene. The highest EC achieved in this period were 39 g m^{-3} h^{-1} for toluene (RE = 85 %), 33 g m^{-3} h^{-1} for ethylbenzene (RE = 84 %), and 20 g m^{-3} h^{-1} for *para*-xylene (RE = 90 %). During this phase, the packing of all bioreactor modules was irrigated and mixed up (on day 124). Upon resuming biofilter operation, all modules tended to dry out but dewatering was particularly strong (from 35 % to 21 %) in the *para*-xylene M2 module, now placed at the biofilter top. Humidity levels in the toluene and ethylbenzene M2 modules were further reduced down to 16 – 18 %.

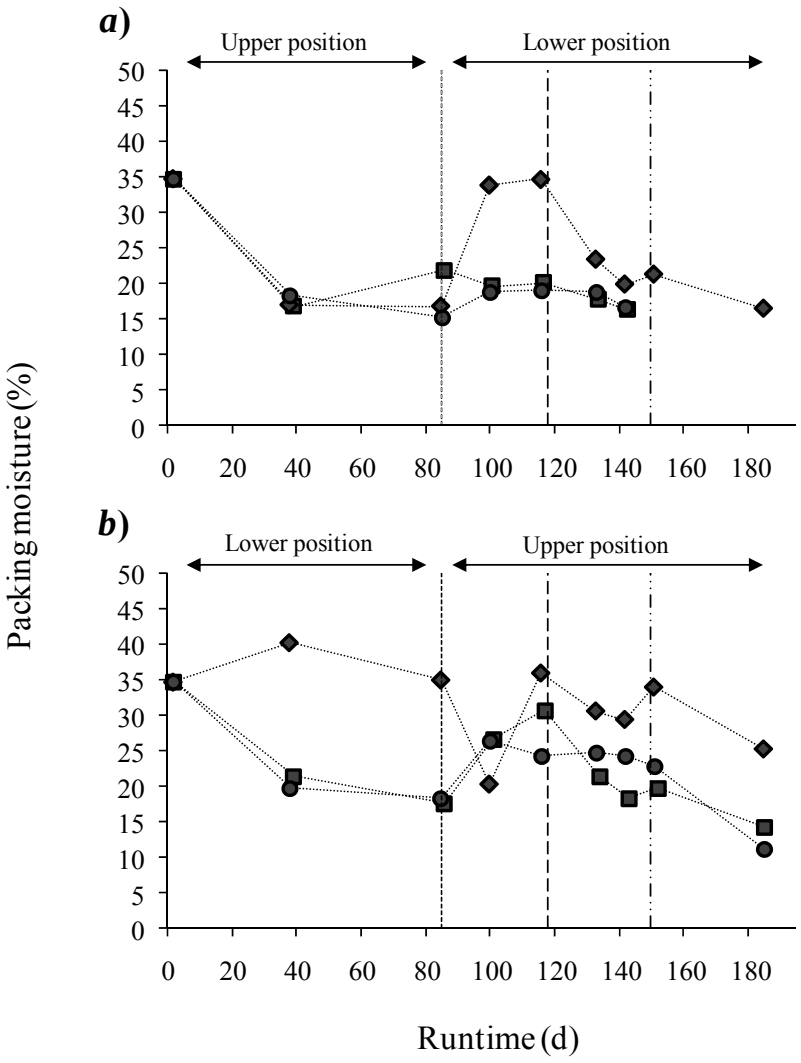

Figure 7.2. Evolution of the moisture content of the packing material from the biofilter modules M1 (a) and M2 (b) in the biofilter degrading toluene (■), ethylbenzene (•), and p-xylene (♦) The following operations are also indicated: watering plus module exchange (dotted line), watering plus bed mixing (dashed line), and watering plus removal of upper module (solid line) only for toluene and ethylbenzene.

A third and final operational stage consisting on a second EBRT reduction down to 44 and 47 s was applied only to the toluene and ethylbenzene biofilters, respectively, by removing the module M1, while the previous operational conditions were maintained for the biofilter fed with *para*-xylene. These conditions were kept for more than 30 days, until the end of the experiments. During this time, the EC and RE in the toluene biofilter progressively dropped from 60 to 40 g h^{-1} m^{-3} and from 80 % to 50 %, respectively. In the case of ethylbenzene, however, the biofilter performance experienced a significant failure and, despite the return to low OLR values, the RE was at the end of the experiments as low as 12 % for inlet substrate concentrations of only 29 – 167 ppm. Despite the fact that the EBRT in the *para*-xylene biofilter was kept at 94 s, its performance tended to deteriorate as well, a process that was temporarily reversed by the application of relatively low OLR (RE = 100 % for OLR of 53 – 67 g m^{-3} h^{-1}, for 10 days). However, the EC and RE at the end of experiments were below 24 g m^{-3} h^{-1} and 64 %, respectively.

7.3.2. Microbial community characterization

The fungal/bacterial biomass ratio in the liquid culture (inoculum), the original packing, and bed samples taken at the end of the biofiltration experiments was estimated from the number of fungal and bacterial ribosomal gene copies per gram of fresh weight (Figure 7.3). Bacterial gene copy numbers remained within the same magnitude order in all packing samples, ranging from $5 * 10^{11}$ to $5 * 10^{12}$ gene copies g^{-1}. Instead, the number of fungal ITS rRNA displayed a larger variability, from $5 * 10^{9}$ gene copies g^{-1} in the *para*-xylene biofilter to $3 * 10^{11}$ gene copies g^{-1} in the toluene biofilter. Microbial gene counts in the liquid enrichment culture were significantly lower than in the biofilter samples ($6 * 10^{7}$ and $2 * 10^{6}$ copies g^{-1} for bacteria and fungi, respectively). In relation to the fungal/bacterial ratio of the original packing, a significant increase in this value was only manifested in the biofilter with toluene. The proliferation of fungi was also visualized macroscopically (results not shown) and microscopically by SEM images on bed samples at the beginning and after prolonged biofilter operation (Figure 7.4). Abundant fungal biomass was primarily observed in the toluene biofilter, depicted as a profuse network of filaments with an approximate width of 5 – 10 µm and therefore compatible with fungal hyphae and mycelial cords.

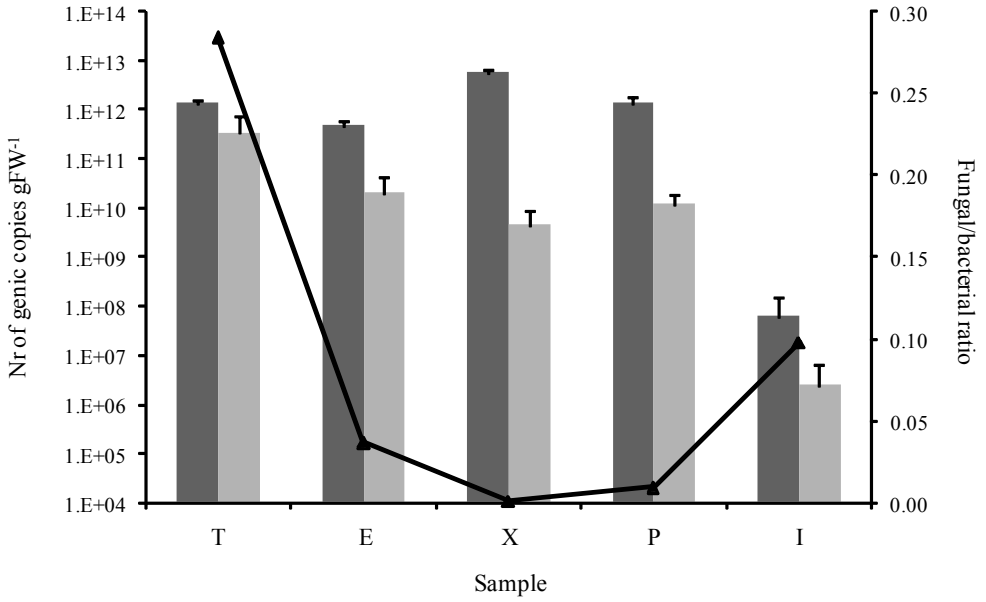

Figure 7.3. Average copy number of bacterial 16S rRNA (dark gray) and fungal ITS1 (light gray) partial ribosomal genes from three independent DNA extracts in different biomass samples (left-side log scale) per gram of sample fresh weight (FW); standard deviations are represented as error bars. The fungal/bacterial ratio is represented by a solid line on the right-site axis.

The microbial community structure of three similar biofilters used respectively for the biodegradation of toluene, ethylbenzene, and *para*-xylene was characterized by DGGE molecular profiling of bacterial 16S and fungal ITS1 rRNA genes (Figure 7.5). All three biofilters were inoculated with the same toluene-degrading enrichment culture obtained from activated sludge. Four predominant bacterial ribotypes were depicted in the inoculum as DGGE bands and were successfully excised and sequenced (Table 7.2). The sequence from band 1 was somewhat related to different members in the *Burkholderia cepacea* species complex, while that of band 2 was identical to the type strain of *Pandoraea pnomenusa*, a species closely related to, and commonly misidentified as, *B. cepacea*. Bands 3 and 4 were very similar (99 % sequence homology) to several uncultured ribotypes belonging to the *Xanthomonadaceae* family that have previously been observed in activated sludge from municipal wastewater treatment plants (Table 7.2). In contrast, the fungal biodiversity from the liquid culture used as inoculum was limited to one single ribotype (band 25), with an ITS1 rRNA sequence that was identical to that of the species type strain of the hypocrealean ascomycete *Acremonium kiliense* (Table 7.3).

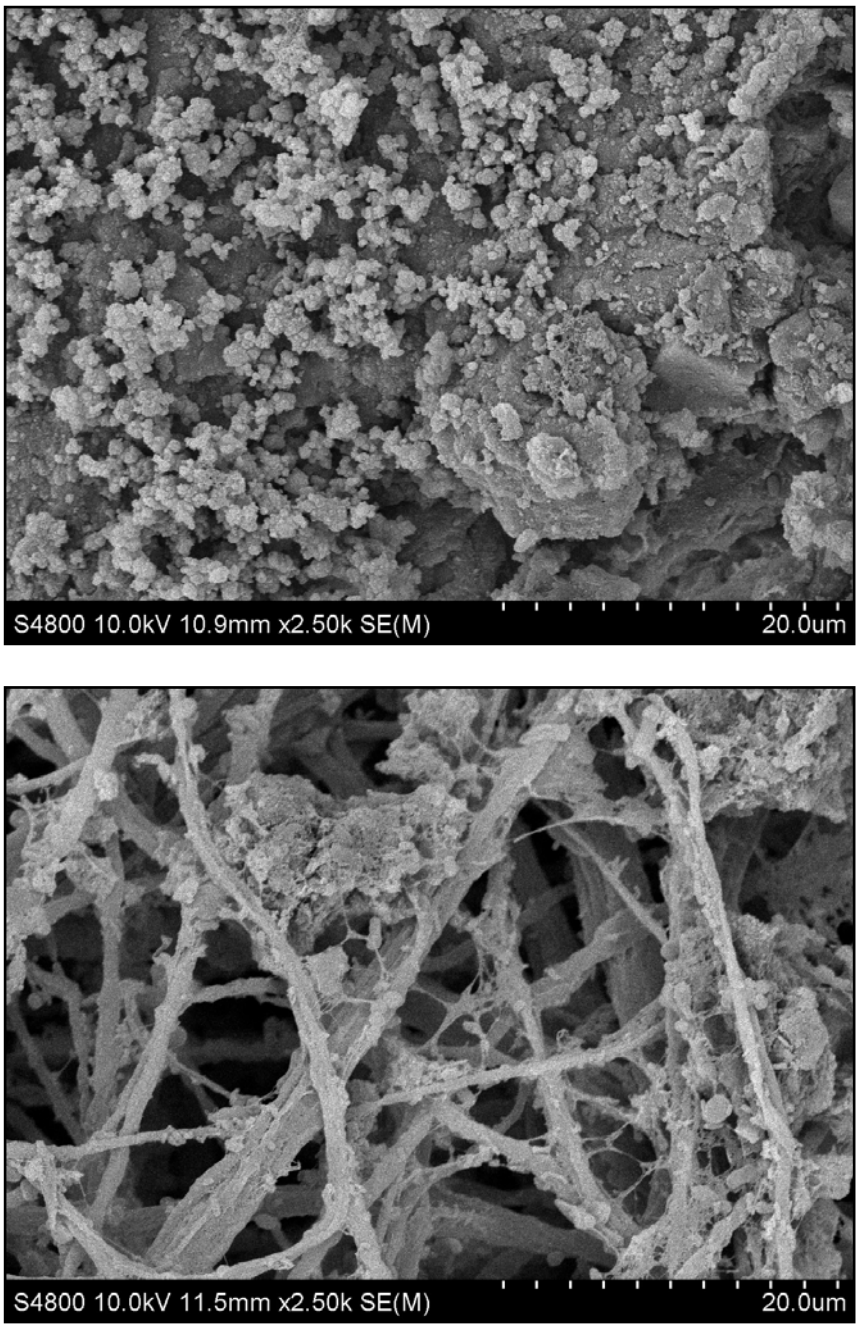

Figure 7.4. Scanning electron microscopy pictures from two packing samples of the toluene-fed biofilter, taken at the beginning of the experimental run (top), and after 185 days of operation (bottom).

Table 7.2. The most closely related sequences found in the GenBank database (NCBI, USA) for the DGGE bands from bacterial 16S rRNA genes obtained from the DGGE profiles from Figure 7.5.

Band	Sample presence	Accession	Order	Reference species, strain or uncultivated microorganism (environmental source)	Accession	H (%)
1	I	JN982532	*Burkholderiales*	*Burkholderia cepacia* ATCC25416^T *B. cenocepacia* J2315^T *B. vietnamiensis* LMG10929^T	HQ849078 AM747720 HQ849107	96 96 96
2	I	JN982533	*Burkholderiales*	*Pandoraea oxalativorans* TA25^T *P. pnomenusa* CCUG38742^T	AB469785 AY268170	100 100
3	I	JN982534	*Xanthomonadales*	Uncultured (activated sludge) *Frateuria aurantia* IFO3245^T	FJ536874 AB091194	99 93
4	I	JN982535	*Xanthomonadales*	Uncultured (activated sludge) *Gynumella flava* YC6842^T	HQ440078 GQ369122	99 94
5, 11	T, E	JN982536	*Burkholderiales*	*Alcaligenes* sp. C4M17 *Pusillimonas noertemannii* T7-7^T	DQ089749 DQ417606	99 98
6	T	JN982537	*Actinomycetales*	*Microbacterium xylanilyticum* S3-E^T *M. hydrocarbonoxydans* DSM16089^T *M.testaceum* DSM20166^T	AJ853908 AJ698726 NR_026163	99 99 99
7	T	JN982538	*Actinomycetales*	*Streptomyces baliensis* NBRC104276^T *S. griseoplanus* NBRC12779^T *S. radiopugnans* R97^T	AB441718 AB184138 912930	97 97 97
8	T	JN982539	*Actinomycetales*	*Streptomyces chungwhensis* AA-98^T *S. ferralitis* strain SFOp68^T *S. paucisporeus* strain 1413^T	AY382292 NR_029087 NR_04324	98 98 98
9, 13, 23	T, E, X, P	JN982542	*Actinomycetales*	*Streptomyces halotolerans* YIM 90017	AY376166	99
10	T, E	JN982540	*Sphingobacteriales*	Uncultured (domesticated horse feces) *Pedobacter bauzanensis*BZ42^T	EU463479 GQ161990	100 96
12, 22	E, P	JN982541	*Actinomycetales*	Uncultured (cattle feedlot) *Aeromicrobium ginsengisoli* GBS39^T	FJ671561 AB245394	99 96
14	X	JN982543	*Sphingobacteriales*	Uncultured (activated sludge) *Lewinella nigricans* ATCC23147^T	FN597784 AM295255	97 86
15, 19	X, P	JN982544	*Cytophagales*	Uncultured (algal mat) *Flexibacter aggregans* IFO15974	HM357047 AB078038	92 90
16, 24	T, E, X, P	JN982545	*Actinomycetales*	*Rhodococcus coprophilus* DSM43347^T	X80626	99
17	X	JN982546	*Actinomycetales*	*Actinomadura madurae* XMU324 *A. nitritigenes* NBRC15918	HM368641 AB364595	96 96
18	X	JN982547	*Thermoleophilales*	Uncultured (contaminated soil) *Thermoleophilum minutum* YS-4^T *T. album* HS-5^T	AM935694 NR_036932 NR_025543	97 82 82
20	P	JN982548	*Rhizobiales*	*Pseudaminobacter* sp. G210 (beach sand) *P. salicylatoxidans* BN12^T	GU199003 NR_028710	98 97
21	X, P	JN982549	*Actinomycetales*	*Ruania albidiflava* AS4.3142^T	DQ343153	96

Notes: The most homologous sequence and the closest phylogenetically relevant match are shown (preferably type strains^T).

The organic packing used in the biofiltration experiments already contained a diverse population of both bacteria and fungi. In relation to the bacterial domain, the sequence from band 19 was distantly related to several, mainly uncultured, ribotypes belonging to the

Cytophagales class and might, thus, belong to a yet undescribed species. Band 20 was similar in sequence (98 % homology) to an undefined *Pseudoactinobacter* sp. as the closest phylogenetically defined match (Table 7.2). The remaining bacterial bands were associated with different species in the *Actinomycetales*: sequences from bands 21 and 22 were distantly related (96 % sequence homology) to the type strain of *Ruania albidiflava* and *Aeromicrobium ginsengisoli*, respectively, while bands 23 and 24 were highly homologous (98 %) to *Streptomyces halotolerans* and *Rhodococcus coprophilus*. The phylogenetic identity of some important DGGE bands from the original packing, the sequences of which could not be directly obtained, was assigned on the basis of an identical migration pattern toward sequenced bands from other related biofilter packing samples. In relation to this, the presence in the original packing of an unknown species in the *Sphingobacteriales* with a 16S rRNA partial sequence identical to an uncultured microorganism from horse feces was established by comparisons of the toluene sample DGGE band pattern (band 10). Several fungi were also detected in the same DNA extract from the original biofilter packing, although fungal diversity depicted by DGGE appears to be comparatively low. Most of the detected species belonged to the *Chaetotyriales*, *Eurotiales*, and *Hypocreales*, with the three predominant bands (38 – 40) assigned to *Cylindrocarpon destructans*, *Exophiala oligosperma*, and *Aspergillus versicolor* on grounds of a high sequence homology (< 99 %) toward reference type strains.

Table 7.3. The most closely related sequences found in the GenBank database (NCBI, USA) for the DGGE bands from fungal ITS1 rRNA genes obtained from the DGGE profiles from Figure 7.5.

Band	Sample presence	Accession	Order	Reference species, strain or uncultivated microorganism (environmental source)	Accession	H (%)
25, 33	I, X	JN982553	*Hypocreales*	*Acremonium killiense* MUCL9724^{T}	FN691446	100
26, 28, 39	T, E, P	JN982550	*Chaetotyriales*	*Exophiala oligosperma* CBS113408 *Exophiala oligosperma* CBS725.88^{T}	AY857531 AY163551	100 99
27, 31, 40	T, E, P	JN982558	*Eurotiales*	*Aspergillus sydowii* CBS593.65^{T}	AY373869	99
29	E	JN982551	*Hypocreales*	Uncultured (watermelon rhizosphere) *Acremonium chrysogenum*ATCC14615^{T}	GQ866190 ACU57672	99 94
32, 37	T, E, P, X	JN982552	*Eurotiales*	Uncultured (airfilter sample) *Aspergillus versicolor* UOA/HCPF8640	GQ999318 FJ878625	99 98
34	X	JN982554	*Chaetotyriales*	*Cladophialophora saturnica*CBS114326	AY857507	100
30, 35	E, X, P	JN982555	*Chaetotyriales*	*Fonsecaea* sp. CBS102252	JN999999	100
36	X	JN982556	*Sordariales*	Unidentified IBL03178 (coffee seedlings)	DQ682601	98
38	E, P	JN982557	*Hypocreales*	*Cylindrocarpon destructans* CBS185.36	AM419062	99

Notes: The most homologous sequence and the closest phylogenetically relevant match are shown (preferably type strains^{T}).

The predominant bacterial populations described at the end of the biofiltration experiments contained indigenous representatives from the original packing, such as *S. halotolerans* or *R. coprophilus*, which were present in all packing samples, but some enriched ribotypes were also found. The toluene and ethylbenzene biofilters presented a relatively low biodiversity and a similar microbial composition profile. Specific ribotypes were closely related to some species encompassed in the genera *Alcaligenes* and *Microbacterium* (bands 5 – 6; sequence homology

> 99 %), as well as to different *Streptomyces* species (bands 7 – 8; sequence homology > 97 %). The biofilter exposed to *para*-xylene displayed a more complex microbial community, from the phylogenetic perspective, that was also more similar to the indigenous microbial population of the packing. Specific ribotypes included the very distinct bands 14 and 18, which had poor sequence homology toward any known microorganism but were relatively similar (97 %) to uncultured ribotypes found previously in activated sludge and polluted soil, respectively (Table 7.2). In relation to the fungi, most of the predominant species found in the biofilters fed with toluene and ethylbenzene were already detected in the original packing material. Those included the previously mentioned *E. Oligosperma* (bands 26 and 28) and *A. versicolor* (bands 27 and 31). Ribotypes related to *A. versicolor* and an uncultured *Aspergillus* species (bands 30 and 32; sequence homology > 98 %) were also found in the ethylbenzene biofilter. The presence of *C. destructans*, an indigenous species in the original packing, was also found in the ethylbenzene biofilter by means of band position matching. Additional *chaethothyrialean* species were found in the ethylbenzene and *para*-xylene biofilters, which included *Cladophialophora saturnica* and an as yet apparently undescribed new species (bands 34 and 35; 100 % sequence homology). Interestingly, the *A. kiliense* detected in the inoculated liquid culture was also found in the *para*-xylene biofilter (band 33).

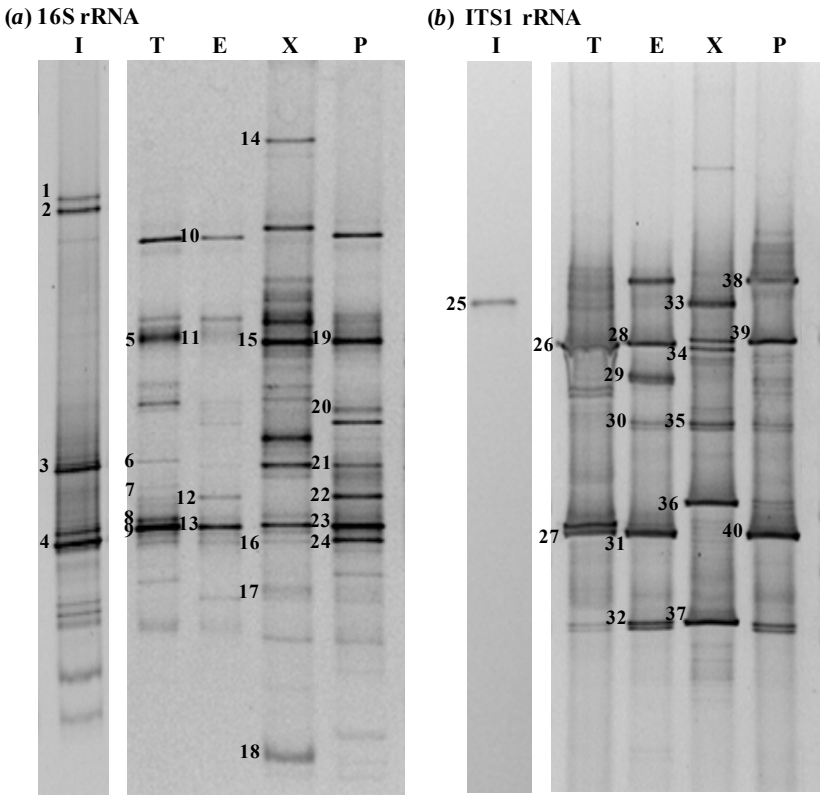

Figure 7.5. DGGE profiles for bacterial 16S (a) and fungal ITS1 (b) rRNA genes from the initial inoculum (I) and packing material used in the biofiltration experiments, and from three biofilters used respectively for the treatment of toluene (T), ethylbenzene (E), and *para*-xylene (X) after 185 days of operation (Figure 7.1). Numbered DGGE bands were successfully excised and sequenced.

7.4. Discussion

In this study, three laboratory-scale biofilters treating vapors of toluene, ethylbenzene, and *para*-xylene were started up and operated for an initial period of 85 days without bed irrigation. The water content in the two modules that composed each biofilter dropped from an initial 32 % and became stabilized to approximately 20 % in the units run with toluene and ethylbenzene (Figure 7.2). These humidity levels were significantly lower than the 40 – 60 % range that has previously been reported as optimal for the biofiltration of alkylbenzenes (Cox et al., 1996; Sun et al., 2002). Yet, a relatively stable biofilter operation and performance was achieved for toluene, with acceptable EC and RE values (Figure 7.1). As reviewed by Kennes and Veiga (2004), significantly higher EC values have previously been reported in biofilters fed with alkylbenzenes but, in general, those elimination rates were sustained for relatively short periods, only, and/or with higher irrigation frequencies. Under identical operational conditions, biofilter performance tended to fail when fed with ethylbenzene indicating that, despite a similar chemical structure, the substrate characteristics had a strong influence on the overall bioreactor performance. In fact, toluene has generally been found to be more easily biodegradable than ethylbenzene and *para*-xylene, by both fungi and bacteria (Mallakin and Ward, 1996; Prenafeta-Boldú et al., 2002). In relation to the biofilter fed with *para*-xylene though, a strong vertical gradient of humidity was observed between the top and bottom modules as the module from the lower position consistently had a water content of 30 – 40 % despite bed homogenization and module reversal (Figure 7.2). Water accumulation in the deeper sections of the biofilter bed is not uncommon, particularly under down-flow operation mode, because of the effect of gravity and the drying effect of the incoming air (Sakuma et al., 2009). Bed homogenization is known to improve operating conditions by reducing bed drying (Znad et al., 2007) but, despite further attempts to re-water the biofilter bed, humidity continued to drop, particularly in the biofilters fed with toluene and ethylbenzene. Such substrate-dependent spatial and temporal humidity gradients point to the fact that the water balance is somehow related to microbial population dynamics which, in its turn, are selected by the organic volatile substrate.

It is now widely accepted that in gas biofilters, microbial community structure and dynamics are strongly influenced by environmental conditions (i.e. bioreactor operational parameters). But we do not yet fully understand to what extent microbial interactions drive the macroscopic process functioning in terms of biodegradation performance and system stability (Cabrol and Malhautier, 2011). Earlier studies on the biofiltration of BTEX compounds have pointed out that operation under relatively dry and/or acidic conditions favors the development of fungi rather than bacteria, and that this effect was generally beneficial for the process (Kennes and Veiga, 2004). However, quantitative evidence supporting this claim has commonly been based on visual macroscopic and microscopic observations (Weber et al., 1995; Prenafeta-Boldú et al., 2001), as well as on culture-dependent microbial counts (García-Peña et al., 2001; Sun et al., 2002), which are inherently biased toward high propagule-producing and fast-growing microorganisms on laboratory media (Cabrol and Malhautier, 2011). The quantification of fungal-specific biomarkers in toluene biofilters demonstrated significant growth of fungi, but bacteria were then overlooked (Prenafeta-Boldú et al., 2008). Here, the culture-independent

quantification of specific fungal and bacterial genes has shown a significant increase in the F/B ratio only in the case of toluene (Figure 7.3). Such an increment was primarily due to the fungal biomass fraction; the number of ribosomal gene copies in fungi increased by one order of magnitude, in relation to the content of the original packing, while the bacterial numbers remained fairly constant. Contradicting the common belief that microbial enrichment in liquid cultures tends to select for bacteria while filamentous fungi are prone to the colonization of solid state-like fermentation systems (Prenafeta-Boldú et al., 2001), the F/B ratio from the biofilter inoculum was significantly higher than that of the original packing; though, the overall microbial gene content in the former was substantially lower.

Certain fungi and bacteria are known to assimilate alkylbenzenes as the sole source of carbon and energy, but the metabolism of these substrates by bacteria is better known, from both enzymatic and genomic perspectives. Several members of the *Pseudomonadales*, *Burkholderiales*, and *Xanthomonadales* are known to assimilate toluene and related substrates (Timmis et al., 2010) and it is therefore not surprising that the predominant bacterial ribotypes found in the inoculum (activated sludge enriched upon toluene additions) were related to these taxa. *Burkholderia* cepacia is in fact a complex of at least nine closely related species, including *B. cenocepacia* and *B. vietnamiensis*, commonly isolated from soil and plant roots with a known ability to degrade several organic pollutants. They are also opportunistic pathogens capable of causing life-threatening respiratory tract infections in predisposed patients (Mahenthiralingam et al., 2005). One bacterial ribotype from the inoculum was closely related to different *Pandoraea* spp. (*Burkholderiales*), commonly associated with activated sludge, but also with lung infection (Coenye et al., 2000). A distinct representative of the *Burkholderiales* closely related to *Pusillimonas noertemannii* was also observed in packing samples from the toluene and ethylbenzene biofilters. This species is able to mineralize substituted salicylates and aromatic acids (Stolz et al., 2005), analogues of some intermediates of the ethylbenzene biodegradation pathway (Gunsch et al., 2005).

Nevertheless, the bacterial ribotypes identified in the biofilter bed samples belonged predominantly to the order *Actinomycetales*. In several aspects, actinobacteria can be considered as the bacterial counterparts of common fungi. Just like fungal hyphae, many actinobacteria form filamentous multicellular structures, the most suitable microbial morphology for the colonization of solid substrates. Consequently, in several cases at least, filamentous growth is related to the biological resistance toward low water activity stress. Because of the need to exploit and protect a spatially defined resource, like many saprotrophic fungi, actinobacteria have developed a complex array of secondary metabolites (antibiotics, volatile compounds, etc.), as well as extracellular enzymes for the hydrolysis of polymeric substrates (McCarthy and Williams, 1992). Moreover, dispersal of such organisms into the environment primarily relies on air-born propagules, which might therefore be more easily be encountered in air biofilters. A few actinobacteria are also known to assimilate aromatic hydrocarbons, like *Microbacterium hydrocarbonoxidans* and related species (Schippers et al., 2005), detected here in the toluene biofilter. Other *Streptomyces* spp. were also present in the DGGE patterns though with lower intensity in the toluene biofilter, in contrast to the very xerophilic *S. halotolerans*, which was found to predominate in all packing samples. It is

interesting to mention that different sequences detected in the original packing could be related to microorganisms that have been related to the ruminant digestive system; this is to be expected considering that animal dung is one of the chief packing components. These included a few ribotypes that could not be phylogenetically assigned (bands 10, 12, and 22), as well as the actinobacteria *R. Coprophilus* (Table 7.2). However, the dominance of these presumably enteric microorganisms, on the basis of DGGE relative band intensity compared to that in the original packing of the biofilter, tended to decrease in most of cases, indicating that they played a minor role in the biodegradation processes.

Interestingly, one single fungus was detected in the toluene-enriched liquid culture used as inoculum for the biofiltration experiments, the ascomycete *A. kiliense* (*Bionectriaceae*), which apparently is responsible for the relatively high F/B ratio of this particular sample. This fungus is a very cosmopolitan fungus that has commonly been isolated from soil, but it has also been claimed that it can biodegrade aromatic hydrocarbons (April et al., 2000). At the end of the experiments, *A. kiliense* was detected in the *para*-xylene biofilter only, indicating that it played a minor role in the biodegradation of toluene and ethylbenzene under biofilter conditions. A fungus related to an unidentified strain in the *Sordariales* isolated from coffee seedlings was strongly enriched in the *para*-xylene biofilter. Several volatile aromatic compounds, including the xylene isomers, have been found to be emitted by green coffee (Holscher et al., 1995) and might, thus, explain the occurrence of this particular strain. Instead, the DGGE profiles indicate that the species *E. oligosperma* was strongly enriched in the biofilter treating toluene and might therefore contribute significantly to the high F/B biomass ratio seen in samples from this biofilter. This species was also detected in the ethylbenzene biofilter though with a lower intensity, which is consistent with the lower F/B ratio observed in this case. *Exophiala oligosperma* appears to be among the most common species isolated from biofilters treating volatile alkylbenzenes (Kennes and Veiga, 2004; Prenafeta-Boldú et al., 2006).

Interestingly, DGGE profiles also indicated that *E. Oligosperma* was already present in the original packing material and might thus be related to its constitutive materials (i.e. sawdust and manure). This fungus is one of the so-called black yeasts, a functional group of fungi that owe its name to their strongly melanized thallus and by an ability to grow either as filaments, budding cells, or by forming meristematic structures. Such physiological flexibility and melanin pigmentation enables members of this group to colonize a wide range of hostile and sometimes very unusual environments, so that many species are in fact considered as extremophilic eukaryotic microorganisms (de Hoog, 1999). In recent years, it has become apparent that black yeast members of the *Chaetothyriales* are consistently isolated from environments that are polluted with aromatic hydrocarbons, and the assimilation of toxic aromatics such as toluene and styrene as sole carbon and energy sources has been demonstrated for an increasing number of species (Prenafeta-Boldú et al., 2006). Besides melanization, these fungi are also characterized by an extremely hydrophobic biomass, which has been of advantage for the selective isolation of these organisms by extraction on mineral oil (Satow et al., 2008). This hydrophobicity could have contributed to the poor bed watering that was observed in the toluene and ethylbenzene biofilters. The *para*-xylene biofilter displayed a more distinct and diverse microbial profile, and the F/B ratio reached the lowest

value measured (Figure 7.3). Also, in contrast to the toluene and ethylbenzene biofilters, *E. Oligosperma* was absent from the dominant fungal population.

However, two other chaetothyrialean black yeasts were detected instead, though with minor intensity: the well-known toluene-growing *C. saturnica* (Badali et al., 2009) and a new *Fonsecaea* species that will soon be described (unpublished data). The latter species was also distinguished in the original packing and in the ethylbenzene biofilter. In contrast to the simpler alkylbenzenes, such as toluene and ethylbenzene, the utilization of the xylene isomers by black yeasts as the sole carbon and energy source remains inconclusive, because of the most commonly reported biodegradation by co-metabolism (Prenafeta-Boldú et al., 2001; Prenafeta Boldú et al., 2002). The comparatively lower fungal biomass and the more complex biodegradation of *para*-xylene might thus explain the poor performance of the biofilter fed with this substrate. It is interesting to contemplate how certain ecological traits (xerotolerance, assimilation of aromatic hydrocarbons, and opportunistic pathogenicity) interact among fungi and bacteria in air biofilters. Among the fungal component, we are mainly concerned with members of the genera *Exophiala* and *Cladophialophora* (*Herpotrichiellaceae*, *Chaetothyriales*). In our study, *E. Oligosperma* appears to be one of the dominant fungi from the original packing and its predominance clearly increased upon exposure to toluene. Besides being a common biofilter species, this fungus has also been found in woody materials and as an agent of opportunistic infections (de Hoog et al., 2003). The same pattern of opportunism and assimilation of alkylbenzenes has been observed with related species, such as *Exophiala xenobiotica, Exophiala lecanii-corni, Phialophora sessilis*, etc. (Prenafeta-Boldú et al., 2006). There are some presumably highly specialized, and also extremely virulent, human pathogens among these genera (de Hoog and Guarro, 2000), which include agents of deep skin lesions (*Cladophialophora carrionii* and *Exophiala spinifera*) as well as infections of the brain (*Cladophialophora bantiana* and *Exophiala dermatitidis*). To our knowledge, though, not a single report has been made on the occurrence of such dangerous pathogens in air biofilters. Recent evidence suggests that a process of speciation might be going on among these fungi, manifested by the occurrence of highly similar sibling species evolving, respectively, toward virulence or to saprotrophy in extreme environments (Badali et al., 2011).

The identified bacterial genera also harbor, or are related to, an important number of opportunistic pathogens which display infection patterns that are similar to those seen among the black yeasts. Several actinobacteria species are associated with subcutaneous lesions (Fahal and Hassan, 1992) and are even connected with dissemination with cerebral involvement (Hobson et al., 1995). These parallels suggest the possibility that phylogenetically very diverse groups of hydrocarbon-degrading organisms may share some common factors predisposing them to particular patterns of human pathogenicity, for example, lipophily, extremotolerance, metabolism of aromatic compounds, etc. Although tentative, the connection appears to be worth exploring as more genomic information becomes available about the groups in question. This information is fundamental for evaluating the biosafety of biofilters treating monoaromatic hydrocarbons and for the development of biotechnological processes with minimal biohazard.

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8. SPANISH PATENT APPLICATION Nº P201132043

Electric arc furnace (EAF) black slag

8.1. Introduction

As a result of the whole research developed during these doctoral studies, sufficient experience has been acquired in order to try the selection of new suitable medium materials from locally available industrial by-products.

Thus, electric arc furnace (EAF) black slag is the major by-product in steelmaking industry using EAF technology. Precisely, the industrial production of one ton of steel produces 120 – 160 kg of black slag waste. The sustainable management of this slag remains a serious concern in those regions like the Basque Country where large amounts are produced every year, accounting for 75 % of Spain's total output. If not recycled, the steel slag is disposed of in landfills, thus increasing the amount of waste to be dumped.

Bearing in mind slag disposal reduction, the use of this by-product material as a packing material in biofilters has emerged as a novel reuse alternative. Consequently, a formal patent application has been applied for a biofilter in treating contaminated gasses with VOCs with a container having a layer of steel slag aggregate, which serves as a filter media.

The black slag obtained from scrap metal melted in electric arc furnaces, after prior selection, crushed and screened to yield a granular material known as steel slag aggregate, holds substantial promise for use as a packing material for conventional biofilters.

Unlike traditional organic packing materials (e.g. compost or peat), this cubical-shape by-product is mechanically strong, resistant to polishing and has a rough surface, which prompts microorganism growth and attachment on its surface. Additionally, possible intrinsic disgusting odours promoted by the organic packing materials themselves are also avoided.

As the patent is under development, more specific details of its behaviour cannot be revealed for confindential reasons.

8.2. Compendio de la Invención

Los autores de la presente invención han desarrollado un método de biofiltración sobre lecho fijo para depurar (es decir, eliminar o reducir) de manera eficiente la cantidad de contaminantes gaseosos biodegradables presentes en una corriente gaseosa contaminada con al menos uno de dichos contaminantes; ejemplos ilustrativos, no limitativos de dichos contaminantes gaseosos biodegradables incluyen COVs, H_2S, y CS_2. Dicho método se basa en la utilización de escoria negra de horno de arco eléctrico o EAFS [del inglés, "Electric Arc Furnace Slag"] como material de relleno del biofiltro, y en el empleo de microorganismos capaces de degradar dichos contaminantes, y proporciona diversas ventajas frente a los métodos tradicionales y frente a otros métodos biológicos descritos en el estado de la técnica, debido principalmente a sus características físico-químicas que permiten una operación en continuo durante largos periodos de tiempo sin sufrir erosión y/o deterioro. Tras una etapa previa de inoculación con microorganismos capaces de degradar el contaminante o contaminantes, este residuo (EAFS) sirve adecuadamente como soporte a los mismos, los cuales forman una biopelícula de microorganismos a su alrededor, posibilitando así la depuración de la corriente gaseosa contaminada.

Así, en un aspecto, la invención se relaciona con un biofiltro que comprende escoria negra de horno de arco eléctrico (EAFS) como material de relleno ("biofiltro de la invención"). Para su puesta en operación, dicho biofiltro comprende, además, microorganismos capaces de degradar el contaminante gaseoso biodegradable que se desea eliminar, por ejemplo, uno o más COVs, H_2S, CS_2, etc. Dichos microorganismos se inoculan en el material de relleno (EAFS) del biofiltro.

En otro aspecto, la invención se relaciona con una instalación para la depuración de corrientes gaseosas contaminadas con al menos un contaminante gaseoso biodegradable que comprende, al menos, un biofiltro de la invención.

En otro aspecto, la invención se relaciona con un método para depurar una corriente gaseosa que contiene al menos un contaminante gaseoso biodegradable, mediante biofiltración, que comprende hacer pasar dicha corriente gaseosa que contiene dicho al menos un contaminante gaseoso biodegradable a través de un biofiltro de la invención que comprende, además, microorganismos capaces de degradar dicho(s) contaminante(s) gaseoso(s) biodegradable(s).

En otro aspecto la invención se relaciona con el uso de escoria negra de horno de arco eléctrico (EAFS) como material de relleno en un biofiltro de lecho fijo para la depuración de corrientes gaseosas, en particular, corrientes gaseosas contaminadas con al menos un contaminante gaseoso biodegradable.

8.3. Justificante de Inventor

Leioa 31/01/2012

JUSTIFICANTE DE INVENTOR

Las/os investigadoras/os ELÍAS SÁENZ, Ana; BARONA FERNÁNDEZ, Astrid; GURTUBAY BUSTINDUY, Luis; GALLASTEGUI RUÍZ DE GORDOA, Gorka Javier; y, ROJO AZÁCETA, Naiara, son inventores de la solicitud de patente P201132043 por BIOFILTRO QUE COMPRENDE ESCORIA NEGRA DE HORNO DE ARCO ELÉCTRICO Y SUS APLICACIONES solicitada el 19/12/2011. Se encuentra en estado de tramitación.

Ricardo Merino
Técnico de Propiedad Industrial e Intelectual UPV/EHU

8.4. Justificante de Solicitud de Patente

MINISTERIO DE INDUSTRIA, TURISMO Y COMERCIO

Oficina Española de Patentes y Marcas

Justificante de presentación electrónica de solicitud de patente

Este documento es un justificante de que se ha recibido una solicitud española de patente por vía electrónica, utilizando la conexión segura de la O.E.P.M. Asimismo, se le ha asignado de forma automática un número de solicitud y una fecha de recepción, conforme al artículo 14.3 del Reglamento para la ejecución de la Ley 11/1986, de 20 de marzo, de Patentes. La fecha de presentación de la solicitud de acuerdo con el art. 22 de la Ley de Patentes, le será comunicada posteriormente.

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8.5. Petición de Informe sobre el Estado de la Técnica (IET)

PARTNERS
Juan Arias
M.Sc. Chemistry
European Patent Attorney
Spanish Patent & Trademark Agent
Francisco Bernardo
M.Sc. Chemistry
European Patent Attorney (CIPI)
Vicente González
M.Sc. Chemistry & Biotechnology
Spanish Patent & Trademark Agent
Fernando Pisón
B.Sc. Electronic Engineering (ICAI)
European Patent Attorney
Delio Carbonell
M.Sc. Chemistry
European Patent Attorney
Agustín Alcaraz
Ph.D. Pharmacy (Molecular Biology)
European Patent Attorney

ASSOCIATES
Alessandro Fernández
Ph.D. Chemistry
European Patent Attorney
Benito Pelaz
Ph.D. Mechanical Engineering
European Patent Attorney
Christine Weimann
Attorney-at-Law
Spanish Patent & Trademark Agent
Community Trademark & Design Attorney

ADVISERS
Cristina Zaccio
M.Sc. Chemistry
Tomás Llamas
Ph.D. Chemistry
María Rodríguez
Ph.D. Chemistry
Paula Camero
Ph.D. Chemistry
Sofía Refolegui
Ph.D. Chemistry
Laura Durán
Ph.D. Chemistry
Esther Martínez
M.Sc. Biology
Irene Vázquez
Ph.D. Pharmacy (Biomedicine)
Gemma Morató
Ph.D. Biology
Lorena Rodríguez
Ph.D. Biochemistry
Noelia Sanz
Ph.D. Molecular Biology
Laura de la Vega
Ph.D. Physics
Aida López
M.Sc. Telecommunications Engineering
Spanish Patent & Trademark Agent
Jaya Artigot
M.Sc. Telecommunications Engineering
Ana Belinchón
Attorney-at-Law
M.Sc. Business Administration
Spanish Patent & Trademark Agent

FORMALITIES COORDINATORS
EP/PCT filings
Cecilia Revilla
M.Sc. Business Administration
Spanish Patent & Trademark Agent
International filings
Beatriz Rodero
M.Sc. Business Administration
National filings
Jorge Sebastián

abg patentes
Madrid
Avenida de Burgos, 16D
Edificio Euromor
E-28036 Madrid (Spain)
Tel. +34 91 417 53 00
Fax. +34 91 417 12 01

abg
patentes
ARIAS, BERNARDO & GONZÁLEZ
Asesoría y Agencia de la Propiedad Industrial
Intellectual Property

UNIVERSIDAD DEL PAÍS VASCO
Rectorado, Barrio Sarriena, s/n
48940 - LEIOA - VIZCAYA

Solo por e-mail

Att: Ricardo Merino

Nuestra ref.: P6900ES00

Madrid, 2 de abril de 2012

Asunto: **Solicitud de patente en España nº P201132043 por "BIOFILTRO QUE COMPRENDE ESCORIA NEGRA DE HORNO DE ARCO ELÉCTRICO Y SUS APLICACIONES", a nombre de UNIVERSIDAD DEL PAÍS VASCO**
Petición de Informe sobre el Estado de la Técnica (IET)

Estimados Sres.:

Nos complace comunicarles que, de acuerdo con sus instrucciones, con fecha 2 de abril de 2012 hemos procedido con la petición del Informe sobre el Estado de la Técnica (IET) ante la Oficina Española de Patentes y Marcas (OEPM) de la solicitud de patente de la referencia.

Adjuntamos el resguardo de petición de dicho IET, así como nuestra factura por los trabajos realizados, la cual esperamos sea de su aprobación.

Les mantendremos informados de cualquier incidencia en la tramitación de la solicitud de patente de la referencia.

Atentamente,

Vicente González Díaz
Agente de Propiedad Industrial
ABG Patentes, S.L.

Adj.: - Petición de I.E.T.
- Factura

9. SCIENTIFIC DISSEMINATION FOR THE GENERAL PUBLIC

Utilización de lodos de EDAR como inóculo de biofiltros para el tratamiento de olores

9.1. Introduction

Specific attention must be paid to strengthening the dissemination of University research outcomes and to information and scientific dissemination, with a view to bringing science and technology closer to society and enterprises.

For that purpose, a clear, concise and understandable mini-review was conducted in terms of type of VOC in the different industries, biofiltration benefits in VOC and odour abatement and details on its configuration and mechanism of operation. Special attention was given to the application of a protocol established for shortening the start-up period in conventional biofilters from the exploitation of wastewater treatment sludge.

The article is hosted on an open access website called http://www.aguasresiduales.info , which is the first Internet portal entirely devoted for Spanish professionals working in sewage treatment activities.

Detailed information of the article could be found in the web link below:

http://www.aguasresiduales.info/main/index.php?md_0=4&md_1=&id=856&_pag=2&navi=Netscape

9.2. Antecedentes

Hasta el año 2000, los procesos de tratamiento biológico han sido empleados principalmente en la depuración de aguas contaminadas y residuos sólidos. La necesidad de alternativas de bajo coste para tratar los olores generados en las Estaciones Depuradoras de Aguas Residuales (EDAR) ha impulsado que dichos métodos biológicos hayan sido aplicados también al tratamiento de corrientes gaseosas contaminadas.

La biofiltración de corrientes gaseosas no es un proceso de filtración en el sentido estricto de la palabra, sino que consiste en una combinación de distintos procesos: absorción, adsorción, degradación biológica y desorción de contaminantes en fase gaseosa. Como todos los procesos de este tipo, consta de una serie de reacciones metabólicas microbiológicas, que conducen a la degradación de los contaminantes presentes en la corriente gaseosa. A través de reacciones oxidativas y en menor lugar reductivas, los contaminantes son transformados en subproductos como por ejemplo, CO_2, vapor de agua y biomasa entre otros.

El presente trabajo se ha basado en el uso de microorganismos capaces de degradar los contaminantes presentes en una corriente gaseosa que atraviesa un lecho fijo en el que se encuentran fijada la biomasa. Los microorganismos crecen en una biopelícula que se desarrolla sobre la superficie del medio poroso que conforma el lecho y/o se encuentran suspendidos en la fase acuosa que rodea el medio. El lecho poroso está constituido por sustancias relativamente inertes, orgánicas (compost, turba, etc.) y/o inorgánicas (roca de lava, perlita, etc.), que presentan una gran área superficial y pueden aportar nutrientes básicos a los

microorganismos. A medida que el gas atraviesa el lecho, los contaminantes presentes en la fase gaseosa se absorben en la biopelícula que rodea al medio, donde son biodegradados por los microorganismos.

Esta tecnología está recomendada para tratar cargas volumétricas altas (≥ 1000 m^3 h^{-1}) que posean bajas concentraciones de contaminantes (≤ 5 g m^{-3}). No obstante, presenta algunos inconvenientes como son: gran demanda de espacio debido a los altos tiempos de residencia empleados para obtener eficacias satisfactorias, difícil control de pH y humedad y limitada vida útil del soporte empleado (se recomienda un cambio de medio cada 2 – 5 años en función de su naturaleza).

A pesar de estos inconvenientes, resulta una tecnología con costes de operación y mantenimiento bajos, que alcanza una buena eficacia de eliminación a bajas concentraciones de contaminantes (eficacias de eliminación superiores al 75 % para compuestos altamente hidrófobos y superiores al 95 % para compuestos parcialmente hidrófobos y compuestos hidrófilos). Globalmente, en el proceso de biodegradación no se generan residuos secundarios que exijan un tratamiento posterior.

El factor determinante en la elección de una tecnología de tratamiento es el económico. Las ventajas económicas de la biofiltración son notorias frente a otras técnicas en un amplio rango de concentraciones; así, los costes totales por cada 1000 m^3 tratados varían en un rango entre 0.4 – 2.4 (€) en biofiltración, frente a 7.5 (€) en procesos de absorción y 11.4 – 14.6 (€) en sistemas de adsorción.

Los contaminantes gaseosos tratados mediante esta tecnología pueden ser tanto orgánicos (tolueno, etilbenceno, etc.) como inorgánicos (NH_3, H_2S, CS_2) y constituyen la fuente energética y/o la fuente de carbono que necesita la población microbiana para su desarrollo.

Los inicios de la investigación por parte del presente Grupo se llevaron a cabo tomando como contaminante de referencia el sulfuro de hidrógeno (H_2S). La utilización de un soporte orgánico peletizado facilitó el arranque de los bioreactores que degradaban corrientes sintéticas constituidas por flujos regulados de aire y H_2S. La operación en continuo con este tipo de soporte peletizado alcanzó eficiencias cercanas al 100 % desde la primera semana de operación. No se llevó a cabo ninguna inoculación siendo la biomasa indígena del soporte la única responsable de la biodegradación, y la estabilidad y eficacia de operación fue la característica que podría definir los biofiltros con este contaminante. La operación no generó pérdidas de carga en el soporte ni ningún deterioro relevante en el mismo tras una operación en continuo de más de 12 meses. Por tanto, la sistemática de arranque y operación permitió obtener resultados para un amplio rango de carga másica (4 – 49 g m^{-3} h^{-1}), generadas por variaciones de caudal de hasta 6 l min^{-1} y una concentración máxima de 500 ppm_v de H_2S.

A partir de los buenos resultados obtenidos con corrientes que contenían H_2S, actualmente se está trabajando en dos líneas de tratamiento, relacionadas con la desodorización de corrientes gaseosas contaminadas con compuestos orgánicos volátiles (COV) (compuestos aromáticos simples como tolueno, etilbenceno y *para*-xileno, denominados TEX) y disulfuro de carbono (CS_2), respectivamente. Estos compuestos son emitidos por un amplio número de sectores industriales, así como por plantas de tratamiento de aguas residuales (Tabla 9.1).

Tabla 9.1. Fuentes de contaminantes y tipos de contaminantes que potencialmente podrían ser tratados

FUENTE	CONTAMINANTE
Compostaje	Olores, amoniaco, hidrocarburos
Fabricación de paneles de control plásticos	Estireno, acetato de butilo
Fabricación de paneles de madera	Estireno, acetona
Fabricación de rayón	Sulfuro de hidrógeno, sulfuro de carbono
Fabricación de envolturas de celulosa	Sulfuro de carbono
Fundición	Etanol, COVs, fenol, amoniaco
Fundición del metal	Estireno, butadieno
Impresión flexográfica	Alcoholes, acetona
Industria alimentaria	Olores, mercaptanos
Industria del lacado	Tolueno, etilbenceno, acetato de butilo
Industria de la fibra de vidrio	Estireno, acetona
Industria de los sabores	Olores, sabores
Industria de los revestimientos	Tolueno, COVs
Industria del mueble	Fenoles, cresoles, xilenos
Industria del reciclaje de residuos plásticos	Compuestos aromáticos clorados, hidrocarburos aromáticos policíclicos
Industria farmacéutica	Olores, alcoholes, acetona + dicloroetano
Industria maderera	Formaldehido, fenol
Industria papelera (pulpa y papel)	Olores, metanol, mezclas de compuestos Sulfurados
Industria petrolífera	Benceno
Industria porcina	Metano
Industria siderúrgica	Benceno, tolueno, p-xileno, hidrocarburos aromáticos policíclicos, (benzo(α)pireno, antraceno, etc.)
Industria tabacalera	Amoníaco, olores, nicotina
Matadero	Olores en general
Panadería/Bollería comercial	Etanol, acetato de etilo, COVs, metano, compuestos alifáticos
Producción de circuitos impresos (PCB)	Butanona
Producción del látex	Xilenos
Resinas plásticas	Estireno
Suelos contaminados	Gasolina
Tratamiento de aguas residuales	Olores en general

Las experiencias se han iniciado utilizando el mismo material de soporte y siguiendo la sistemática aplicada en el sulfuro de hidrógeno. En el caso del tratamiento de las corrientes contaminadas con disulfuro de carbono, tras un largo periodo de arranque, la biomasa indígena presente en el mismo soporte orgánico peletizado ha resultado capaz de metabolizar el contaminante, alcanzando elevadas eficacias de eliminación de CS_2.

Sin embargo, en el caso del tratamiento de los TEX, los microorganismos no presentaban la capacidad de degradar este tipo de compuestos, lo que se ha traducido en periodos de operación muy largos sin ninguna efectividad de degradación. Ante esta situación se tomaron

muestras de lodos de depuradora (obtenidos de la etapa aerobia de tratamiento), con el fin de lograr un inóculo adaptado o aclimatado específicamente a los TEX.

El trabajo se ha planteado con la idea de responder a las siguientes preguntas:

- ¿Influye el origen del inóculo? ¿Qué es más recomendable utilizar durante la aclimatación: la fase sólida, la fase líquida (sobrenadante) o el lodo completo?
- ¿Es imprescindible adaptar el inóculo al contaminante?
- ¿Es útil sistematizar los ensayos de seguimiento de la etapa de aclimatación arranque?

Por ello, y con la idea de encontrar respuesta a cada una de estas cuestiones, se ha llevado a cabo el diseño de una sistemática de aclimatación a estos tres contaminantes, empleando para ello lodo de distintas plantas depuradoras, etapa que se describe a continuación.

9.3. Ensayos de aclimatación

Para conseguir la aclimatación se han tomado muestras en distintas plantas depuradoras, tanto de procesos aerobios como de lodos residuales de tratamiento de aguas. Cantidades prefijadas de cada lodo con un contenido similar de sólidos volátiles se disuelven en una solución estándar de nutrientes con el fin de favorecer el medio de desarrollo de los microorganismos.

Este medio semilíquido se agita y alimenta en continuo con un flujo gaseoso que contiene los mismos contaminantes que luego se desean tratar en la corriente gaseosa real. Una imagen descriptiva de estos ensayos se muestra en la siguiente figura (Figura 9.1).

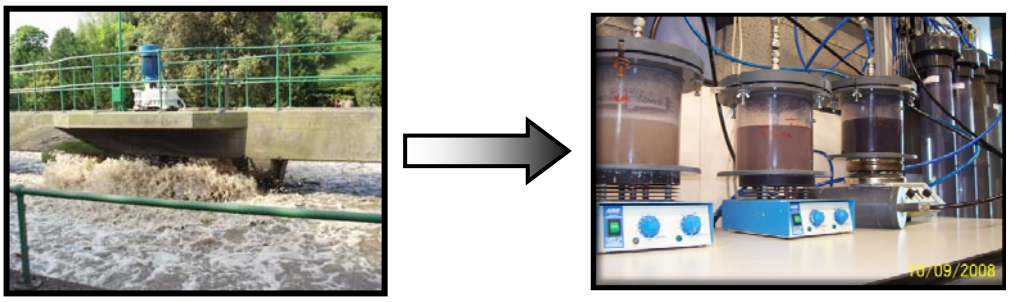

Figura 9.1. Ensayos de activación de lodo de depuradora para su utilización como inóculo.

En general, la operación a bajas cargas contaminantes favorece el desarrollo y aclimatación de la biomasa. Por ello, se seleccionaron los intervalos de concentración de contaminante y flujo de gas en la aclimatación recogidos en la Tabla 9.2.

Tabla 9.2. Condiciones de operación de los ensayos de aclimatación.

Variable	Rango
Concentración entrada de COV (ppm_v)	50 – 100
Eficacia de eliminación (%)	> 50
Caudal ($l\ min^{-1}$)	1
Tiempo de residencia (s)	90 – 110

Los parámetros de seguimiento de los ensayos de aclimatación han sido: pH, contenido en sólidos totales (ST) y volátiles (SV), concentración de CO_2, concentraciones de entrada y salida de contaminantes, contenido de células vivas y muertas (%) y densidad celular ($cfu\ l^{-1}$).

En los sistemas de tratamiento biológicos se ha asociado frecuentemente el contenido de sólidos volátiles (SV) con la biomasa presente en el medio líquido. En todos los ensayos llevados a cabo en este estudio, se ha observado un crecimiento de la densidad de sólidos volátiles hasta alcanzar un valor asintótico en torno a 3 $g\ l^{-1}$ (Figura 9.2). La relación de SV/ST también se estabiliza en valores porcentuales del 50 % a partir del día 200 de aclimatación.

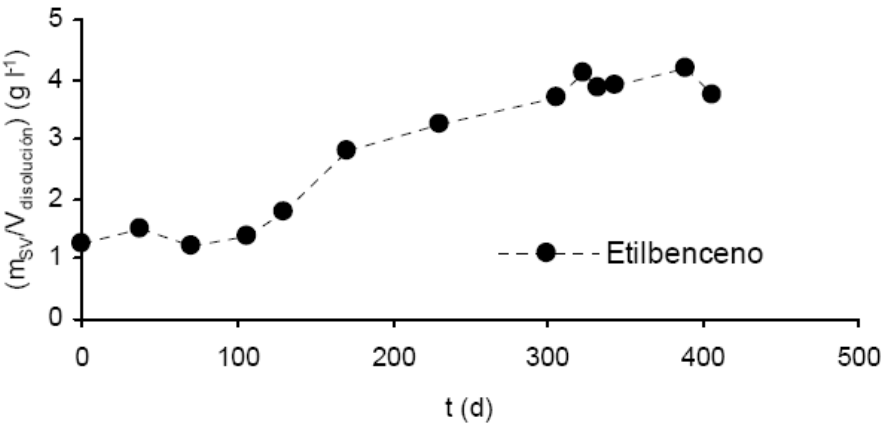

Figura 9.2. Evolución de la densidad de sólidos volátiles en ensayos de aclimatación con etilbenceno.

Se ha diseñado de forma paralela un ensayo denominado “de velocidad de degradación”, basado en inyectar una cantidad conocida de contaminante a una cantidad prefijada de biomasa y cuantificar su desaparición (degradación) a lo largo del tiempo mediante cromatografía de gases. Este ensayo permite predecir el estado de la biomasa, considerando que se encuentra aclimatada cuando la pendiente de los ensayos (velocidad de degradación) es la misma independientemente de la concentración de contaminante eliminado.

Una imagen descriptiva de estos ensayos se muestra en la Figura 9.3. A la hora de decidir qué fase del lodo de depuradora se debería utilizar para la obtención del inóculo (la fase completa, la fase sólida o la fase sobrenadante) se realizaron los mismos ensayos con las tres fases, llegando a la conclusión de que la mejor opción era no separar la fracción sólida de la líquida.

Pese a que las tres muestras mostraron un ratio de células vivas/muertas similar tras 7 días de aclimatación en discontinuo, la fase sólida no fue capaz de degradar el contaminante presente en los ensayos de velocidad. Por otro lado, la fase sobrenadante (ρ_{SV} = 0.38 g l^{-1}) presentó una velocidad de degradación un 40 – 60 % inferior a la de la fase completa (ρ_{SV} = 1.41 g l^{-1}), probablemente debido a la menor densidad microbiana (número de células vivas por unidad de volumen) en el medio.

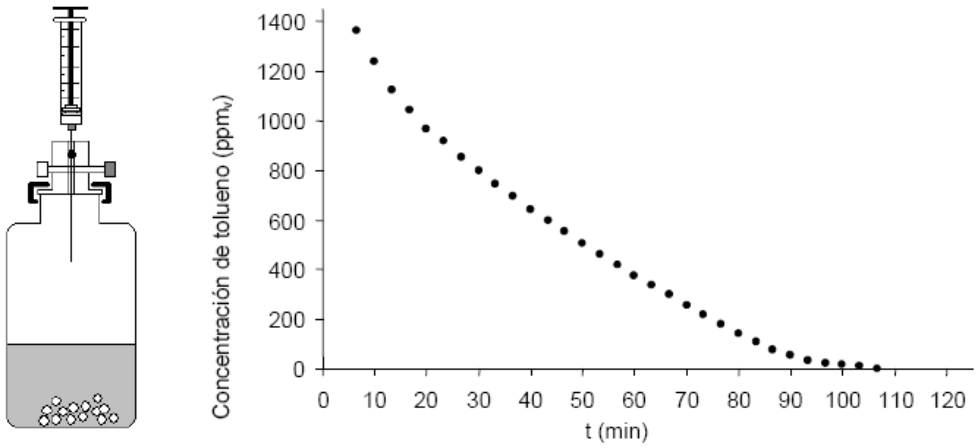

Figura 9.3. Ensayo de velocidad de degradación. Izquierda: fotografía de uno de los sistemas empleados. Derecha: perfil de desaparición de tolueno a lo largo del tiempo.

Por lo tanto, se concluyó que el lodo completo es una opción eficaz y cómoda como inóculo para el arranque de bioreactores. A partir de la biomasa aclimatada y con objeto de conseguir la biodegradación de TEX se llevó a cabo la instalación y arranque de biofiltros, etapa que de describe a continuación.

9.4. Arranque de biofiltros

La puesta en marcha de los biofiltros ha sido la prueba clave para saber si la sistemática seguida en la etapa de aclimatación ha sido adecuada y si se ha logrado el objetivo principal: conseguir biomasa adaptada al contaminante.

Para ello se ha llevado a cabo el riego del soporte con la biomasa aclimatada (Figura 9.4), y posteriormente se han seleccionado y aplicado las variables y parámetros de operación que se incluían en la Tabla 9.2. La operación en continuo de los bioreactores se ha iniciado con cargas másicas inferiores a los 30 g m^{-3} h^{-1} en una instalación piloto con varios biofiltros que operan en régimen continuo (Figura 9.5).

Figura 9.4. Procedimiento para puesta en marcha de la planta de biofiltración.

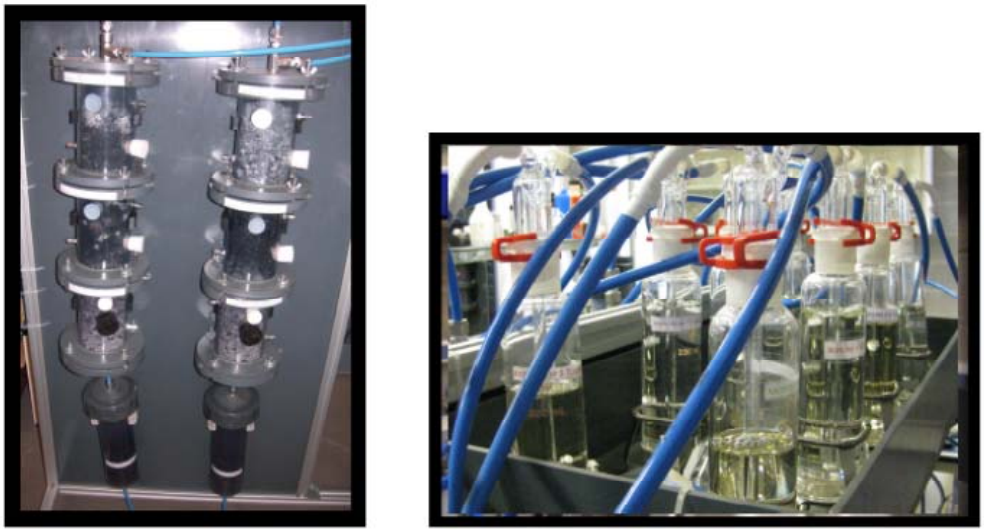

Figura 9.5. Plantas piloto de biofiltración. Izquierda: Sistema modular de dos de los biofiltros con una cámara de carbón activo inferior como sistema de seguridad. Derecha: Detalle del sistema de borboteo para lograr la corriente contaminada.

Durante la operación en continuo no se ha aportado ningún tipo de riego externo de agua y/o disolución de nutrientes, siguiendo la sistemática del biotratamiento del H_2S. Este hecho ha permitido que la humedad relativa del lecho no sea superior al 10 – 15 %, lo que ha favorecido la formación mayoritaria de hongos en detrimento de las bacterias.

Tras tres meses de operación, los soportes de los biofiltros han desarrollado una amplia biomasa visible en su superficie, de gran colorido (Figura 9.6). La eficacia de los distintos biofiltros durante esta etapa sin riego no ha superado en ningún caso el 20 – 30 %. La primera hipótesis planteada es que la falta de un nivel de humedad mínimo en el medio no ha permitido la supervivencia de los microorganismos adaptados. Por otro lado, la abundancia de sustrato fácilmente asimilable que aporta el soporte orgánico podría igualmente retardar el arranque. Esta fuente de energía es más accesible para los microorganismos que la constituida por el contaminante alimentado en forma gaseosa.

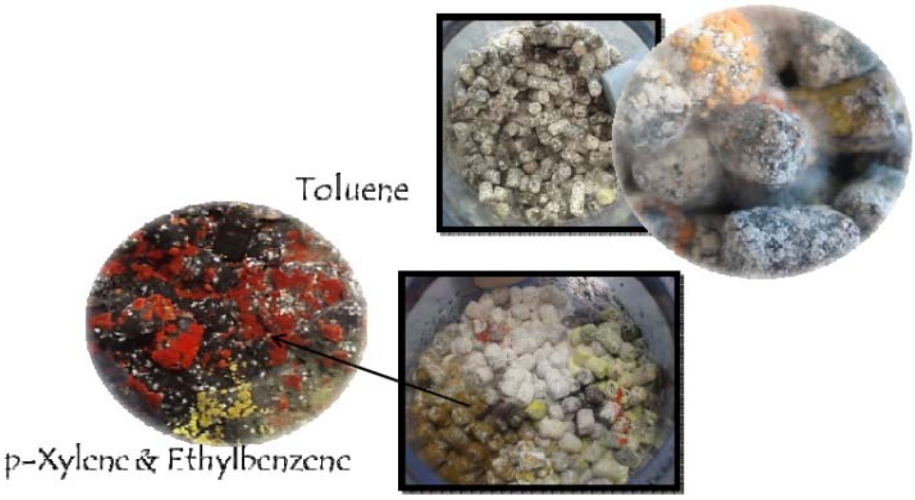

Figura 9.6. Colonización de microorganismos sobre el material soporte de todos los biofiltros alimentados con COV.

A la vista de los resultados obtenidos durante el periodo de sequedad, se ha establecido un protocolo de riego con medio nutriente, con el fin de ajustar la humedad del soporte a los niveles óptimos descritos en la bibliografía y comprobar su influencia en la eficacia de los biofiltros. Los bioreactores han alcanzado eficacias de eliminación del 100 % a los pocos días tras el primer riego, lo que confirma que la etapa de aclimatación es eficaz en el desarrollo de biomasa y que ésta anida de forma eficiente sobre el soporte.

Por tanto, se puede concluir que la biomasa indígena presente en el material de soporte orgánico utilizado en este trabajo es susceptible de ser enriquecida para tratar compuestos como H_2S o CS_2. Por otra parte, en los casos en los que la biomasa no es capaz de metabolizar los compuestos de interés, la sistemática de aclimatación de biomasa a partir de lodo de depuradora aquí descrita es una alternativa válida para obtener un inóculo eficaz en el arranque de biofiltros, aconsejando utilizar como inóculo el lodo completo de EDAR. Por último, cabe destacar que el riego con nutrientes tras el arranque del bioreactor ha permitido el desarrollo mayoritario de bacterias sobre hongos consiguiéndose minimizar las pérdidas de carga, favoreciendo la difusión del contaminante hasta la biomasa activa.

10.OTHERS

10.1. Congresses

Chemical Reaction Engineeting XI. Green chemical reactor engineering

Title: Biomass growth and control in batch experiments for biofilter operation

Authors: Elías, A., Gallastegui, G., Barona, A., Ibarra, G.

Year: 2007

Place: Bilbao (Spain)

Type of participation: Oral

2nd International Congress on Biotechniques for Air Pollution Control

Title: Biofilter response to biomass reactivation for VOC treatment

Authors: Elías, A., Barona, A., Gallastegui, G., Larrañaga, M., Fernández, M.

Year: 2007

Place: A Coruña (Spain)

Type of participation: Poster

NOSE 2008. International Conference on Environmental Odour Monitoring and Control

Title: Start-up of biofilters continuous acclimation of biomass inoculation purposes

Authors: Elías, A., Barona, A., Gallastegui, G., Garcia, A., Ibarra, G.

Year: 2008

Place: Roma (Italy)

Type of participation: Oral

3rd International Meeting on Environmental Biotechnology and Bioengineering

Title: Control of biomass activation for VOC treatment in biofilters: influence on the start-up and subsequent operation

Authors: Elías, A., Barona, A., Gallastegui, G., Ibarra, G.

Year: 2008

Place: Palma de Mallorca (Spain)

Type of participation: Oral

3rd International Congress on Biotechniques for Air Pollution Control

Title: Biomass growth and control in batch experiments for biofilter operation

Authors: Elías, A., Barona, A., Gallastegui, G., Gurtubay, K., Rojo, N.

Year: 2009

Place: Delft (The Netherlands)

Type of participation: Poster

i-SUP 2010. 2nd International Conference on Innovation for Sustainable Production

Title: An initial insight into the biodegradation of synthetic materials in biofilters

Authors: Elías, A., Barona, A., Gallastegui, G., Gurtubay, K., Rojo, N.

Year: 2010

Place: Bruges (Belgium)

Type of participation: Poster

12th annual Ontario-Québec Biotechnology Meeting

Title: Determination of kinetic parameters for biodegradation of toluene and p-xylene mixtures in a conventional biofilter

Authors: Gallastegui, G., Ávalos Ramirez, A., Elías, A., Jones, J.P., Heitz, M.

Year: 2010

Place: Montréal (Canada)

Type of participation: Oral

Water & Industry 2011 IWA Specialist Conference Chemical Industries

Title: Response of an organic waste biofilter for treating ethylbenzene

Authors: Gallastegui, G., Rojo, E., Elías, A., Gurtubay, K., Ibarra, G., Barona, A.

Year: 2011

Place: Valladolid (Spain)

Type of participation: Participant

ISACS-4. Challenges in Renewable Energy

Title: Materials engineering for energy applications

Authors: Ruiz Morales, J.C., Canales, J., Borges, E., Esparza, P., Marrero, D., Elías, A., Gallastegui, G.

Year: 2011

Place: Boston (United States of America)

Type of participation: Poster

4th International Congress on Biotechniques for Air Pollution Control

Title: Biodegradation kinetics of p-xylene and toluene mixtures in a biofilter packed with inert material

Authors: Gallastegui, G., Ávalos-Ramirez, A., Elías, A., Barona, A., Ibarra, G., Jones, J.P., Heitz, M.

Year: 2011

Place: A Coruña (Spain)

Type of participation: Oral

4th meeting of the ISHAM working groups on Black Yeasts and Cromoblastomycosis

Title: Naturally enriched black yeast in biofilter packed with an organic material applied for the treatment of tolueno, ethylbenzene and p-xylene vapors

Authors: Prenafeta-Boldú, F.X., Guivernau, N., Gallastegui, G., Viñas, M., Elías, A.

Year: 2011

Place: Curitiba (Brasil)

Type of participation: Participant

16th International Congress on Project Engineering

Title: Aproximación a la problemática del aire interior

Authors: Fernández, I., Montes, J., Roggero, S., Gallastegui, G., Barona, A.

Year: 2012

Place: Valencia (Spain)

Type of participation: Poster

16th International Congress on Project Engineering

Title: Evaluación de la lixiviación natural de una escoria negra de acería sometida a un proceso de envejecimiento acelerado por carbonatación

Authors: Gallastegui, G., Barona, A., Elías, A., Rojo, N., Gurtubay, L.

Year: 2012

Place: Valencia (Spain)

Type of participation: Poster

4th International Congress Eurosoil

Title: Carbonation ageing of EAF slag: implications for leaching behaviour

Authors: Gurtubay, L., Batiz, L., Elías, A., Barona, A., Gallastegui, G., Rojo, N., Gómez-Nubla, L., Fernández-Ortiz de Vallejuelo, S., Madariaga, J.M.

Year: 2012

Place: Bari (Italy)

Type of participation: Poster

10. OTHERS

10.2. Stays abroad

Česká republika

Institution: Institute of Chemical Technology (ICT) Prague.

Responsible: Prof. Jan Paca, DSc.

Year: 2009

Duration: 2 weeks

Activity: Training period learning mechanisms of microbial degradation of nitroaromatic compounds. Presentation of the lecture "Biofiltration research" for Ph.D. students of ICT Prague.

Canada

Institution: Université de Sherbrooke. Faculté de génie. Département de génie chimique.

Responsible: Professeure titulaire Michèle Heitz, Ph.D., ing.

Year: 2010

Duration: 26 weeks

Activity: Training period learning experimental techniques developed in the BIOCOM biofiltration-lab for the determination of kinetic parameters. Research subject was the biofiltration of toluene and *para*-xylene and for that purpose 3 bench-scale biofilters were set up. Determination of kinetic parameters for biodegradation of toluene and p-xylene mixtures was successfully carried out.

France

Institution: École des mines d'Alès. Centre de recherche Louis Leprince Ringuet. Laboratoire de génie de l'environnement industriel et des risques industriels et naturels.

Responsible: Enseignant-chercheur Luc Malhautier, Ph.D.

Year: 2011

Duration: 14 weeks

Activity: Research subject was the biofiltration of dimethyl sulphide. The study was focused on the optimization of several key operating parameters as nutrient availability, irrigation periods, pressure drop and sulphate content in the lixiviate.

11. CONCLUSIONS

- ✓ A standardised methodology or protocol was established for acclimating inocula from a sewage sludge. Thus, certain strategies for successfully acclimating biomass have been proposed.
  - o Concerning the prior separation of sludges phases, the use of the whole sample is recommended.
  - o Regarding the discussion between continuous versus discontinuous acclimation mode, the latter is recommended for rapidly obtaining acclimated sludge samples. The great advantage of the continuous system was the absence of daily maintenance, although a longer operating time was required.
  - o Regarding the decision on the prior addition of a readily degradable carbon source (i.e. glucose), the biomass response to contaminant biodegradation was delayed and, therefore, it is not recommended for obtaining a specific microbial community.
- ✓ The ABONLIR^{TM} organic packing material rendered a negligible pressure drop after 550 days of continuous operation and periodic irrigation. The suitability of the material in *para*-xylene biofiltration was confirmed.
- ✓ A periodic irrigation strategy every 25 days at a 5/1 ($kg_{Material}/L_{Nutrients}$) ratio, rendered a considerable increase in the overall removal efficiency of the bioreactor treating *para*-xylene. Based on the absence of active biomass in the leaching solution, the microbial population was concluded to be robust enough (i.e. attached strongly enough) to withstand this maintenance strategy.
- ✓ The peak elimination capacities (EC_{MAX}) recordered in biofilters filled with the organic material were 170 g m^{-3} h^{-1} for ethylbenzene, 138 g m^{-3} h^{-1} for toluene and 128 g m^{-3} h^{-1} for *para*-xylene, for long-term operation (more than 400 days).
- ✓ Regarding biofilters treating ethylbenzene and toluene, the recommended moisture content of the organic packing material ranged from 15 to 30 %. This range was considerably lower than others published in the literature, which is consistent with the aforementioned irrigation strategy. A sudden decrease in the performance of both biofilters occurred when the moisture content was not within the recommended range.
- ✓ Regarding the bacterial and fungal community characterisation of biofilters treating toluene, ethylbenzene and *para*-xylene under xerophilic conditions for 185 days and inoculated with the same toluene-degrading enrichment culture:
  - o Toluene and ethylbenzene biofilters had relatively low biodiversity and a similar microbial composition profile.
  - o By contrast, the biofilter exposed to *para*-xylene had a more complex microbial community, which was also more similar to the indigenous microbial population identified in the packing material.
- ✓ When the mixture of *para*-xylene and toluene was fed into a biofilter:

- The presence of toluene led to a remarkable inhibition effect of *para*-xylene biodegradation.
- On the other hand, the presence of *para*-xylene enhanced the effect of toluene biodegradation.

✓ The specific growth rate in mixed biofilters (*para*-xylene + toluene) ranged from 0.012 to 0.068 h^{-1}, with the highest values being those corresponding to mixtures with lower *para*-xylene levels.

✓ Based on the expertise and technical skills developed through the ABONLIR™ experience, other wastes (industrial inorganic by-products) have been tested as innovative packing materials in conventional biofilters. In this preliminary study, the EAF slag generated in the steel industry was selected because it would provide complementary benefits to the organic material for further applications.

✓ As a final conclusion, the recommended average range of operating parameters for efficiently treating toluene, ethylbenzene and *para*-xylene in biofilters packed with the proposed support material is as follows:

o	EBRT	≥ 150 s.
o	Inlet load	≤ 120 g m^{-3} h^{-1}
o	Moisture content in the packing material	[15 – 30 %] (5/1 $kg_{Material}/L_{Nutrients}$)
o	Irrigation frequency	25 days
o	Replacement frequency	≥ 1 year
o	Average removal efficiency	≥ 70 %.

12.APPENDIX

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