Universidad de Valladolid

ESCUELA DE INGENIERÍAS INDUSTRIALES

DEPARTAMENTO DE INGENIERÍA QUÍMICA Y TECNOLOGÍA DEL MEDIO AMBIENTE

TESIS DOCTORAL:

Comparative evaluation of conventional and innovative biotechnologies for odour abatement in wastewater treatment plants

Presentada por la Ingeniera Química Raquel Lebrero Fernández
para optar al grado de
Doctora por la Universidad de Valladolid

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UNIVERSIDAD DE VALLADOLID

ESCUELA DE INGENIERÍAS INDUSTRIALES

Secretaría

La presente tesis doctoral queda registrada en el folio número _____ del correspondiente libro de registro número _____

Valladolid, a _____ de_____ de 2011

Fdo. El encargado del registro.

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Certifica que:

La ingeniera RAQUEL LEBRERO FERNÁNDEZ ha realizado bajo su dirección el trabajo "*Comparative evaluation of conventional and innovative biotechnologies for odour abatement in wastewater treatment plants"*, en el Departamento de Ingeniería Química y Tecnología del Medio Ambiente de la Escuela de Ingenierías Industriales de la Universidad de Valladolid. Considerando que dicho trabajo reúne los requisitos para ser presentado como Tesis Doctoral expresan su conformidad con dicha presentación.

Valladolid, a _____ de_____ de 2012

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Reunido el tribunal que ha juzgado la Tesis Doctoral titulada "*Comparative evaluation of conventional and innovative biotechnologies for odour abatement in wastewater treatment plants"* presentada por la ingeniera Raquel Lebrero Fernández y en cumplimiento con lo establecido por el Real Decreto 778/1998 del 1 de Mayo de 1998 acuerda conceder por_____ la calificación de _____.

Valladolid, a _____ de_____ de 2012

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Acknowledgements (Agradecimientos)

En primer lugar, quiero agradecer a Raúl todo lo que me ha enseñado durante estos años, su apoyo y paciencia, su dedicación y disponibilidad las 24 horas del día, los 7 días de la semana.

A los profesores del departamento, con cuyo apoyo he tenido la suerte de contar durante los cuatro años de doctorado.

A todos los chicos/as Pana: al grupo VOC & Odour Treatment: Guillermo, Jose y Juan Carlos, y a las chicas de algas: Esther kinki, Cynthia, Marta y Melanie. Y como no a Mary, por escucharme y ayudarme siempre.

Por supuesto a todos mis compañeros, con los que he pasado horas interminables en el laboratorio, en el cuarto, en la sala del café, en barbacoas, casas rurales, fiestas… A los que ya acabaron: Sali, Teresa, Eva, Andreita, Alex, Jose, Facundo… a Elisa, porque parte de esta tesis es suya! Gracias por todo lo que me has enseñado de microbiología. Y a los que aún estáis dando guerra: a las chicas Kati, Rayito, Sarita, Rebeca, Cati, Cris, Flor, Iris, Ana, Camila, Lara, Silvia, Begoña… y a la *FAF* (Danilo, Oscar Don Perfecto, Joao, Theo, Pablo, Mou,…) y a todos los que formamos parte de esta enorme familia. Gracias por compartir los buenos momentos y estar ahí en los más difíciles.

A los técnicos del laboratorio, y en especial a Araceli por todos los análisis, por su ayuda y disponibilidad en todo momento, y por cuidar de todos.

To all the members of the Water Research Center, and especially to Richard Stuetz, for their support during my research stay in Sydney. To Gio, thanks for your friendship, and all the good friends from everywhere who made my stay unforgivable.

Also to the members of the EnVOC Group in Gent, for their help with lab and no-lab tasks during my research stay in Belgium. To Diëgo, for your help and happiness when working, and to Herman van Langenhove, for giving me the opportunity to develop there part of my thesis.

A María G. Lira Rangel y a Ana Celina Gondim Santos, las mejores koalas, gracias por vuestra ayuda y los buenos ratos del laboratorio. Ha sido un placer trabajar con vosotras!

A todos mis amigos/as, en especial a Mariate por las largas conversaciones, los paseos y los momentos de diversión.

Y por supuesto a mi familia, a mis padres y mis hermanas Cris y Patri, gracias por vuestro apoyo, sin vosotros esto no habría sido posible.

Y a JAVI, por estar siempre a mi lado.

Resumen

Como resultado de una legislación ambiental cada vez más estricta, del acercamiento de las zonas residenciales a las Estaciones Depuradoras de Aguas Residuales (EDARs) y del aumento de las expectativas ciudadanas con respecto a los estándares de calidad ambiental exigidos a las compañías que explotan estas EDARS, el número de quejas por contaminación odorífera ha crecido de manera substancial en los últimos años. A pesar de no ser una causa directa de enfermedad, la exposición prolongada a emisiones odoríferas afecta negativamente a la salud humana, además de ser perjudiciales para el medio ambiente. Por lo tanto, la minimización y el tratamiento de dichas emisiones se han convertido en uno de los principales retos de las EDARs. Son muchas las tecnologías que han sido implementadas para el control de las emisiones odoríferas, tanto físico-químicas como biológicas. No obstante, estas últimas son cada vez más empleadas por ser más respetuosas con el medio ambiente y presentar menores costes de operación y elevadas eficacias de eliminación.

Sin embargo, a pesar de las ventajas arriba mencionadas, las eliminaciones de compuestos odoríferos hidrofóbicos en las tecnologías biológicas suelen verse reducidas debido a limitaciones de transferencia desde la fase gaseosa a la biopelícula donde tiene lugar la degradación. Por otro lado, la mayor parte de los estudios existentes emplean compuestos modelo (H_2S, NH_3), o bien corrientes sintéticas con un único compuesto orgánico volátil (COV) a alta concentración, mientras que las emisiones odoríferas son mezclas complejas de numerosos compuestos a concentraciones traza (mg m^{-3}).

En la presente tesis se realiza una comparación sistemática de la eficacia de diferentes sistemas biológicos (tanto convencionales como innovadores) en el tratamiento de emisiones odoríferas, centrándose en la fracción más hidrofóbica de estas emisiones. Además, se evalúa la influencia de parámetros clave en el rendimiento de desodorización del proceso, la estabilidad y las dinámicas microbianas.

En el primer estudio comparativo se operaron un biofiltro (BF) y un sistema de difusión en lodos activos (ASD) para tratar una corriente de H_2S (24-43 mg m^{-3}), butanona (4-6 mg m^{-3}) y tolueno (0.4-0.6 mg m^{-3}) a tiempos de residencia entre 94 y 32 s. Las eficacias de eliminación (RE) fueron similares para ambos biorreactores, con concentraciones de H_2S a la salida inferiores a 1.4 mg m^{-3} y eliminaciones de butanona y tolueno superiores al 95%. Se observó además que el tratamiento simultáneo de agua residual en el sistema de lodos activos no sólo no perjudicó el rendimiento del sistema para el tratamiento de olores, si no que el aporte de fuentes

de carbono adicionales fue necesario para mantener la actividad microbiológica del proceso. El reducido espectro de fuentes de carbono alimentado al sistema no afectó a la diversidad bacteriana, que se mantuvo elevada durante todo el periodo experimental. Adicionalmente al estudio del rendimiento de ambos procesos en condiciones estacionarias, se evaluó la robustez frente a fluctuaciones de temperatura (8-30 ºC), incrementos de 3 y 6 veces en la carga nominal de contaminantes, paradas en la alimentación de 5 días y fallos en el sistema de riego del BF y en el de control del pH del ASD. El análisis cuantitativo de robustez demostró la fiabilidad de ambas tecnologías en el tratamiento de emisiones odoríferas procedentes de plantas depuradoras (H_2S, butanona, tolueno y alfa-pineno fueron empleados como compuestos modelo a un tiempo de residencia del gas de 50 s), no obstante el ASD presentó una mayor robustez frente a los fallos comunes de operación.

Aunque los BFs se han empleado durante décadas para el tratamiento de corrientes odoríferas, son escasos los estudios orientados a evaluar las eficacias de tratamiento individuales para cada compuesto en corrientes reales. Por otro lado, la información relativa a las dinámicas de formación y emisión de los contaminantes odoríferos también es limitada pero resulta necesaria para un correcto diseño y operación del biorreactor. Por ello, se realizó un estudio con el objetivo de analizar el comportamiento de un BF de compost durante el tratamiento de una corriente odorífera “real” procedente de un lodo de depuradora, representativa de las emisiones en las etapas de espesamiento, centrifugado y transporte de fangos. Se seleccionaron 8 compuestos de diferente naturaleza (cetonas, terpenos, hidrocarburos, derivados sulfurados y ácidos volátiles). Las RE del limoneno, acetona, butanona y benceno fueron superiores al 99% incluso a tiempos de residencia de 40 s, mientras que el tolueno y el trisulfuro de dimetilo mostraron eliminaciones por encima del 80% a un tiempo de residencia de 60 s. Estos valores de RE se mantuvieron estables a pesar de las fluctuaciones en las concentraciones de entrada observadas. Por otro lado, las reducidas eliminaciones de sulfuro de dimetilo y ácido acético en el biofiltro se atribuyeron a la formación de estos compuestos en zonas anaerobias del material de empaque.

A pesar de los resultados satisfactorios obtenidos para el biofiltro y de las numerosas ventajas de esta biotecnología (sencillez de operación, amplia experiencia de diseño y operación, bajos costes de operación), los elevados requerimientos de espacio derivados de sus altos tiempos de residencia (> 30 s) y la dificultad en el control de los parámetros de operación limitan su aplicación, especialmente en plantas de tratamiento con restricciones de espacio. Por otro lado, los sistemas de lodos

activos son económicamente viables exclusivamente en plantas que cuentan con sistemas de aireación por difusión. Una alternativa potencial a estos procesos biológicos la constituyen los biofiltros percoladores (BTF), que presentan bajos costes de operación y menores necesidades de espacio debido a sus bajos tiempos de retención. En este contexto, el efecto del tiempo de residencia en el tratamiento de una corriente odorífera de metil mercaptano, tolueno, alfa-pineno y hexano (concentraciones de entrada de 22, 0.22, 0.23 y 0.28 mg m^{-3} respectivamente) se estudió en un BTF empacado con espuma de poliuretano a tiempos de residencia de 50, 30, 11 y 7 s. Los resultados confirmaron los altos rendimientos de estos sistemas a bajos tiempos de residencia, con RE> 95% para metil mercaptano, tolueno y alfa-pineno, y RE >70% para el hexano. Sin embargo, a 7 s de tiempo de residencia se observó un deterioro de la actividad microbiológica, y por lo tanto del rendimiento del BTF. La adición de aceite de silicona permitió estabilizar el proceso, y tras la re-inoculación del BTF únicamente se detectó hexano en la corriente de salida, con eliminaciones estables del ~ 80%.

Las limitaciones en el transporte de los compuestos odoríferos hidrofóbicos dan lugar a menores eficacias de desodorización en sistemas biológicos de tratamiento, por lo que su diseño y escalado debe estar basado en datos de transferencia de materia. El modelo desarrollado en la presente tesis permitió caracterizar el potencial de transferencia de un BTF basándose en coeficientes globales de transporte (K_La). Los altos valores de K_La observados en el sistema (35-113 h^{-1}) corroboran los elevados rendimientos de eliminación descritos con anterioridad. Por otro lado, la estimación de los coeficientes individuales situó en la fase líquida la principal resistencia al transporte.

La biodegradación de compuestos orgánicos volátiles con alta hidrofobicidad sigue siendo por tanto la principal restricción de sistemas biológicos como los BTFs. En este sentido, la adición de una fase orgánica no-acuosa a los BTFs convencionales (sistemas bifásicos) ha demostrado incrementar la transferencia de dichos compuestos de la fase gas a los microrganismos, aunque su aplicación al tratamiento de corrientes odoríferas aún no ha sido estudiada. Para evaluar la influencia de una fase orgánica, se operaron paralelamente dos BTFs, uno de ellos con un 30% v/v de aceite de silicona, y se analizó la eficacia de biodegradación de una mezcla de butanona, tolueno, alfa pineno y hexano a tiempos de residencia comprendidos entre 47 y 6 s. Ambos biorreactores alcanzaron RE de butanona, tolueno y alfa-pineno > 96%, independientemente del tiempo de residencia, mientras que el sistema bifásico presentó, en general, mayores eficacias de eliminación de hexano que el BTF

convencional. Además, la adición de aceite de silicona resultó en una operación más estable, con una mayor resistencia ante fluctuaciones en las concentraciones de entrada y periodos de inanición. Por otro lado, los fallos en el sistema de recirculación de líquido provocaron un importante deterioro del rendimiento del sistema bifásico, posiblemente debido a disminución en la capacidad de transferencia del sistema.

Los biorreactores de membrana (MBR) aplicados al tratamiento de corrientes gaseosas han emergido recientemente como una alternativa compacta y capaz de superar las limitaciones de transferencia gracias a la elevada permeabilidad y afinidad de algunas membranas por los compuestos más hidrofóbicos. Sin embargo, no existen estudios orientados al tratamiento de corrientes odoríferas en estos MBR. Para ello, se estudió el comportamiento de un MBR de membrana plana para el tratamiento de una mezcla de COVs (acetona, tolueno, limoneno y hexano) a concentraciones comprendidas entre 1.3 y 3.2 mg m^{-3} y tiempos de residencia entre 60 y 7 s. Mientras que la acetona y el tolueno presentaron RE > 93%, las REs de hexano y limoneno fueron muy inferiores. Para aumentar el transporte y degradación de hexano, se inoculó la membrana con un consorcio hidrofóbico embebido en aceite de silicona y adaptado a la degradación de hexano. Sorprendentemente, la re-inoculación del sistema dio lugar a un incremento en la RE del limoneno, alcanzando valores superiores al 90% a tiempos de residencia de 7 s, mientras que las REs de hexano no se vieron afectadas. Un análisis abiótico posterior del MBR reveló limitaciones en el transporte del limoneno y el hexano, por lo que la selección adecuada de un material afín a los COVs más hidrofóbicos se identificó como un elemento clave en el rendimiento de los MBR.

Finalmente, se realizó un estudio comparativo de tres biotecnologías (BF, BTF y MBR de fibra hueca) alimentadas con una corriente sintética de 5 mg m^{-3} de metil mercaptano y entre 0.7 y 0.9 mg m^{-3} de tolueno, alfa-pineno y hexano. REs > 97% fueron registradas tanto en el BF como en el BTF para el metil mercaptano, tolueno y alfa-pineno, siendo algo inferiores (>88%) para el hexano. La operación del MBR estuvo caracterizada por una gran inestabilidad, con eliminaciones elevadas para metil mercaptano y tolueno (>95%), muy variables en el caso del limoneno (39-99%) y reducidas para el hexano (~45%). Esta inestabilidad estuvo correlacionada con los aumentos de caída de presión en el MBR debidos al crecimiento y acumulación de biomasa en el mismo, llegando a alcanzar los 200 cm de columna de agua. Por otro lado, mientras que las caídas de presión en el BTF se mantuvieron inferiores a 30 cm de columna de agua, valores superiores a 1 m fueron registrados en el BF como consecuencia de una compactación prematura del lecho.

Abstract

During the last decades, the number of public complaints related to malodorous emissions has increased noticeably due to the enforcement of the environmental regulations, the encroachment of residential areas on wastewater treatment plants (WWTPs), and the increasing public expectations on privatized water companies. Despite not being a direct cause of disease, the prolonged exposition to odorous emissions negatively affect human health, besides being harmful for the environment. Thus, minimization and abatement of odours has become one of the main challenges for WWTPs utilities worldwide. Several technologies have been implemented for odour control, both physical-chemical and biological techniques. Nevertheless, biological treatment has overcome physical-chemical technologies due to their environmentally friendly profile, their lower operating cost and the higher removal efficiencies (RE).

In spite of the above mentioned advantages, biological techniques usually present low REs for the hydrophobic fraction of the odorous emission, due to mass transfer limitations from the gas phase to the biofilm where the odorants are biodegraded. In addition, most of the studies are focused on the removal of a model odorant (H_2S, NH_3), or they mimic odorous emissions by means of a synthetic stream with single or few volatile organic compounds (VOCs) at high concentrations. These conditions are not representative of WWTPs odorous emissions, which are complex mixtures of several compounds at trace level concentrations (mg m^{-3}).

In the present thesis, a systematic comparison of the removal performance of some biological systems for odour abatement was performed, particularly focused on the most hydrophobic fraction of these emissions. The influence of the key operating parameters on the removal performance, the process stability and the microbial dynamics were also evaluated.

In the first comparative study, a biofilter (BF) and an activated sludge diffusion system (ASD) where operated to treat a H_2S (24-43 mg m^{-3}), butanone (4-6 mg m^{-3}) and toluene (0.4-0.6 mg m^{-3}) polluted stream at empty bed residence times (EBRTs) ranging from 94 to 32 s. Similar REs were observed in both bioreactors, with H_2S outlet concentrations always lower than 1.4 mg m^{-3}, and butanone and toluene REs > 95%. It was also observed that the simultaneous treatment of wastewater in the ASD system did not have a detrimental effect on the process performance but the supply of additional carbon sources was necessary to maintain the microbial activity of the process. The limited carbon source spectrum fed to the system did not affect the bacterial diversity, which remained high over the complete experimentation period. In

addition, the robustness towards temperature fluctuations (8-30 ºC), step increases of 3 and 6 times the nominal load of pollutants, starvation periods of 5 days, and failures in the irrigation system of the BF and in the pH control system of the ASD was also evaluaed. The quantitative analysis of the robustness showed the reliability of both technologies for odorous emissions treatment (H_2S, butanone, toluene and alpha-pinene were employed as model odorants at gas residence times of 50 s), although the ASD showed a higher robustness towards common operational failures.

Biofilters have been employed for decades for odorous emission treatment, but there is still a limited number of studies focused on the evaluation of the individual odorant REs. Moreover, there is a lack of information on the odorant formation and emission dynamics, in spite of being necessary for a proper bioreactor design and operation. For this reason, the performance of a compost-based BF was assessed for the treatment of a “real” odorous emission from WWTP sludge, representative of the emissions from sludge thickening, centrifuge and transport activities. Eight compounds of different nature were selected (ketones, terpenes, hydrocarbons, sulfur derived compounds and volatile fatty acids). Limonene, acetone, butanone and benzene REs always exceeded 99% even at EBRTs of 40 s, whereas REs > 80% were recorded for toluene and dimethyl trisulfide at an EBRT of 60 s. A stable operation was observed in spite of the fluctuations in the inlet odorant concentration. On the other hand, the low REs recorded for dimethyl sulfide and acetic acid were attributed to their formation in anaerobic zones of the packing material.

Despite the satisfactory results obtained for the BF and the numerous advantages of this biotechnology (easy operation, wide design and operation experience, low operating costs), the high land requirements derived from the high EBRT (> 30 s) and the complex control or the operating parameters have limited their application, especially in WWTPs with space limitations. Conversely, ASD systems are an economically viable alternative in WWTPs with land restrictions but provided with a diffused aerated tank. A potential alternative to these biological systems are biotrickling filters (BTFs), which present low operating costs and lower land requirements due to their lower retention times. In this context, the effect of the EBRT on the treatment of an odorous emission of methyl mercaptan, toluene, alpha-pinene and hexane (inlet concentrations of 22, 0.22, 0.23 y 0.28 mg m^{-3}, respectively) was studied in a BTF packed with polyurethane foam at EBRTs of 50, 30, 11 and 7 s. The results confirmed the high performance of BTFs at low EBRTs, with REs > 95% for methyl mercaptan, toluene and alpha-pinene, and RE > 70% for hexane. However, at 7 s of residence time, an irreversible damage in the microbial activity, and therefore in the BTF

performance, was observed. The addition of silicone oil allowed for a rapid stabilization of the process, and after the BTF re-inoculation only hexane was detected in the outlet stream, achieving stable REs of ~ 80%.

The mass transport limitations of hydrophobic odorants result in lower deodorization efficiencies in biological systems, therefore their design and scale-up should be based on mass transfer data. The model herein developed allowed for an accurate characterization of the mass transfer potential in BTFs based on global mass transport coefficients (K_La). The high K_La values observed in the system (35-113 h^{-1}) corroborated the high removal performances previously described. On the other hand, the estimation of the individual coefficients revealed that the main resistance for VOC transfer was in the liquid film regardless of the EBRT.

The biodegradation of highly hydrophobic VOCs is still the main restriction of biological technologies such as BTFs. In this context, the addition of an organic, non-aqueous phase to conventional BTFs (biphasic reactors) has resulted in an increased mass transfer of hydrophobic VOCs from the gas phase to the microbial community, although their application to odour abatement has not been evaluated yet. In order to assess the influence of an organic phase, two BTFs were operated in parallel, one of them containing 30% v/v of silicone oil in the recycling solution. The biodegradation efficiency of a mixture of butanone, toluene, alpha-pinene and hexane was recorded at EBRTs ranging from 47 to 6 s. Both bioreactors achieved butanone, toluene and alpha-pinene REs > 96%, regardless of the EBRT, while the biphasic system showed in general higher REs for hexane than the conventional BTF. Besides, the addition of silicone oil resulted in a more stable operation, with a higher robustness towards fluctuations in VOC inlet concentration and starvation periods. Conversely, a failure in the liquid recycling system lead to a significant deterioration of the two-phase BTF performance, likely due to a reduction in the mass transfer potential of the system.

Membrane bioreactors (MBR) applied for waste gas treatment have recently emerged as a compact alternative able to overcome mass transfer limitations due to the high permeability and affinity of certain membranes for hydrophobic VOCs. However, there is no single study devoted to the treatment of odorous emissions in MBRs. To assess the odorant biodegradation performance of MBRs, a flat MBR was fed with a VOC mixture (acetone, toluene, limonene and hexane) at concentrations ranging from 1.3 to 3.2 mg m^{-3} and EBRTs from 60 to 7 s. While high REs were achieved for acetone and toluene (>93%), hexane and limonene REs were much lower. In order to increase mass transport and hexane biodegradation, the membrane

was inoculated with a hydrophobic consortium embedded in silicone oil and adapted to hexane biodegradation. Surprisingly, the re-inoculation of the system resulted in higher limonene REs, reaching values >90% at an EBRT of 7 s, while no improvement in hexane RE was recorded. A subsequent abiotic analysis of the MBR revealed limonene and hexane mass transport limitations, thus the selection of a membrane material with high affinity for the target hydrophobic odorants is of key importance for a proper MBR performance.

Finally, a comparative study of three biotechnologies (BF, BTF and hollow fiber MBR) was developed to assess their abatement efficiency of a synthetic emission of 5 mg m^{-3} of methyl mercaptan and 0.7-0.9 mg m^{-3} of toluene, alpha-pinene and hexane. Removal efficiencies > 97% were recorded in the BF and the BTF for methyl mercaptan, toluene and alpha-pinene, and slightly lower REs were observed for hexane (>88%). On the other hand, the operation of the MBR was characterized by an instable behavior, with high REs for methyl mercaptan and toluene (>95%), highly varying limonene REs (39-99%) and low hexane REs (~45%). This instability was correlated with the increases in the MBR pressure drop (up to 200 cm of water column) caused by an excessive biomass growth and accumulation. Conversely, BTF pressure drops were always lower than 30 cm of water column, whereas pressure drops higher than 1 m were recorded in the BF as a result of a premature packing material compaction.

List of publications

The following publications are presented as part of this thesis. Five of them are published in international journals indexed in ISI Web of Knowledge (articles I to V), and two of them (articles VI and VIII) have been sent for publication.

Article I. Lebrero R., Rodriguez E., García-Encina P.A., Muñoz R. (2011). *A comparative assessment of biofiltration and activated sludge diffusion for odour abatement.* Journal of Hazardous Materials, 190: 622-630

Article II. Lebrero R., Rodriguez E., Martín M., García-Encina P.A., Muñoz R. (2010). *H2S and VOCs Abatement Robustness in Biofilters and Air Diffusion Bioreactors: A Comparative Study*. Water Research, 44: 3905-3914.

Article III. Lebrero R., Rangel M.G.L., Muñoz R. (2012). *Characterization and biofiltration of a real odorous emission from wastewater treatment plant sludge*. Environmental Management, sent for publication.

Article IV. Lebrero R., Rodriguez E., Estrada J.M., García-Encina P.A., Muñoz R. (2012). *Odor abatement in biotrickling filters: effect of the EBRT on methyl mercaptan and hydrophobic VOC removal.* Bioresource Technology, 109: 38-45.

Article V. Lebrero R., Estrada J.M., Muñoz R., Quijano G. (2012). *Toluene mass transfer characterization in a biotrickling filter*. Biochemical Engineering Journal, 60: 44-49.

Article VI. Lebrero R., Rodriguez E., Perez R., Garcia-Encina P.A., Muñoz R. (2012). *Abatement of odorant compounds in one a two-phase biotrickling filters under steady and transient conditions*. Applied Microbiology and Biotechnology, available On-line.

Article VII. Lebrero R., Volckaert D., Perez R., Muñoz R., Van Langenhove H. (2012). *A membrane bioreactor for the simultaneous treatment of acetone, toluene, limonene and hexane at trace level concentrations*. Water Research, sent for publication.

Article VIII. Lebrero R., Gondim A.C., Perez R., Muñoz R. (2012). *Comparative assessment of a biofilter, a biotrickling filter and a hollow fiber membrane bioreactor for odour abatement*. Manuscript.

Contribution to the articles belonging to the thesis

Article I. During the execution of this work I was responsible of the start-up and operation of the experimental set-up, results evaluation and article writing under the supervision of Dr. Raúl Muñoz. Dr. Elisa Rodríguez was responsible of the microbiological analysis together with Dr. Pedro Garcia-Encina. I collaborated in sample preparation and results discussion.

Article II. In this work I was in charge of the start-up and operation of the experimental set-up, results evaluation and article writing under the supervision of Dr. Raúl Muñoz. Dr. Elisa Rodríguez was responsible of the microbiological analysis together with Dr. Pedro Garcia-Encina, and I helped with the analytical part and results discussion.

Article III. During this research study I co-supervised Maria G. Lira Rangel in the start-up and operation of the experimental set-up and I performed part of the experimentation, the results evaluation and I wrote the article under the supervision of Dr. Raúl Muñoz

Article IV. During the execution of this work I was responsible of the start-up and operation of the experimental set-up with the assistance of Jose M. Estrada, results evaluation, and article writing under the supervision of Dr. Raúl Muñoz. Dr. Elisa Rodríguez was responsible of the microbiological analysis together with Dr. Pedro Garcia-Encina. I helped with the analytical part and results discussion.

Article V. The experimental data was obtained in collaboration with Jose M. Estrada, the model was developed by Dr. Guillermo Quijano and both of us wrote the article under the supervision of Dr. Raúl Muñoz.

Article VI. In this work I carried out the start-up and operation of the experimental set-up, results evaluation, and article writing under the supervision of Dr. Raúl Muñoz. Dr. Elisa Rodríguez was responsible of the microbiological analysis together with Dr. Pedro Garcia-Encina. I collaborated in the analytical procedures and results discussion.

Article VII. In this work I performed the experimental part with the assistance of Diëgo Volckaert and I wrote the article under the supervision of Dr. Herman Van Langenhove and Dr. Raúl Muñoz. Dr. Rebeca Pérez was responsible of the microbiological analysis. This work was developed in the EnVOC Research group, in the Faculty of Bioscience Engineering, Gent University (Belgium).

Article VIII. During this research study I co-supervised Ana Celina Gondim in the start-up and operation of the experimental set-up and I carried out some of the experimentation. I was also responsible of the results evaluation and article preparation under the supervision of Dr. Raúl Muñoz

Chapter 1

Introduction

1.1 The problematic of odours

The term “odour” refers to the physiological stimulus of the olfactory cells in the presence of specific molecules (known as odorants) that varies between individuals and with environmental conditions such as temperature, pressure and humidity [1].

Odorous emissions have traditionally occupied a secondary role in environmental concern since they represent a lower risk for human health and natural ecosystems when compared to solid and liquid emissions. However, despite not being a direct cause of disease, long-term exposure to such odorants can have a negative effect on human well-being, causing nausea, headaches, insomnia, loss of appetite, respiratory problems, irrational behavior, weakness, etc. When detected in residential areas, they lead to a negative perception of the quality of life, and they can even create a significant economic cost for housing surrounding odour sources. In addition, they can pose a severe occupational risk within confined spaces in wastewater treatment plants (WWTPs), such as sludge handling and pumping facilities, reaching lethal concentrations [2-4].

As a result of the encroachment of residential areas on WWTPs, the general higher environmental standards and the increasing public expectations on private water companies’ duties, the number of public odour complaints has substantially increased during the last decades. This increase in public complaints has in turn lead to the enforcement of stricter environmental regulations [3]. Therefore, the minimization and abatement of unpleasant malodorous emissions has become one of the main challenges for WWTPs worldwide, increasingly concerned about their public image.

1.1.1 Odour characterization

Odorous emissions from WWTPs are complex mixtures of compounds including H_2S and highly malodorous sulfur derived compounds such as mercaptans or organic sulfides, NH_3 and nitrogen-containing compounds (amines, indole, skatole), and a wide range of oxygenated compounds such as aldehydes, alcohols, organic acids or ketones (Figure 1.1) (**Chapter 5**). The degree of nuisance depends on the sensorial properties of each compound, their concentration and the synergistic/antagonistic sensorial effects established within the mixture components. In addition to their complexity, malodorous emissions are also characterized by their low odorant

concentrations (within the range of µg L^{-1} - mg L^{-1}) and their high emitted flow rates (between one and five thousands higher than wastewater flow rates) [5-7]. Nevertheless, trace level concentrations of certain odorants can induce a significant nuisance due to their low detection threshold (minimum concentration of an odorant that is perceivable by human sense of smell). In general, sulfur derived compounds present the lowest detection thresholds, in the order of ppb (Table 1.1).

Table 1.1 Odour descriptor, detection threshold and typical concentration of selected odorants emitted from WWTPs [8-10]

Compound	Odour descriptor	Detection threshold (ppb)	Concentration in WWTPs (ppb)
Sulfur derived compounds			
Hydrogen sulfide	Rotten eggs	0.5	0 - 28700
Methyl mercaptan	Rotten cabbage	0.02	0.03 - 8
Dimethyl disulfide	Rotten cabbage/putrefaction	0.026	54.5
Dimethyl trisulfide	Rotten cabbage	1.2	9.7
Nitrogen derived compounds			
Ammonia	Irritating, pungent	38	18-1023
Trimethyl amine	Fishy	0.4	0.15 - 3.16
Indole	Fecal, nauseating	0.1	-
Skatole	Fecal, nauseating	1	-
Volatile fatty acids			
Acetic acid	Vinegar	1020	44.8
Butanoic acid	Rancid	0.3	5.6
Propanoic acid	Rancid/pungent	28	3.3
Ketones			
Butanone	Sweet	250	1540
Acetone	Pungent, fruity	20000	190
Aldehydes			
Acetaldehyde	Green sweet	0.1	-
Propionaldehyde	Sweet, ester	11	-
Benzaldehyde	Pleasant, almond-like	50	15.4
Aromatics			
Toluene	Solventy/hydrocarbon	2100	140
Benzene	Solventy/hydrocarbon	1400	6.3
p-Xylene	Sweet	50	11.5
Terpenes			
Alpha-pinene	Fragant/fruity	6	1.8
Limonene	Fragant/fruity	10	21.5

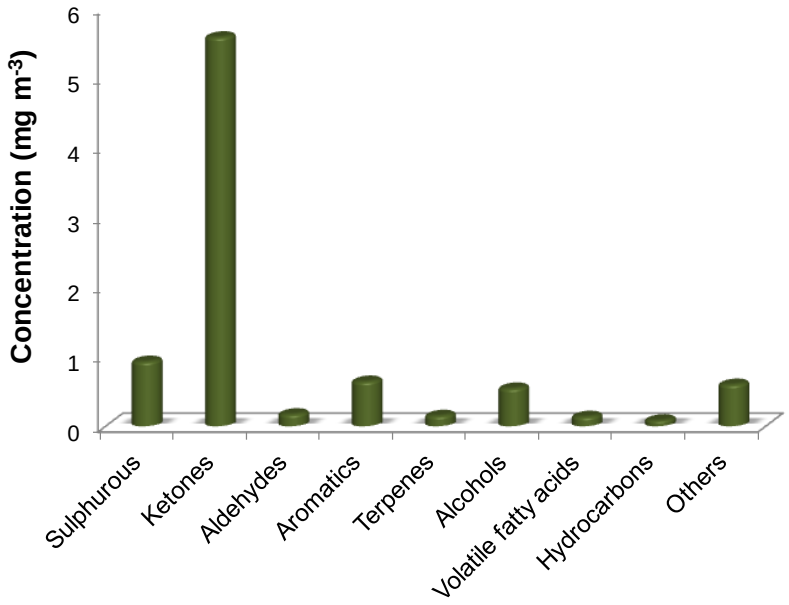

Figure 1.1 Odorant concentrations emitted from a WWTP [9]

The subjective nature of human odour perception and the chemical complexity of odorous emissions from WWTPs make odour characterization a rather difficult task, which has resulted in odour measurement being the cornerstone of odour management [11-12]. Sensorial odour perception constitutes, together with analytical measurements, the most common approach for odour characterization.

Sensory measurements characterize odours in terms of their perceived effects employing the human nose as odour detector, and therefore provide an estimation of the total effect of the sample in the perception of the affected population. Sensorial measurements are highly costly, time consuming, very sensitive to the receptor characteristics and less precise and repeatable than analytical measurements, but they accurately describe the odour effect on receptors. The odour concentration is commonly determined by dynamic olfactometry and is expressed in terms of "odour units" (OU), which, according to the European Standard EN-13725 is a unit volume of air (usually 1 m^3) containing a compound at its odour identification threshold.

On the other hand, analytical measurements characterize odours in terms of their chemical composition. Traditionally, single odorants such as hydrogen sulfide or ammonia were commonly used for online monitoring as surrogate markers, and a broad variety of specific, high sensitivity and selectivity gas sensors have been developed for these surrogates (chemical, electrochemical, catalytic or optical detectors). However, these specific sensors only provide a partial characterization of the odorous emissions since the surrogate marker is not always responsible for the entire odour nuisance. Besides, the low concentration of the chemical compounds

present in the odorous emission (ppb and ppt concentrations) often challenges the sensitivity and separation capacity of gas chromatography, very often below the detection limits of most common GC detectors (mass spectrometry and flame ionization).This enforces the pre-concentration of the odorants either by cryogenic trapping or via adsorption into porous polymers or carbon-based adsorbents [5] (**Chapter 5)**. Analytical techniques are more objective, repeatable and accurate than sensorial odour measurements, although they provide little information about the real odorant impact on human receptors (Table 1.2).

Table 1.2. Advantages and limitations of analytical and sensorial odour monitoring techniques ([5, 13-14]).

Analytical techniques		**Sensorial techniques**	
Identification and quantification of the chemical compounds		Quantitative and qualitative characterization of the sensorial component of the odour	
• Objectivity • Repeatability • Reproducibility • Accuracy • Useful for identification of odour sources	• No indication of the actual odour nuisance • Detectability limit of some compounds • Impossible to determine the odorous potential	• Determination of odour concentration (OU m^{-3}) • Description of the odour effect on receptors	• Highly costly • Time consuming • Subjective • Sensitive to receptor characteristics

Due to their dual sensorial and chemical nature, the characterization of odorous emissions from WWTPs does not necessarily involve a unique or universal technique. The last decade has brought about a paramount development both in sensorial and chemical measurement, which will certainly contribute to improve both the design and monitoring of odour abatement units and the assessment of odour impact [5].

1.1.2 Odour legislation

In a context of increasing public concern and complaints and higher life quality standards, the development of uniform and clearer environmental regulations concerning odour emissions and impact becomes mandatory. These laws and directives must set standards concerning acceptable odour concentrations and a tolerable degree of annoyance. However, this goal has been severely hindered in the past by the lack of reliable olfactometry standards, the subjective nature of odour perception and the absence of consistent dose-effect relationship for most of the pollutants present in odour emissions. International regulations have traditionally focused on the odorants causing nuisance and on complying with specific regulatory

limits for those toxic pollutants present in the malodorous emissions, based on health and safety issues. Former standards such as minimum distance and maximum emission regulations do not meet the current requirements for population protection since they do not take nuisance level into consideration. Therefore, more sophisticated standards based on maximum impact and maximum tolerable annoyance have been introduced [11, 15].

Nowadays, different approaches to the legislation of environmental odours can be found: a qualitative approach, where odour is regarded and assessed as a nuisance; a quantitative approach if odour standards are provided to assess odour data obtained from ambient air measurements, and/or prediction and operational requirements, which include setback distances, specific operating procedures and odour control equipment [16].

In most European countries, Canada or Australia, odour regulations are evolving to take directly into account not the emission but the actual impact in the environment, and are based on the maximum impact odour concentration associated with a limit time during which a higher impact concentration is not tolerable. The concentration limit usually depends on the land use and/or the industrial activity sector. Some regulations take into account the nuisance indirectly, for example several regulations in France set limits in terms of odour flow depending on the height or diffuse odour concentration and on the distance to the closest house. In other European countries such as Switzerland, odour regulation policies do not set emission or impact limits and are based on local population questioning and complaints. Likewise, in the United States, ambient air quality is controlled via H_2S concentration standards or dilution to threshold (D/T) limits, which is a measure of the number of dilutions needed until the odorous emission is not detectable [15-17] (Table 1.3).

In Spain, one of the first regulations concerning environmental odorous pollution is the Law 16/2002, of July 1st, of Integrated Prevention and Control of the Pollution. The main objective is to avoid, reduce or control the atmospheric, water and soil pollution by establishing new integrated prevention and control system. However, the first specific legislation focused on odorous emissions was the Draft Bylaw of Odours Emissions of the Generalitat de Catalunya, which establishes maximum levels of odour immision concentrations, based on the UNE-EN 13275 methodology, and anticipates fines for those activities (cattle farming, waste managing, industries) exceeding those limits.

Table 1.3. Current odour regulation and policies

	Limits	Receptor	Observations
AUSTRALIA			
New South Wales	2/7 OU m^{-3} <0.5% hour $year^{-1}$	Urban/rural areas	Odour regulation policies are responsibility of each state. Very individual approaches
Queensland	0.5/2.5 OU m^{-3}	Short/tall stacks based on modeling	
South Australia	2/10 OU m^{-3}	Urban/rural based on modeling	
ASIA			
Japan	Odour standards for 22 odorants		
South Korea	1000/500 OU 20 OU 10 OU	Stack concentration in industrial/other areas Industrial areas Other areas-facility boundary	
EUROPE			
Austria	1 OU at 8% detection / 3 OU at 3% detection Minimum distances	Any Farm animals	
Belgium	<12% of mildly annoyed population		
Denmark	17 OU m^{-3} 1 OU m^{-3}	Sensitive receptor, pig farm Urban area	
Germany	1 OU m^{-3} <15% time <10%	Industrial areas Residential areas	
Netherlands	<12% of mildly annoyed population Eliminate severe odour nuisance (8-10 OU) by 2010 Minimum distance setbacks in farm animals		
Switzerland	No emission/impact limits, based on public complaints		
United Kingdom	No general valid odour emission standards		
CANADA			
Ontario	1 OU m^{-3}, average 10 min	Any	No federal legislation: responsibility relies on individual provinces/territories
Manitoba	2 OU m^{-3} 7 OU m^{-3}	Residential Industrial	
UNITED STATES			
California	Complaints of >10 persons in a period of < 90 days		Individual states generally have a responsibility for odour emission
Colorado	8 D/T 16 D/T	Residential/commercial Other areas	
Connecticut	8 D/T	Any	
Illinois	9 D/T 25 D/T 17 D/T	Residential/recreational Industrial Others	
Vermont	No odour detectable	Any	
Wisconsin	Odour complaints by 66% of persons		

It is also worth noting the existence of local regulation policies such as the Municipal Bylaw of the Council of Lliçà de Vall (Barcelona), which includes limits of odour concentration as a function of the emission flow rate and the odour threshold of the emitted odorants together with immision concentration limits for diffuse sources.

Similarly, the Municipal Bylaw of the Council of San Vicente de Raspeig (Alicante) for odour control is based on perception indices.

The increasing public concern, together with the coming technological breakthroughs in odour measurement and best available technologies will certainly result in more stringent limits in the near future. The combination of annoyance surveys within malodours-affected population and the development of robust instrumentation for continuous odour monitoring will lay the basis for the establishment of accurate concentration-nuisance relationships. The development of these combined odour concentration-odour nuisance maps will certainly allow the introduction of objectivity into the assessment of odour impact and the establishment of reasonable odour limits in environmental odour legislation [17-18].

1.2 Odorous emissions from WWTPs

1.2.1 Odour formation and composition

Odour emissions from WWTPs are composed of stripped components present in receiving wastewaters, volatile by-products generated in the complex biochemical reactions occurring within the different treatment steps and chemical reagents used during the treatment process. The complete depletion of O_2 and nitrate, together with the presence of a well developed fermentative and sulfate oxidizing microbial community, result in an intense formation of volatile fatty acids, aldehydes, amines, H_2S and sulfur derived compounds. This occurs both in transport and treatment systems such as rinsing mains, primary settlers and sludge handling units. Discharges into the sewer system from household and industrial sources introduce into the wastewater a large number of aliphatic, aromatic or chlorinated hydrocarbons, as well as odorants from human excretion such as skatole, ammonia, etc. Chemicals from industrial sources are especially problematic due to their inherent toxicity and high reactivity, which can often lead to the formation of even more malodours compounds than their parent chemical compounds. Moreover, chemical reagents such as hypochlorite or ozone used in physical/chemical scrubbers for off-gas treatment might also generate residual odours as well as odorous reaction products such as aldehydes [2, 19-20].

1.2.2 Main odour sources

Odour impact only occurs when the odorants are transferred from the liquid to the gaseous phase. This mass transfer, usually referred to as odour emission rate (OER), is determined by the Henry's law constant and concentration of the odorants, the temperature, the pH and the volumetric mass transfer coefficient. These OERs can be either calculated from direct flow rate and concentration measurements or estimated from empirical equations based on wastewater odour emission potentials. Direct OER measurements are highly dependent on the type of odour source being evaluated, which can be classified as active or passive depending on the presence or absence of an external airflow, respectively. While in active sources such as biofilters or aeration tanks, odorants are transported by the active airflow emitted, in passive sources odorants diffuse from the wastewater and are swept by the natural movement of ambient air (wind) [2, 21]. Depending on their extension and geometry, odour sources can also be classified as punctual (locally restricted emissions such as biofilters), linear (elongated sources such as receiving wastewater channels), superficial (extensive emission areas such as primary settlers) and mobile (changing position) sources [22].

The knowledge of the main odour sources and their contribution to the overall problem is crucial for the development of an odour abatement strategy. In general, odorous emissions are more significant in areas with a high turbulence or a high wastewater-air interfacial area such as weirs, inlet works, sedimentation tanks, etc., where a high mass transfer value governs gas-liquid mass transport. The primary sedimentation tank is usually pointed out as the major odour source in WWTPs, with their high odour emission factors also associated with the first stages of the wastewater treatment process: raw wastewater and wastewater pre-treatments (grit chambers, screenings). Hence, the odour potential tends to decrease along the depuration process (Figure 1.2) [7, 23-24].

On the other hand, anaerobic conditions are inherent to sludge handling processes such as sludge thickening, centrifugation or anaerobic digestion. Due to the high organic matter concentrations (in the range of 5-20% w/w) and the absence of O_2 or NO_3^-, these units also exhibit high odorant and odour concentrations (especially sulfur and nitrogen reduced compounds and volatile fatty acids). Special attention must given to the appropriate ventilation of these facilities, since very toxic (even lethal) atmospheres can built up in this particular section of the WWTPs [7, 23].

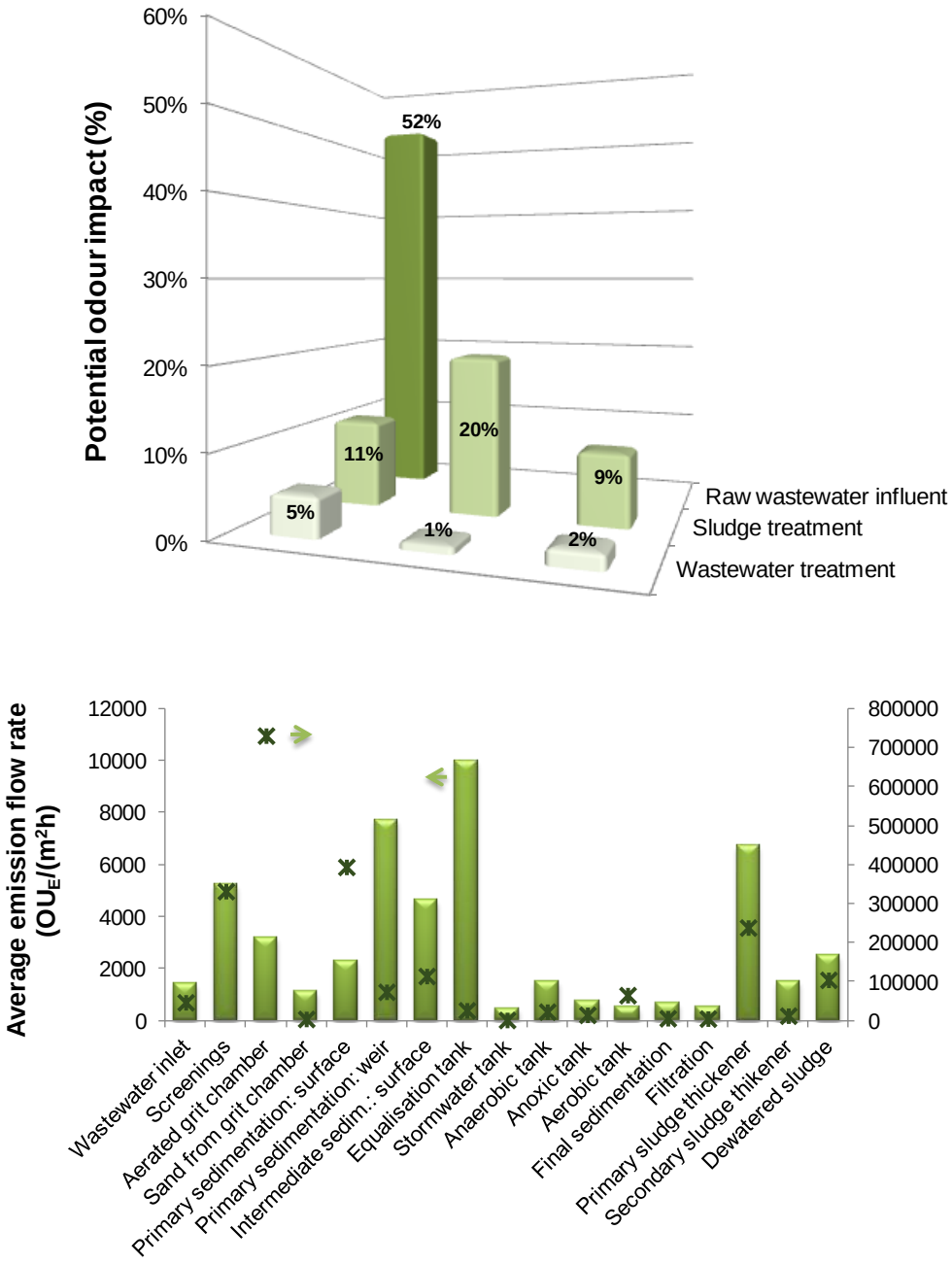

Figure 1.2. (a) Percentage of the potential odour impact at a small WWTP [9] and (b) Average (left axis) and maximum (right axis) area-related odour emission rates in the different units of WWTPs ([23] .

1.3 Odour Control

Odour control in WWTPs involves both prevention of odorant formation and emission, impact minimization and “end of the pipe” odour-abatement technologies. Any assessment of odour control techniques must consider odour prevention before impact minimization or treatment, since it often constitutes a more simple and economical alternative than odour containment and treatment.

1.3.1 Odour Prevention and Impact Minimization

Odour prevention involves a combination of both efficient design and operation practices and odour containment via installation of process covers. In this context, prevention of odour formation in sewers is crucial since a large number of odours that are emitted from WWTPs are associated to the formation of septic wastewaters in sewer networks. Although physical modifications in the network to reduce malodorous emissions would require a significant capital investment, the proper design of gravity sewers is among the most common approaches to minimize odour formation in receiving wastewaters. Moreover, *in-situ* control technologies can be applied to minimize the formation of malodours by preventing the production of sulfides (through addition of NO_3^-, O_2, ozone to the wastewater) or their stripping from the wastewater by precipitation with ferric salts [25-27].

In WWTPs, prevention of odour formation mainly involves good operation practices such as the frequent cleaning of grit chambers or screening units, minimization of sludge retention time in thickeners or dewatering units, operation under adequate aeration and mixing rates, etc. Reductions in odour emissions can also be achieved by a correct plant design, minimizing heights in weirs and by process covering. Process covering not only allows for prevention in odour emissions but also facilitates their subsequent treatment, which is particularly relevant in passive sources such as primary settlers or sludge thickeners. However, and despite the broad range of measures available for odour emission minimization, a complete reduction of odorant formation and release is not always possible.

In specific areas such as diffused emissions or sources with highly variable odour flow rates, the implementation of end-of-the-pipe odour abatement techniques might be technically complex, besides being limited by the high investment and operational costs. A temporary alternative to minimize odour impact in such scenarios consists on the use of turbulence inducing structures and facilities, buffer zones or masking/neutralizing agents. While passive impact reduction by high barrier fences, trees or buffer zones is based on a dispersion-enhanced odour dilution, nuisance reduction by masking or neutralizing agents relies on odour superimposing or neutralization by chemical reactions. These air spray or surface application products are masking agents, counteractants, neutralizers and surfactant enhanced absorption agents, mainly composed of terpenes, aliphatic and aromatic hydrocarbons, aldehydes and alcohols. However, little is known concerning the real efficiency and mechanisms

of action of the available commercial products. As a matter of fact, recent studies have shown that most of them do not possess the claimed odour neutralizing potential or even increase the odour concentration in the emission when applied [28-30].

1.3.2 Odour Treatment Technologies

When prevention of odorant emissions or impact minimization is not sufficient to mitigate odour nuisance, odour abatement technologies must be applied. The selection of the most cost-effective end-of-the-pipe treatment technology for each specific odour contamination case is determined by the nature and concentration of the odorants, the level of efficiency required, the type of odour sources (active or passive), the air flow rates to be treated, etc. Based on the mechanisms underlying odour removal, two major treatment methods, physical-chemical and biological, are distinguished.

1.3.2.1 Physical-Chemical Methods

- ***Chemical Scrubbers***

Chemical scrubbers are among the most commonly employed abatement techniques in WWTPs mainly due to the extensive experience in their design and operation, their short gas retention time (as low as 1-2.5 s), low pressure drops and high robustness when properly operated [30-31]. Odorants are transferred from the malodorous air into an aqueous solution (often sulfuric acid or sodium hydroxide depending on the target odorants) where they are destroyed by chemical oxidation. Chemical scrubbers are implemented in packed towers (either in counter current or cross flow configurations) or atomized mist absorbers, using sodium hydroxide, potassium permanganate or hydrogen peroxide as the oxidant (Figure 1.3). The bed height ranges between 1 and 1.5 m, with pressure losses from 100 to 400 Pa m^{-3}_{bed} and typical recirculation rates ranging from 150 to 170 L min^{-1} $(m^3h^{-1})^{-1}_{treated}$ [30].

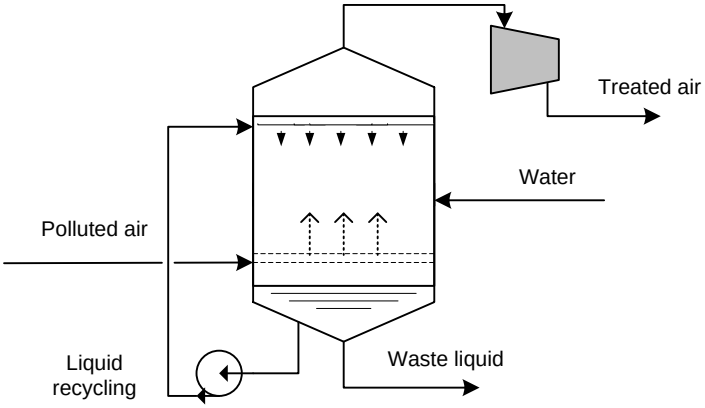

Figure 1.3. Physical-chemical technologies for odour abatement: chemical scrubber

Removal efficiencies of up to 98% for H_2S and 90% for mercaptans are commonly achieved under good operation practices, although more specific data on the abatement of other odorants are needed. Moreover, chemical scrubbing is only cost-effective for the treatment of low odorant and VOC concentrations since high odour concentrations result in excessive chemical requirements (Table 1.4) [30, 32-33].

Table 1.4 Oxidants commonly used in chemical scrubbers [30]

Oxidant	Reaction	mg L^{-1} oxidant/ mg L^{-1} H_2S
NaOCl	$H_2S + 4NaOCl + NaOH \rightarrow Na_2SO_4 + 2H_2O + 4NaCl$ $H_2S + NaOCl \rightarrow S^o + NaCl + H_2O$	8-10
$KMnO_4$	$3H_2S + 2KMnO_4 \rightarrow 3S + 2KOH + 2MnO_2 + 2H_2O$ (acid pH) $3H_2S + 8KMnO_4 \rightarrow 3K_2SO4 + 2KOH + 8MnO_2 + 2H_2O$ (basic pH)	6-7
H_2O_2	$H_2S + H_2O_2 \rightarrow S^o\downarrow + 2H_2O$ (pH<8.5)	1-4

- ***Adsorption***

The efficiency and experience acquired in the off-gas treatment of industrial effluents has also contributed to the wide implementation of activated carbon, silica gel or zeolite-based odour adsorption systems in WWTPs. In this process, odorants are adsorbed onto a fixed bed of adsorbent by intermolecular forces [30, 34] (Figure 1.4). This process is especially convenient for the mitigation of odour impact when hydrocarbons, mercaptans or other oxygenated organic compounds rather than H_2S are the main odorants in WWTP emissions. Adsorption systems are operated at empty bed residence times (EBRTs) ranging from 2 to 3 s, with common pressure drops of 1400-1750 Pa. Carbon lifespan varies widely depending on the adsorbent and the characteristics of the emission, but usually ranges between 3 and 9 months [35].

Among the above mentioned adsorbents, activated carbon obtained by carbonization followed by activation at high temperature of organic precursors (anthracite, coal, wood, etc.) is the most widely used option [36]. Recent advances in the field of catalysis have also resulted in the development of a new generation of high-performance sludge-based activated carbons capable of adsorbing up to 10 times more H_2S and NH_3 than commercially available activated carbons [37]. Adsorption-based systems provide an excellent performance for the treatment of highly hydrophobic odorants, with typical odour and VOCs removals from 90 to 99%.

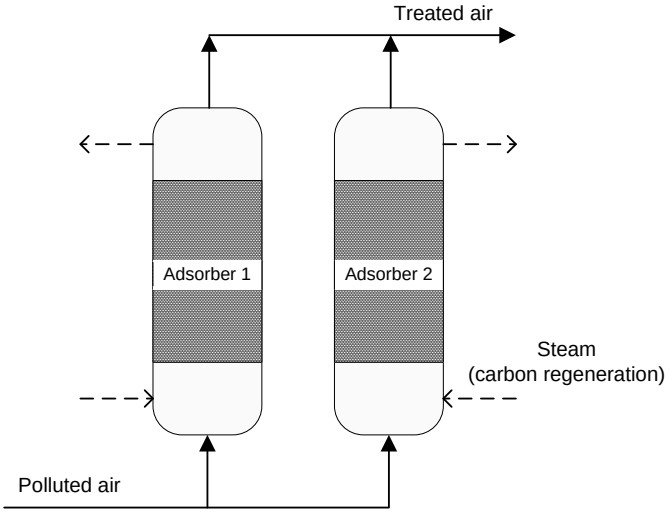

Figure 1.4. Physical-chemical technologies for odour abatement: activated carbon adsorption

- ***Incineration***

Incineration consist of the complete oxidation of the odorous compounds at high temperatures using an additional source of heat (natural gas or oil are commonly used as fuels) since the combustion process cannot be self-maintained by the low odorant concentrations present in the emissions from WWTPs. The air emission must be heated at temperatures up to 800ºC for 3 to 4 s. Typical pressure drops vary from 1000 to 1200 Pa. The high cost and availability of energy, coupled with the low odorant concentrations typical in odorous emissions from WWTP, make thermal incineration an inappropriate technique for odour treatment from an economic and environmental viewpoint. However, it is still employed in landfill or industrial applications due to the high odorant removal efficiencies (99.9%) [35].

In catalytic oxidation, the odorous compounds are completely oxidized at moderate temperatures (which depend on the nature of the compound) and pressures in the presence of a solid catalyst (e.g. platinum or palladium catalysts). Compared to standard incineration, catalytic incineration requires lower temperatures, thus reducing

the quantity of fuel needed (and therefore the environmental impact) and the costs of the construction materials. However, due to the complexity of odorous emissions, a complete oxidation usually involves an excess of oxygen and a combination of several catalysts to achieve acceptable odour removal efficiencies. On the other hand, some odorous compounds such as sulfur derivates can poison the catalyst, thus reducing its lifetime [38].

- ***Photolysis***

Photolysis is based on the transfer of electromagnetic energy (UV radiation) from a source of radicals to the odorous compounds being oxidized. During photolysis, the odorant is disintegrated by UV light releasing radicals (e.g. the breakdown of the S-H bound in sulfur compounds), which then react with oxygen or other oxidants (O_3, H_2O_2, OH, O^-) [34].

$$H_2S + hv \rightarrow HS^{\bullet} + H^{\bullet}$$
$$CH_3SH + hv \rightarrow CH_3S^{\bullet} + H^{\bullet}$$

This method can be employed directly to the air emission without any preliminary transfer of the odorant to a secondary phase since radiation is able to deeply penetrate the gas stream. In the particular case of photocatalysis, the UV light is combined with a semiconductor and a photo-catalyst (TiO_2, CdS), which allows the oxidation of both organic and inorganic compounds. The mechanism is based on the excitation of the electrons of the semi-conductor material, inducing areas with both an excess (reducing area) and a deficit of electrons (oxidizing area) [38].

- ***Ozonization***

Ozone is commonly used as an oxidant in the aqueous phase due to its low oxidation kinetics in the gas phase (which requires its combination with UV radiation). Hence, malodorous compounds (H_2S or COVs) must be firstly transferred to the liquid phase in a scrubber column and subsequently oxidized either by ozone molecules or by ozone radicals:

$$HS^- + O_3 \rightarrow S^o + OH^- + O_2$$
$$HS^- + 4O_3 \rightarrow SO_4^{2-} + 4O_2 + H^+$$

Radical concentration depends on the pH, the presence of neutralizers such as CO_3^{2-} and the concentration of the odorous compound in the aqueous phase. Amines, phenols and alkenes exhibit the higher oxidation rates. However, ozonization requires the in situ production of ozone due to its high instability, turning it into a highly energetic and inefficient process. Although odour concentration is significantly reduced at very short residence times (few seconds), many authors support that oxidation is not complete and an odour masking effect is produced instead. Besides, the high costs and the difficult control of ozone in-situ generation reduce the applicability of ozonization [30, 38].

1.3.2.2 Biological Treatment

Since the late 1970s, the extensive research carried out in the microbiology of VOC biodegradation has demonstrated that most odorant are biodegradable to some extent by bacteria, fungi, yeasts and even microalgae, and therefore subjected to removal in bioreactors. Physical-chemical treatment systems are been gradually replaced by biological-based odour technologies such as biofilters, biotrickling filters and bioscrubbers due to their higher cost-effectiveness and environmental friendliness. This is derived from the reduced energetic and/or chemical requirements and the absence of expensive adsorbent materials. Biotechnologies also have a more environmentally friendly profile since pollutants are finally converted into innocuous compounds such as CO_2, H_2O, and biomass at ambient pressure and temperature [39-40].

Microbiological fundamentals of biological oxidation

The biodegradation of odorants is based on their use as carbon and/or energy source by the microorganisms present in the bioreactor. On one side, VOCs such as terpenes, aldehydes or hydrocarbons are used as a carbon source to produce new biomass (maintenance and cellular reproduction) and as a energy source to support microbial metabolism (by oxidation to H_2O and CO_2 using as electron acceptor the oxygen from the odorous emission). On the other side, odorants such as H_2S or NH_3 are used by the microbial community as an energy source (by oxidation to sulfate and nitrate, respectively) for maintenance and/or cellular growth based on the assimilation of inorganic carbon from the media. The energy produced in those oxidation processes is either used by microorganisms immediately or stored as high-energy phosphate bonds (mainly as ATP or polyphosphates). The ability of microorganisms to use the

odorants as raw material for their growth and maintenance makes possible their biological removal from wastewater and composting plants' emissions. The presence of an aqueous media to sustain all the metabolic reactions, together with the availability of dissolved macronutrients (nitrogen, phosphorous, sulfur, potassium, etc.) and micronutrients (usually heavy metals for enzyme synthesis) is mandatory in biodegradation processes.

Bacteria and fungi are the main groups of microorganisms responsible for odorant biodegradation in most biological techniques (Figure 1.5). Bacteria present high growth and biodegradation rates, a high resistance to toxicity and are able to degrade a wide spectrum of gaseous pollutants. However, their optimum pH is near neutrality and they require high water activity. Fungi, on the other side, possess a narrower spectrum of biodegradable odorants (likely due to the fact that they have been less studied), but tolerate a low pH (2-5), low humidity and nutrients limitation, conditions typically found in biological systems (biofilters and biotrickling filters). Besides, fungi are able to synthesize proteins (namely hydrophobins) that facilitate the direct transport of hydrophobic odorants from the gas phase to the fungi hyphae.

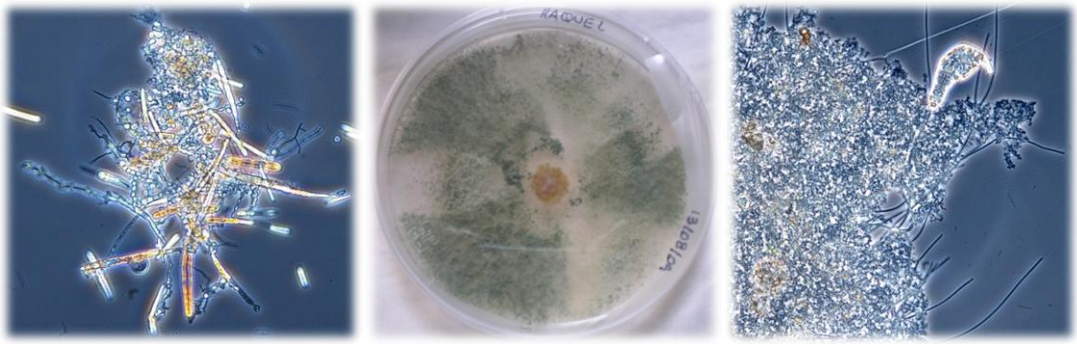

Figure 1.5. Bacteria and fungi growth in bioreactors

Both oxygen and odorants are transferred from the gas to the aqueous phase containing the microorganisms (both in suspension or in a biofilm), and diffuse through the cell walls and/or membranes to be biodegraded inside the cells (Figure 1.6). This biodegradation is carried out in a series of catabolic reactions, globally called metabolic routes, which results in the decomposition of the odorants in metabolites belonging to the central routes of the microbial metabolism. The metabolic routes for odorant biodegradation depend on both the type of contaminant to be degraded and the type of microorganism.

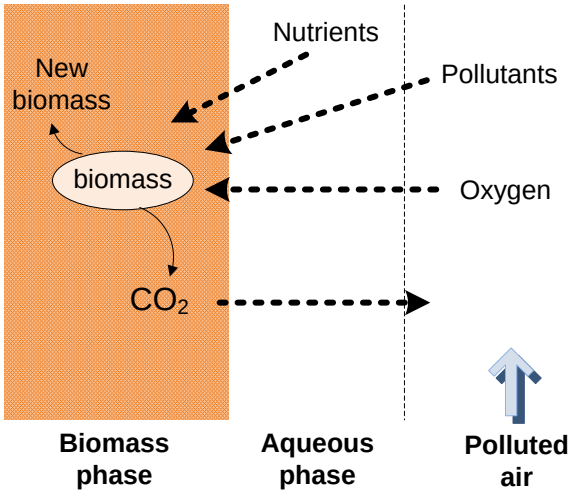

Figure 1.6. Odorant biodegradation mechanisms in bioreactors

An odorant can be biodegraded through mineralization, partial oxidation or co-metabolic degradation. Thus, while odorant mineralization is the optimum scenario (transformation of C into CO_2 and biomass, N into NO_3^-, H into H_2O, S into SO_4^{-2} and chloride to HCl), a partial oxidation entails the generation of a by-product waste stream to be treated (formation of acetone from isopropanol, catechol from benzene, etc). However, partial oxidation is more common under high pollutant concentrations, situation hardly found in odour treatment. Co-metabolic pollutant degradation is typical in the biodegradation of very recalcitrant compounds (usually branched compounds or very stable ring structures), unable to induce the synthesis of the enzymes needed for their destruction. The degradation of such compounds occurs by enzymes generated to degrade a secondary carbon source present in the media (monooxygenases and dioxygenases). As a rule of thumb, the biodegradability of a compound is related with its structure: lineal, short chain compounds such as alcohols, aldehydes, ketones, esters, organic acids or amines are considered easily biodegradable, while hydrocarbons and phenolic compounds support lower biodegradation rates due to their higher stability. Finally, halogenated compounds and polycyclic hydrocarbons are considered extremely recalcitrant due to their high resistance to microbial degradation.

The distribution of carbon fluxes during odorant biodegradation is determined by the thermodynamics of the biodegradation process, which is related with the pollutant structure (bonds stability). In the case of pollutants moderately stable such as toluene, approximately 50-60% of the carbon is converted into CO_2, 30-40% into biomass and 5-10% into extracellular metabolites. The biomass yield increases with the biodegradability of the compound. In the particular case of H_2S degrading bacteria, the

inorganic carbon needs are determined by the overall cellular yields (0.15-0.2 g biomass (g H_2S oxidized)$^{-1}$ [41].

Physical fundamentals of biological odorant oxidation

The transport of odorants from the air emission to the aqueous phase constitutes the first step in any biological odour abatement process. In general, the volumetric odorant flowrate $\left[N_{g/l} = kg\ m^{-3}\ h^{-1}\right]$ from the gas to the aqueous phase is described according to the equation:

$$N_{g/l} = K_L^{g/l}\ a \left(\frac{C_g}{K_{g/l}} - C_l\right)$$

Where $K_L^{g/l}\ a$ (h^{-1}), C_g (g m^{-3}), C_l (g m^{-3}) and $K_{g/l}$ (dimensionless) are the volumetric mass transport coefficient gas/water, the odorant concentration in the gas phase, the odorant concentration in the aqueous phase and the odorant partition coefficient between the gas and the aqueous phase, respectively.

The volumetric transport coefficient is a function of the diffusivity of the odorant in water, the gas turbulence within the bioreactor, the type and size of the packing material in the particular case of packed towers or the bubble size in aerated sludge diffusion systems. For instance, $K_L^{g/l}\ a$ values of 700 h^{-1} and 500-1400 h^{-1} are typical in activated sludge diffusion systems at an EBRT of 30 s and in biotrickling filters at 5-10 s of EBRT, respectively [42] (**Chapters 4 and 6**). On the other hand, the partition coefficient gas-water can be estimated based on the Henry´s constant and the equation of state of ideal gas. It is also worth noting that hydrophobic odorant transport, and therefore their biodegradation, is limited by the low concentration gradients available for mass transfer from the gas phase as a consequence of their high partition coefficients (alkenes, terpenes or sulphur-derived compounds) [43].

1.3.2.2.1 Conventional biotechnologies

- ***Biofiltration***

The origin of biofiltration dates back to the early 50s, with the first successful applications and patents in the field of odour abatement reported in Germany and the United States [44]. These first biofilters were simple open-soil media systems, which were designed and operated according to empirical considerations [44]. As

environmental legislations became increasingly stringent worldwide in the 1970s, and residential and urban areas started to encroach on odour sources, the application of biofilters for odour mitigation expanded significantly [44-45]. From the 1980s, the application of biofiltration extended to the removal of volatile organic and inorganic compounds (VOCs and VICs, respectively) from industrial waste gases [44, 46]. A 3rd generation of enclosed biofilters capable of providing a more controlled environment in terms of moisture content, pH, T and metabolite accumulation was developed in the late 1990s [34]. Today, based on the extensive experience acquired in full scale facilities and the sustained research effort carried out over the last two decades, biofiltration has become a cost-effective and environmentally friendly alternative to other physical-chemical methods such as incineration, adsorption or absorption (Figure 1.7).

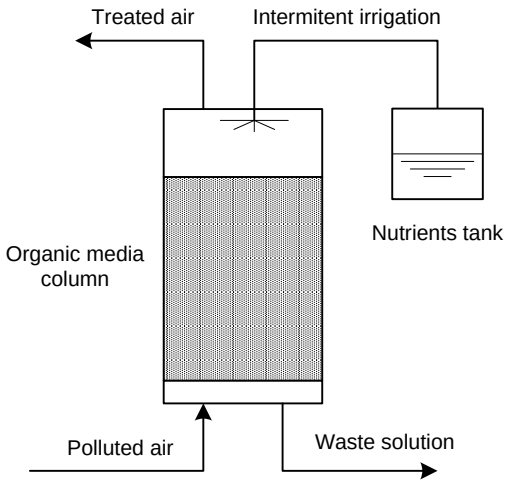

Figure 1.7. Biological treatment technologies for odour abatement: biofilter

In biofilters, the malodorous-laden air is forced through a fixed bed hosting the microbial community responsible for odorant mineralization. Odorants must first diffuse from the gas phase into the biofilm before being biodegraded. Several factors such as the gas-biofilm contact time (characterised as EBRT), the filtration packing media, moisture content and temperature must be considered when designing biofilters (Table 1.5). The optimization of these parameters provides both the optimum scenario for an efficient mass transfer and a suitable environment to sustain the activity of the microorganisms responsible for odorant mineralization.

Table 1.5 Typical parameters for biofilter design and operation [30, 47].

Parameter	Typical range
Empty bed residence time (EBRT)	15-60 s
Height	0.9-1.5 m
Volumetric gas flow rates	<200000 $m^3 h^{-1}$
Superficial gas velocity	35-400 $m^3 m^{-2} h^{-1}$
Inlet gas humidity	90%-100% RH
Water content	40%-70% RH
Temperature	15-40ºC
Inlet gas concentration	0-1000 ppm_v

Biofilter media is very often pointed out as the key design parameter in biofilters, determining the removal efficiencies, the pressure drop (and therefore the operating costs) and biofilter lifespan [48-49]. In this context, the maximum economically-tolerable pressure drop across a biofilter bed (due to compaction and thus increased energy requirements) ranges between 600 and 825 Pa m_{bed}^{-1} (6-8.4 cm H_2O m_{bed}^{-1}) [49]. Indeed, packing media replacement represents 47% of the total operation costs, while the energy consumption accounts for 18% [35]. Adequate packing materials must exhibit a high porosity (0.4-0.9) to reduce the pressure drop across the bed, a high specific surface area (300-1000 $m^2 m^{-3}$) for biomass attachment and odorant adsorption, an elevated structural strength and a low bulk density, a good moisture retention and pH buffering capacities for acidic end-products, low cost and local availability, and the ability to retain microorganisms and to provide them with a suitable growth environment. Organic media (compost, soil, peat, wood chips, bark, sludge, etc.) provide an extra C and nutrients source necessary to maintain microbial activity, likely challenged by the low C concentrations present at the biofilm-water/air interface as a result of the extremely low odorant concentrations. On the other hand, inorganic materials such as ceramic, plastics, lava rock, activated carbon, etc. provide an extra structural stability, which increases the biofilter lifespan (typically ranging from, 1 to 2 years) [50-51].

The contact time between the gaseous emission and biofilter media (EBRT) is also considered as a key operational variable determining process performance, especially when dealing with hydrophobic VOCs [52-53]. Depending on the odorant nature and concentrations, the EBRT can vary from several seconds to few minutes [53-55]. The maintenance of an adequate moisture content (40-60%), pH (~7), or the

accumulation of toxic metabolites in the filter media are also crucial issues to ensure a reliable and efficient operation.

Biofiltration is best suited for the treatment of moderate to high polluted airflow rates at low concentrations, although high VOC concentrations (up to 1000 ppm_v) and flow rates up to 170 000 m^3/h have been successfully treated [56]. Most of the laboratory scale studies reported in literature focused on the abatement of a single pollutant or simple mixtures of few selected compounds from a synthetic stream at high concentrations (in the range of g m^{-3}), conditions rarely found in real malodour air emissions. Under these "ideal" conditions, biofilters have shown high removal efficiencies for odorous sulfur compounds, amino-acids, oxygenated aliphatics, aromatics and chlorinated compounds [40, 57-60]. However, the performance of full-scale biofilters is significantly affected by the varying environmental conditions (temperature, humidity, etc.) and discontinuous pollutant supply (system maintenance or breakdowns), and the elimination of hydrophobic VOCs might be also limited by the low concentration gradients available for mass transfer. In **Chapters 1 and 2**, the abatement performance of a mixture of H_2S, butanone, toluene and alpha-pinene at trace level concentrations (mg m^{-3}) in a compost-based biofilter was evaluated under steady and transient operating conditions.

Field biofiltration studies always report high H_2S removal efficiencies, (90%-100%) and odour abatements over 80% [61-62]. However, the VOC removal performance in pilot and full scale plants varies widely from 20% to 95%, being usually lower than 90% even for easily biodegradable VOCs [49, 53, 63-64]. Moreover, the odour abatement efficiency of biofilters has been rarely assessed on an individual odorant elimination basis [65], which together with the lack of information on the dynamics of odorant formation, have hindered the proper design of biofilters. In **Chapter 5,** the dynamics of the chemical composition of the odorous emissions produced during sludge handling and the VOC abatement performance of a compost-based biofilter treating this emission under different EBRTs was evaluated.

- ***Biotrickling filtration***

During the 1990s biotrickling filtration emerged as an innovative technology for waste gas treatment, and the number of research studies and industrial applications has increased significantly over the last two decades [40]. In biotrickling filters, the odorous emissions are passed through a packed bed of microorganisms immobilized

onto a support material (usually inorganic packing such as plastics, resins, polyurethane foam (PUF), ceramics, rocks, etc.) and continuously irrigated by a recirculating nutrients-containing aqueous solution (Figure 1.8). Hence, the odorants are initially absorbed in the recycling solution and further degraded within the biofilm. The presence of this mobile liquid phase allows for a better control of the operating parameters such as pH, temperature or the concentration of toxic by-products.

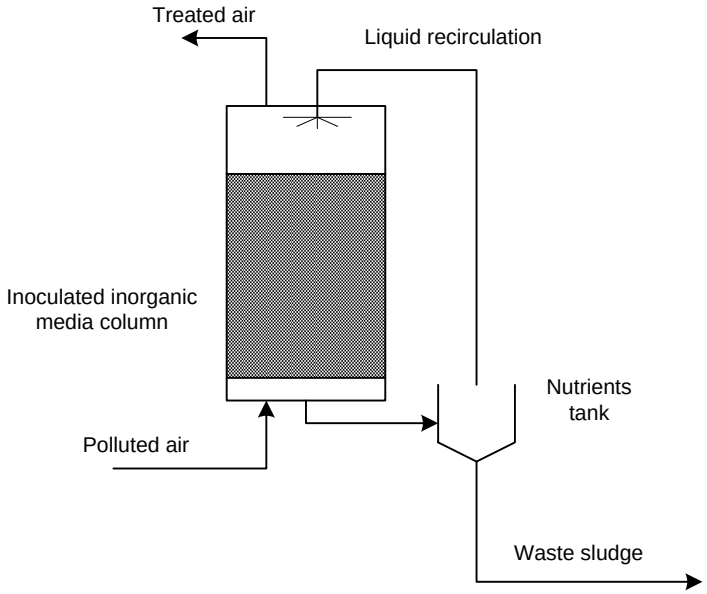

Figure 1.8. Biological treatment technologies for odour abatement: biotrickling filter

Inert packing materials usually exhibit a high porosity and specific surface areas (between 100 and 400 m^2 m^{-3}) to avoid high pressure drops (often ranging from 100-400 Pa m_{bed}^{-1}) and filter clogging, with a typical lifetimes for most conventional packing materials ranging from 8 to 10 years [34, 40].

Other key operational parameters in biotrickling filters are the gas (G) and liquid (L) flow rates and their ratio (G/L). The liquid flow rate determines the wetting of the packing material and therefore the bioreactor's performance, besides influencing the operating costs and biofilm thickness. Typical liquid recirculating rates range from 0.5 to 10 m h^{-1}. Nevertheless, some studies have shown that the influence of the liquid flow rate is not relevant when operating at the low pollutant concentrations commonly found in real odorous emissions [66]. Regarding the gas flow rate, typical EBRTs for the removal of VOCs range from 15 to 60 s, with EBRTs over 1 min needed for treating more recalcitrant compounds and lower than 5 s for the removal of easily biodegradable odorants [40, 67-68].

Overall, the results reported in literature indicated the effective co-treatment of high concentrations (in the order of g m^{-3}) of H_2S and sulfur-derived compounds and VOCs, with removal efficiencies >99% at EBRTs ranging from 35 to 120 s [54, 67, 69], whereas lower EBRTs usually support a lower abatement performance [70]. Under full scale operation, H_2S eliminations of up to 100% at EBRTs comparable to those of physical-chemical technologies have been recorded (less than 5 s) [68, 71]. The low bioreactor volume resulting from these low EBRTs offers a competitive advantage for its implementation in facilities limited by space requirements. However, the co-treatment of mixtures of odorants in biotrickling filters is still a rather unexplored area and the performance of these bioreactors under steady and fluctuating conditions needs to be systematically tested (**Chapter 6**).

- ***Bioscrubbers***

This process takes place in two separated but interconnected units: an absorption tower where a preliminary odorant transfer to an aqueous solution sprayed over the malodorous air occurs, followed by a suspended growth bioreactor where the absorbed pollutants are degraded (Figure 1.9). Being based on air-aqueous mass transport, this biotechnique presents a reasonable good performance for soluble odorants [40]: H_2S removal efficiencies of up to 99% were reported by Hansen and Rindel using a conventional bioscrubber treating the ventilation air from Damhusaaen WWTP's headworks (Denmark) [72]. Absorption tower packing materials must favour the mass transfer from the air to the aqueous phase and, at the same time, maintain a low pressure drop with gas velocities ranging between 1 and 3 m s^{-1}. Operating parameters, such as pH, temperature or nutrient concentration are easily controlled in this particular biotechnology [34].

While bioscrubbers offer a higher operational stability and a better control of the operating parameters than biofilters, they provide lower specific surface areas for gas-liquid mass transfer (thus hindering the abatement of the hydrophobic odorants). Besides, additional liquid and sludge wastes are generated [73].

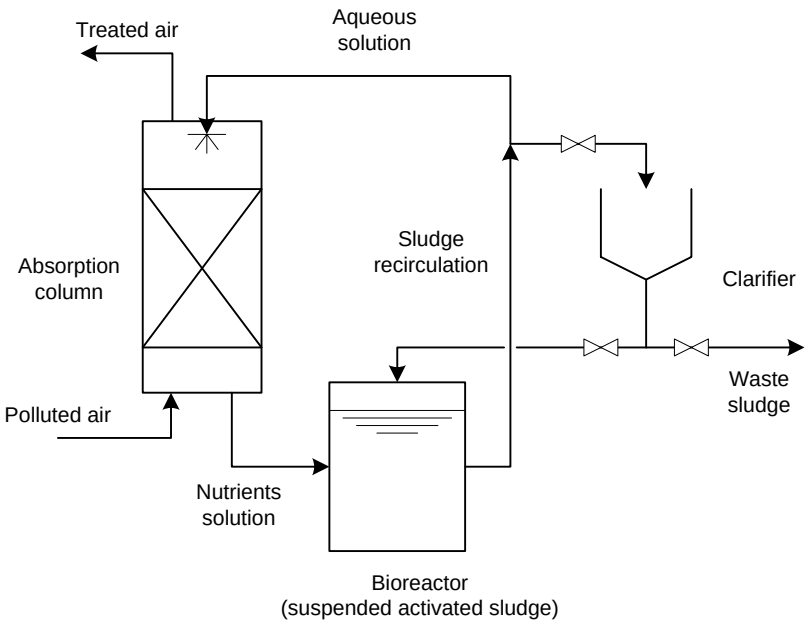

Figure 1.9. Biological treatment technologies for odour abatement: bioscrubber

- ***Activated sludge diffusion reactors***

Since the 1950's, more than 50 WWTPs have implemented activated sludge diffusion (ASD) as an odour control technique in the USA. In ASD systems the odorous air is collected from the source, transferred via blowers through a delivery pipe work system and sparged into the aeration tank through submerged nozzles. The odorants diffuse into the culture medium together with the O_2 needed for wastewater oxygenation and are subsequently biodegraded by the activated sludge community [74-75] (Figure 1.10). This biotechnique is especially advantageous in plants with diffusion-based aeration systems and space limitations, due to the usage of existing facilities which in turn involves minimal capital costs. Besides, ASD avoids typical operational problems associated to conventional odour abatement biological technologies such as media plugging, excess biomass accumulation, gas short-circuiting, moisture control or by-products accumulation and media acidification.

ASD systems have demonstrated high H_2S removals in pilot scale (96-100% at inlet concentrations between 5 and 150 ppm_v) and full-scale plants (92-99% at inlet concentrations up to 100 ppm_v), with odour detectability reductions ranging from 50 to 100% [74-76] (Ref. Bart). In spite of the advantages of ASD for odour treatment and the positive results often observed in the operating systems, the use of this technology is still hindered due to design limitations: concerns about corrosion, diffusers plugging, restrictions in the odorous air flowrates to be treated and unexpected negative effects

on wastewater treatment performance. Moreover, the performance of ASD systems has been scarcely assessed, and there is a lack of data regarding their effectiveness for odorous VOC abatement and process robustness. The removal performance of a mixture of H_2S, butanone, toluene and alpha-pinene in a laboratory-scale activated sludge diffusion system and a compost-based biofilter and their robustness towards operational fluctuations and process upsets was comparatively evaluated in **Chapters 1** and **2**.

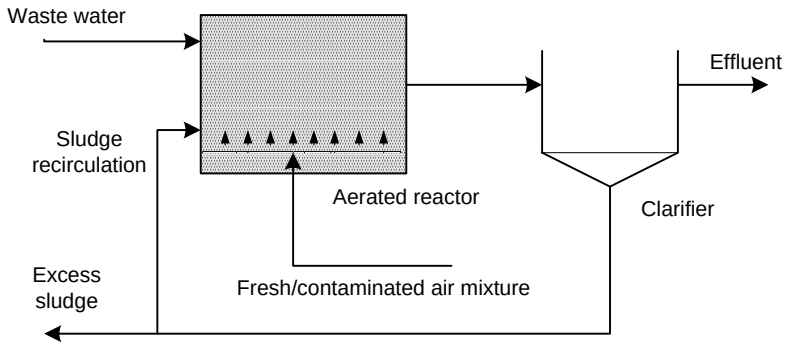

Figure 1.10. Biological treatment technologies for odour abatement: activated sludge diffusion system

1.3.2.2.2 Emerging biotechnologies

- ***Membrane bioreactors***

Advanced membrane bioreactors represent a promising alternative to conventional biotechnologies to overcome most of their limitations such as the low removal efficiencies for poorly water soluble volatile compounds, control of biomass overgrowth, media acidification, drying and/or compaction, accumulation of toxic metabolites, etc. [77]. Membrane bioreactors for waste gas treatment (MBRWG) combine the selective extraction of the target gaseous pollutants and O_2 from the contaminated air emission (circulating through one side of the membrane) with their biodegradation by a microbial community attached on the other side of the membrane (or in suspension) in contact with a discrete aqueous phase containing the nutrients required for microbial growth [77]. Hence, the membrane acts as an interphase between the air emission and the microbial community, and the gaseous pollutants either diffuse through the membrane pores (porous or microporous membranes) or permeate via solution-diffusion mechanisms (dense membranes or composite membranes) (Figure 1.11). The presence of a biofilm or a culture suspension on the other side of the membrane increases the local concentration gradients (due to the

rapid consumption of the gaseous pollutants and O_2) and therefore the overall mass transfer rates [78].

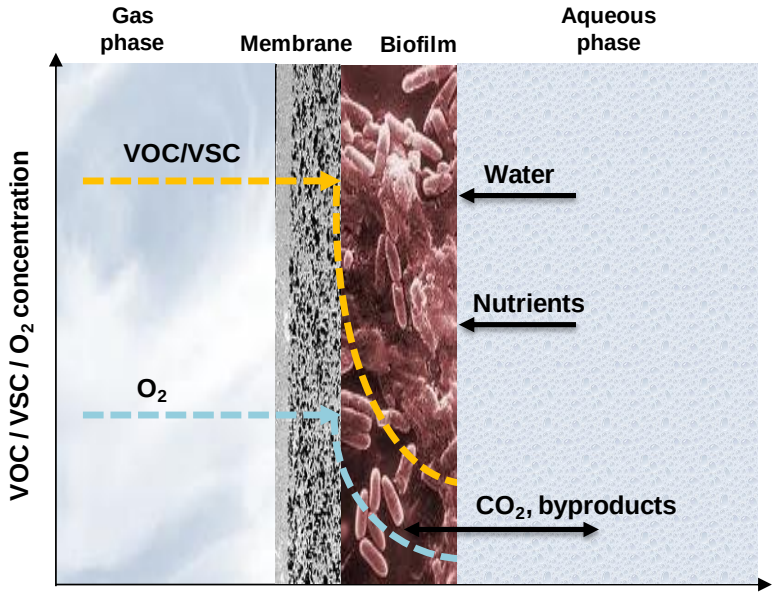

Fig. 1.11. Mass transfer mechanisms underlying VOC oxidation in gas-phase membrane bioreactors.

In addition, this technology is available in several bioreactor configurations (flat-plate, hollow fiber, tubular) and provide air-liquid interfacial areas as high as 20000 m^2 m^{-3} [79]. The presence of a discrete water phase in advanced MBRWG apart from providing the water and nutrients necessary for microbial growth, maintains a suitable pH and removes by-products. The high selectivity of some hydrophobic membrane materials such as polydimethylsiloxane or polyolefin can enhance the mass transfer of poorly water soluble compounds as a result of the increased concentration gradients mediated by these materials. In addition, the gas and liquid flow rates can be varied independently without problems of flooding or foaming [77, 80].

The first laboratory-scale studies with membrane bioreactors were reported in 1986 but, to date, no full scale investigation in membrane reactors for waste gas treatment has still been performed [79]. Laboratory studies of MBRWG have demonstrated a good performance for the biodegradation of a wide range of VOCs (toluene, propene, benzene, dimethyl sulfide, trichloroethylene, NOx, etc.) with different hydrophobicity but at high concentrations (g m^{-3}) [77]. However, research on the performance of MBRs for the removal of mixtures of VOCs is scarce, and the performance of MBRWGs at the trace level concentrations typically found in WWTPs odorous emissions has not been evaluated. In this context, the results reported in literature for MBRWGs cannot be directly applied to odour abatement. Besides, it has

been hypothesized that the low substrate concentrations in odorous emissions is one of the main limitations of MBRWGs since they might not be able to sustain an active microbial population [77]. In **Chapter 9**, the performance of a flat MBRWG for the treatment of a mixture of VOCs (acetone, toluene, limonene and hexane) at trace level concentrations was investigated. Moreover, in **Chapter 10** a comparative evaluation of a hollow-fiber MBRWG, a biofilter and a biotrickling filter for the treatment of a mixture of methyl mercaptan, toluene, alpha-pinene and limonene at concentrations ranging 1-6 mg m^{-3} was performed.

- ***Two-phase partitioning bioreactors***

Another novel approach for the abatement of the hydrophobic odorant fraction present in odorous emissions is based on the addition of a non-aqueous phase (NAP) to the bioreactor. This technology, namely two-phase partitionning bioreactors or TPPBs, has been mainly implemented in suspended growth reactors and biotrickling filters [81]. In this regard, the addition of an organic liquid phase with a high affinity for the target VOC results in higher VOC mass transfer rates as a result of the increased driving forces, and therefore in an enhanced biodegradation performance in mass transport limited scenarios. In TPPBs, the aqueous concentration remains low due to an active microbial consumption and to the high substrate affinity for the non-aqueous phase (which mediates a high abatement performance). Besides, certain microorganisms can grow inside the liquid non-aqueous phase and directly take up the pollutants without prior transfer to the aqueous phase, which dramatically increases the concentration gradients available for mass transfer and consequently the VOC abatement performance [82] (Figure 1.12).

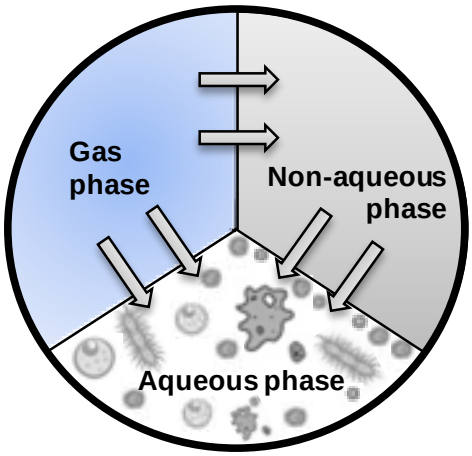

Figure 1.12. Mechanism transfer in two-phase systems with liquid NAP

Moreover, the addition of a second phase can also buffer process microbiology against VOC loading surges and starvation periods, by temporarily lowering the VOC concentration in the aqueous phase or acting as a VOC reservoir. The decrease in the aqueous VOC concentration also reduces any potential toxic effect towards the microbial community growing in suspension in the aqueous phase [40, 81].

The NAPs used in TPPBs should be inexpensive, readily available, immiscible in water, non-biodegradable, non-toxic to the microbial community and exhibit a high affinity for the target pollutants. The selection of an adequate NAP is a complex task since solvent toxicity and biodegradability are microorganism-specific, silicone being one of the few NAPs fulfilling most of the above described selection criteria [43, 81].

The successful applications of TPPBs has been recorded in bioscrubbers, biotrickling filters and stirred tank bioreactors at high inlet VOCs concentrations (0.5–20 gm^{-3}), much higher than those typically found in WWTP odorous, with most of the studies conducted with single VOC streams [43, 81, 83-85]. In addition, there is a lack of information on the long-term stability of TPPBs, which is essential to determine potential degradation or losses of the non-aqueous phase during continuous operation. In **Chapter 8**, the performance of a one- and a two-liquid phase biotrickling filters for the removal of butanone, toluene, alpha-pinene and hexane at trace level concentrations and different EBRTs was comparatively assessed. Besides, the robustness of TPPBs for odorant abatement was evaluated.

1.3.3 Modeling and design of odour control biotechniques

Predictive mathematical modeling is a useful tool to reduce the need for extensive pilot and field testing prior to scale-up. To date, biofilter and biotrickling filter modeling has been based on fundamental and general mass balance, and microbial kinetic equations, but the need for simplifying assumptions results in limitations in the applicability of such models. The major uncertainty in model development is often the determination of the model constants (diffusion, Monod and maximum growth rate, etc.) and the characterization of the packing properties (biofilm distribution on the media surface, pores geometry, etc.) [66, 86-87]. Although significant progress has been made in the field of biofilter modeling, there is still no single model accepted as a standard for biofiltration [87]. Indeed, the fact that most models are finally based on the fitting of all unknown parameters, their mathematical complexity is high or their specific applicability is often limited to very specific experimental conditions, model-based

design is still not regarded as sufficiently reliable, and instead, experience-based guidelines and pilot-testing are employed [87].

Current biofilter and biotrickling filter design and operational practices are based on the experience gained over the years. The few guidelines available today are still based on typical gas retention times and sulfur loadings, the fundamental know-how on odorant mass transfer, diffusion or biological reaction being rarely applied [30]. During biofilter design, it is a common practice to design at low EBRTs when treating highly hydrophilic odorants (H_2S or NH_3), thus neglecting the mass transfer resistance from the gas phase to the biofilm [64]. However, when H_2S or NH_3 are not the main surrogate in the odorous emission it is recommended to focus the design and operation on the removal of VOCs as the rate-limiting parameter to achieve an effective odour abatement [53]. If transport is limiting (either from the gas to the biotic phase or through the biofilm), the EBRT must be longer than that suggested by simply considering the biological degradation rate [64]. In this scenario of mass transfer limitations, the design and scale-up of bioreactors should be based on mass transfer data, since this is the rate limiting step in the odour abatement process. In **Chapter 7**, a simple and reliable mathematical model able to characterize the mass transfer in a biotrickling filter operated under typical VOC treatment conditions was developed. The model did not require any particular assumption, data linearization or complex mathematical resolution as previously reported models [66, 88-90] and accurately described the VOC concentration profile in the gas and liquid phases.

1.3.4 Comparative analysis of odour abatement technologies

Technologies based on physical-chemical principles, such as chemical scrubbing or activated carbon filtration, are reliable and well established techniques. Chemical scrubbing constitutes nowadays the most commonly implemented technology in the odour control market due to its high performance, low cost compared to their physical-chemical partners and extensive design guidelines [31-33]. Despite the high removal efficiencies achieved for H_2S, chemical scrubbers present serious limitations in the elimination of the hydrophobic fraction of the odorous emissions (VOCs with high Henry's law constants) as they are finally based on odorant transfer to an aqueous solution of oxidant [52]. In addition, the high chemical requirements and the hazardous nature of the chemical reagents employed and the by-products generated, represent a serious challenge to its supremacy in a world increasingly concerned about sustainable development ([30-31, 33, 35]. Investment and operating

cost ranging from 3-12 € (m^3 $h^{-1})^{-1}$ and 0.27 € (1000 $m^3_{treated})^{-1}$, respectively, are commonly reported for such odour abatement systems [31, 35].

Activated carbon filtration provides a high VOC removal performance (90-99%), however its efficiency is severely limited by the high moisture content prevailing in WWTPs malodorous emissions and by the high costs of the adsorbents [52]. Moderate investment cost (4-14 € (m^3 $h^{-1})^{-1}$) and high operating costs (0.45 € (1000 $m^3_{treated})^{-1}$) derived from the short lifespan of the packing material and the needs for specific management procedures (regeneration at high temperatures or disposal as hazardous waste) are characteristic of adsorbent-based technologies [30] [35]. The efficiency of activated carbon filtration is compound specific and must be tested before use.

Despite their high odour removal efficiencies (98-99.5%), incineration technologies do not represent an attractive odour abatement alternative due to its high investment and operating costs (up to 350 and 120 € (m^3 $h^{-1})^{-1}$, respectively), the potential production of toxic dioxins and furans and its high CO_2 footprint [52]. Moreover, this technology is not well socially accepted nowadays.

The high energy requirements and costs involved during the operation and construction of the above discussed physical-chemical technologies have resulted in the development of more cost-effective and environmentally friendly alternatives for odour abatement in WWTPs. Recent studies confirmed the technical viability of biological processes for odour treatment in WWTPs and therefore the reliable replacement of existing physical-chemical technologies by this low-cost, high performance biotechniques [53, 68]. In this context, it must be highlighted that the commonly reported limitations for biological systems such as microbial activity deterioration under high H_2S concentrations (>50 ppm), the large space requirements, VOC mass transfer limitations or poor efficiencies at intermittent odorous emissions can be easily overcome by the use of acclimated biomass and the selection of the most appropriate bioreactor configuration in each specific scenario [34, 43, 52]. The low robustness of biological technologies for odour treatment has been also pointed out as one of their major drawbacks compared with their physical-chemical counterparts. However, recent studies at both laboratory and full-scale with individual pollutants (H_2S, toluene, CS_2, etc.) at high concentrations have demonstrated that biological systems are able to rapidly recover from process fluctuations and/or operational upsets [91-94]. The robustness of an activated sludge system, a biofilter, a biotrickling filter and a two-phase biotrickling filter for odour treatment was evaluated in **Chapters 2 and 8**, and the ability of biotechnologies to withstand surges in odorant concentration, temperature

fluctuations, starvation and shut-down periods, liquid recycling stoppages, etc. was demonstrated.

Among the available biotechnologies for odour abatement, biofilters are the preferred option due to its satisfactory performance both in H_2S and VOC treatment, reasonable investments cost (5-28 € $(m^3\ h^{-1})^{-1}$) and low operating costs (0.21 € (1000 $m^3_{treated})^{-1}$) [35]. Due to the absence of a continuous water layer covering the biofilm, biofilters are capable of providing higher abatement efficiencies for the hydrophobic fraction of the odorous emissions than their biological counterparts (biotrickling filters and biological scrubbers). The main limitations of biofilters in WWTPs are however the requirement for large areas (imposed by the relative high EBRTs and air pressure drops) and the operational problems derived from media structural stability and bad operational practices.

The use of the existing aeration tank as odour-abatement unit results economically attractive in WWTPs where diffused aeration is already practiced and space availability constitutes the main limitation. Full scale applications have demonstrated high odour and VOC removal performances, being especially effective for treatment of moderate to high strength odours.

Bioscrubbers allow higher air loading rates than biofilters, reducing the required space while ensuring high elimination efficiencies for H_2S and soluble VOCs [72]. Besides, the physical separation between odorant absorption and treatment avoids problems such as bed drying, acidification, clogging or toxic by-products accumulation. However, poor odour abatement efficiencies are observed when malodours are mainly composed of poorly soluble VOCs. This factor, together with the high investment costs (23-92 € $(m^3\ h^{-1})^{-1}$ without auxiliary equipment) derived from its complex configuration, have restricted its widespread implementation [72].

When H_2S concentrations are high or when low residence times (low unit footprint) are required, biotrickling filters constitute the best treatment option. They present low pressure drops as a result of their structured packing material and reasonable investment costs (10-41 € $(m^3\ h^{-1})^{-1}$). They also exhibit the lowest operating costs among the available technologies for odor treatment (0.11 € (1000 $m^3_{treated})^{-1}$) mainly because of the higher lifespan of its packing material, lower liquid recirculation rates compared to those of chemical scrubbers, and absence of chemical requirements [35]. Overall, the removal of an odorant different from the surrogate H_2S must be tested since parameters such as the recirculation flow rate (which increases both air pressure drop and operational costs) and odorant hydrophobicity can severely limit biotrickling

filter performance [62]. In this context the addition of an organic liquid phase into the biological process has been shown to enhance the mass transfer of hydrophobic VOCs ([43].

In brief, a state-of-the-art odour management strategy must involve a preliminary evaluation of the potential odour prevention and impact minimization alternatives followed by a detailed characterization of the malodorous emission, which is crucial for a successful design and operation of the dedicated odour control units. Nowadays, biological treatment processes have become a real technological alternative to conventional physical-chemical processes, both in terms of odour abatement efficiencies and process economics. The design of cost-efficient abatement processes targeting hydrophobic odorants and the development of innovative odour control strategies in sewer networks constitute the two major challenges to be faced by environmental engineers in the next decade.

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Chapter 2

Scope and Extent of the Thesis

2.1 Justification of the thesis

The environmental and health impact of odorous emissions from WWTPs has resulted in the last decades in an increasing number of public complaints and the enforcement of stricter environmental legislations. Therefore, malodours have become a major concern for wastewater treatment plant operators due to the increasing negative public image of WWTPs. Biological technologies have emerged in the last two decades as a cost-effective solution for odour abatement, with elimination efficiencies comparable to those provided by their physical-chemical counterparts, lower operational costs and a more environmentally-friendly profile. However, despite the merits of biological techniques and the large number of technological advances in the field of biological odour abatement, they still face some important limitations such as a poor deodorization efficiency when the odorous emission contains hydrophobic odorants with low odour thresholds, the high space requirements of biofilters (particularly relevant in WWTPs with low space availability), or the lack of studies under realistic operating conditions (i.e. low odorant concentrations in the range of μg m^{-3}, mixtures of volatile sulfur and organic compounds, non-steady operation, etc.). An improved odorant mass transport from the air emission to the aqueous phase containing the degrading microbial community will result in lower gas residence times, and therefore to lower space requirements, and higher abatement efficiencies of the hydrophobic odorant fraction. In summary, there is a need for the development of low-cost, compact bioreactors devoted to overcome the above mentioned limitations.

2.2 Main objectives

The overall objective of this thesis was the comparative evaluation of the performance of several biotechnologies for the abatement of odorants of different nature and hydrophobicity, assessing the influence of the key design and operating parameters on the abatement efficiency, process stability and microbial dynamics. More specifically, the objectives were:

1. Study of the influence of the empty bed residence time on the abatement performance of different biotechnologies (biofilter, biotrickling filter, activated sludge diffusion system, membrane bioreactor and two-phase partitioning biotrickling filters) treating synthetic mixtures of odorants at trace level concentrations and real odorous emissions.

2. Evaluation of process performance (robustness) under process fluctuations and operational upsets (surges in odorant inlet concentration, process shut-down, starvation periods, failure in the irrigation systems, variation in temperature and pH, etc.).
3. Assessment of the influence of the addition of a non-aqueous phase on the VOC abatement capacity of biotrickling filters.
4. Determination of the mass transfer potential of an activated sludge diffusion system, a biotrickling filter and a membrane bioreactor.
5. Development of a mathematical model based on VOC mass transport in biotrickling filters under transport limiting conditions.
6. Analysis of the dynamics of the microbial populations and its potential correlation to the macroscopic odorant abatement.

2.3 Experimental development

This thesis focused on the systematic evaluation of both conventional and innovative biotechnologies for the treatment of odorous emissions from WWTP. H_2S and methyl mercaptan were selected as model sulfur-derived compounds at concentrations ranging from 5 to 45 mg m^{-3}. High removal efficiencies are commonly observed for these soluble odorants, which are responsible for media and/or aqueous phase acidification. Acetone or butanone, toluene, alpha-pinene or limonene and hexane were chosen as model ketones, aromatic hydrocarbons and terpenes commonly present in odorous emissions from WWTPs, exhibiting a wide range of hydrophobicity. The concentration of these volatile organic compounds in the synthetic odorous emission was in the mg m^{-3} level.

For the achievement of the first objective, several biotechnologies were evaluated: a biofilter and an activated sludge diffusion system for the treatment of H_2S, butanone, toluene and alpha-pinene (**Chapter 3**, Article I), a biotrickling filter for methyl mercaptan, toluene, alpha-pinene and hexane removal (**Chapter 6**, Article IV), a flat membrane bioreactor fed with a mixture of volatile organic compounds (acetone, toluene, limonene and hexane) (**Chapter 9**, Article VI) and a biofilter, a biotrickling filter and a hollow fiber membrane bioreactor for the treatment of methyl mercaptan, toluene, alpha-pinene and hexane (**Chapter 10**, Article VIII). **Chapter 5** aimed at studying the abatement efficiency of a compost-based biofilter fed with a real odorous emission from wastewater treatment plant sludge (Article III).

The second objective was accomplished in **Chapters 4 and 8** where the ability of a biofilter and an activated sludge diffusion system (Article II), and of a conventional and a two-phase biotrickling filter (Article VII) to cope with common process fluctuations and operational upsets was evaluated. **Chapter 8** was devoted to fulfill the third objective (Article VI) by studying the influence of a non-aqueous phase (i.e. silicone oil) on the steady and transient performance of a biotrickling filter treating butanone, toluene, alpha-pinene and hexane. The evaluation of the mass transfer potential of some biotechnologies (objective 4) was performed in **Chapters 4 and 6** (Articles II and IV), where the volumetric mass transfer coefficients of an activated sludge diffusion system and a biotrickling filter were evaluated at different EBRTs. Moreover, a simple, universal mathematical model for mass transfer characterization in biotrickling filters was developed in **Chapter 7** (Article V), using toluene as a model VOC.

Finally, the structure (evenness and richness) and/or the composition of the microbial communities present in the bioreactors (objective 6) was investigated by denaturing gradient gel electrophoresis (DGGE) of 16S rRNA gene fragments (**Chapters 3, 4, 6, 8 and 9**, corresponding to Articles I, II, IV, VI and VII).

Lebrero R, Rodriguez EM, García-Encina PA, Muñoz R (2011)
Journal of Hazardous Materials 190: 622-630

Chapter 3

A comparative assessment of biofiltration and activated sludge diffusion for odour abatement

Journal of Hazardous Materials 190 (2011) 622–630

Contents lists available at ScienceDirect

Journal of Hazardous Materials

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A comparative assessment of biofiltration and activated sludge diffusion for odour abatement

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ARTICLE INFO

Article history:
Received 10 January 2011
Received in revised form 21 February 2011
Accepted 24 March 2011
Available online 31 March 2011

Keywords:
Activated sludge diffusion system
Biofiltration
Hydrophobicity
Odour treatment
VOC biodegradation

ABSTRACT

The deodorization performance of a biofilter and an activated sludge diffusion (AS) system was comparatively evaluated in terms of removal efficiency (RE) and process stability at empty bed residence times (EBRT) ranging from 94 to 32 s. Both bioreactors were fed with a synthetic odorous emission containing H_2S, butanone and toluene at 23.6–43.3, 4.3–6.3 and 0.4–0.6 mg m^{-3}, respectively. While the outlet H_2S concentration was always lower than 1.4 mg m^{-3}, the REs for butanone and toluene remained higher than 95% in both bioreactors regardless of the EBRT. The continuous supply of wastewater in the AS unit did not affect removal and appeared to be a requirement for efficient pollutant abatement. Despite the narrow carbon source spectrum treated, the AS system maintained a large bacterial diversity over time. Therefore, the results obtained confirmed the potential of AS systems as a robust and efficient biotechnology for odour treatment in WWTPs.

1. Introduction

Emissions of malodorous gases from Wastewater Treatment Plants (WWTPs) have a negative impact on the nearby local population. The increasing number of malodours-related complaints and the recent enforcement of stricter environmental regulations have ranked minimization and abatement of malodorous emissions among the top priorities in the design and operation of WWTP utilities worldwide [1,2]. The key relevance of the problem, both in terms of compliance to regulations and good public image, initially triggered the implementation of physical/chemical off-gas abatement technologies such as chemical scrubbing, activated carbon filtration and incineration. However, WWTP operators became rapidly aware of the merits of biological treatment processes. Nowadays, biological technologies are the preferred option due to their high efficiency, lower operating costs and absence of hazardous end-products [3,4].

Biofiltration is indisputably the most commonly employed biotechnology for odour treatment in WWTPs [5,6]. In biofilters, the odorous emission is forced through an organic/inorganic packed bed supporting the microbial community responsible for odorant removal. Despite their cost-effectiveness, the widespread implementation of biofilters is often restricted by their large footprint (high empty bed residence times, EBRT, and low packed media heights in order to minimize pressure drops) and by the gradual compaction of packing media. In addition, the technical difficulties to control key parameters such as pH and moisture content within the packed bed, and to avoid the accumulation of inhibitory by-products can also limit biofilter performance [7].

In this context, activated sludge diffusion (AS) system represents a cost-effective alternative to media-based odour treatment bioreactors. In AS systems, the malodorous emission is directly sparged into the aeration tank as the air needed to satisfy the biological oxygen demand of the wastewater [8]. Odorants diffuse into the mixed liquor together with O_2, being subsequently degraded by the AS community [3,9]. AS systems possess all merits of their biological counterparts (environmental friendliness, low operating cost) while overcoming most of their major limitations (packing media compaction, moisture control or accumulation of toxic metabolites in biofiltration, etc.). In addition, the use of the existing aeration tank as odour-abatement unit renders them economically attractive in plants with land limitations. Despite AS systems have been used for over 30 years with high H_2S removal efficiencies (REs), their widespread implementation is still limited by the lack of reliable data concerning its performance during the treatment of odorous volatile organic compounds (VOCs) [9–11].

This work was conducted to systematically compare the performance of a conventional biofilter and an AS system for the treatment of a model WWTP malodorous emission containing four representative odorants with a large range of hydrophobicities. Butanone, toluene, and α-pinene were selected as model VOC odorants representing soluble, moderately soluble and hydrophobic VOCs associated to WWTP emissions [12]. Likewise, H_2S was also selected as model sulphur odorant for being widely present in

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doi:10.1016/j.jhazmat.2011.03.090

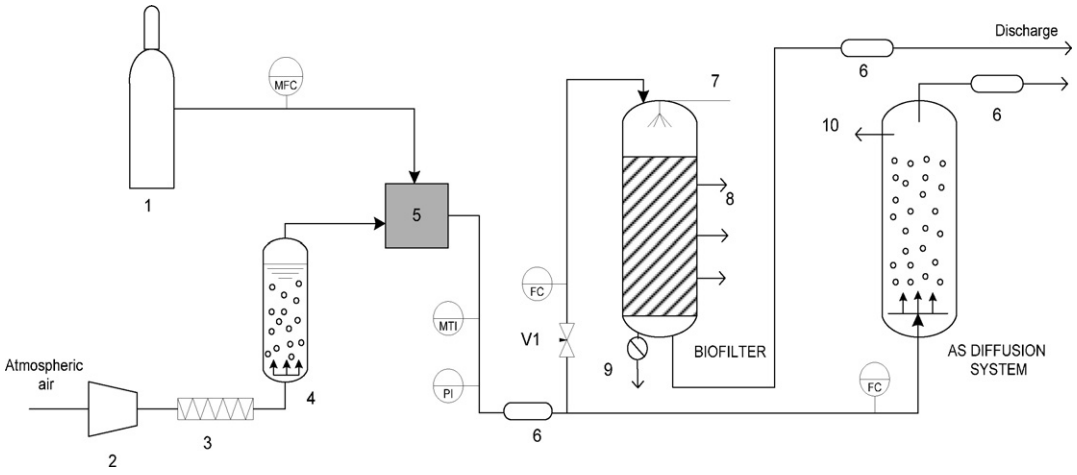

Fig. 1. Schematic representation of the experimental setup. (1) H_2S and VOC reservoir, (2) Compressor, (3) Activated carbon filter bed, (4) Humidifier, (5) Mixing chamber, (6) Gas sampling bulb, (7) Irrigation system, (8) Gas sampling ports, (9) Leachate port, (10) Liquid sampling port, MFC: Mass flow controller FC: Flow controller MTI: Temperature and moisture indicator PI: Pressure indicator VI: Needle valve.

sewage work emissions. The long term performance of both systems along with their detailed characterization at different EBRTs was herein studied. The capacity of these technologies to cope with process fluctuations is reported elsewhere [13].

2. Materials and methods

2.1. Microorganisms and culture conditions

Aerobic bacterial sludge collected at Valladolid WWTP was used here as inoculum. A *Pseudomonas fluorescens* NCIMB 11671, purchased from the National Collection of Industrial and Marine Bacteria (Aberdeen, Scotland), was also added on day 132 to enhance α-pinene biodegradation. A SO_4^{2-}-free mineral salt medium (MSM) was used for biofilter irrigation and as a wastewater matrix to feed the AS unit [14]. MSM was composed of ($g\,l^{-1}$): $Na_2HPO_4{\cdot}12H_2O$, 6.15; KH_2PO_4, 1.52; NH_4Cl, 0.81; $MgCl_2{\cdot}6H_2O$, 0.17; $CaCl_2$, 0.038; and 10 $ml\,l^{-1}$ of a trace element solution containing ($g\,l^{-1}$): EDTA, 0.5; $FeSO_4{\cdot}7H_2O$, 0.2; $ZnSO_4{\cdot}7H_2O$, 0.01; $MnCl_2{\cdot}4H_2O$, 0.003; H_3BO_3, 0.03; $CoCl_2{\cdot}6H_2O$, 0.02; $CuCl_2{\cdot}2H_2O$, 0.001; $NiCl_2{\cdot}6H_2O$, 0.002; $NaMoO_4{\cdot}2H_2O$, 0.003. The final pH of medium was 7.0.

2.2. Chemicals

Butanone, toluene and α-pinene were purchased from Sigma-Aldrich with a purity higher than 99.9%. All other chemicals and reagents were purchased from PANREAC with a purity of +99% (Barcelona, Spain).

2.3. Experimental Set-up

The experiments were carried out in a lab-scale plant consisting of two jacketed column bioreactors operated in parallel: a biofilter and an AS system (Fig. 1). Both bioreactors (120 cm height × 10 cm i.d.) were made of clear PVC with a working volume of 8.5 L and operated at 20 °C. The biofilter was packed with 8.5 L of a mixture of compost and perlite while the AS unit was initially filled with 7.5 L of MSM. The characterization of the packing material was carried out according to standard methods [15] (Table 1).

The odorous stream was prepared by mixing either a concentrated H_2S/butanone/toluene stream or a concentrated H_2S/butanone/toluene/α-pinene stream from calibration cylinders (Abello Linde S.A., Spain) with pre-humidified H_2S and VOCs-free air (ambient air filtered through a 1.6 L activated carbon bed and humidified in a 1 m water column). A mass flow controller (Aalborg, Denmark) was used to accurately dose the concentrated mixture. The final concentrations ranges were 23.6–43.3, 4.3–6.3, 0.40–0.60 and 0.12–0.15 $mg\,m^{-3}$ for H_2S, butanone, toluene and α-pinene, respectively, corresponding to concentrations ranges of 17–31, 1.5–2.2, 0.1–0.2 and 0.02–0.03 ppm, respectively. The concentrations selected were within the typical concentration range of VOC emissions from WWTP according to Zarra et al. [12]. The odorous emission was then equally split and fed to the biofilter from the top of the reactor (downflow configuration) and to the AS system via three ceramic spargers located at the bottom of the bioreactor.

Prior to process start-up, a test was conducted to assess abiotic H_2S and VOC removal. Inlet and outlet H_2S and VOC concentrations were periodically monitored in both bioreactors for 94 h at an EBRT of 94 s in the absence of biofilter packing material and microbial activity to asses for pollutant adsorption and photolysis in the experimental set-up.

Both bioreactors were inoculated with 1–L of concentrated ($17\,g\,L^{-1}$) return AS resuspended in MSM. The systems were first operated for approximately 121 d to evaluate the influence of the EBRT (94, 74, 55, 48, and 32 s) on RE using H_2S, butanone and toluene as model odorants. During this period, the AS system was operated in the absence of glucose addition at an infinite sludge retention time (SRT) (no biomass withdrawal). However, due to biomass aggregation and compaction on day 95 (corresponding to an EBRT of 48 s), the SRT in the AS system was set up at 25 d by daily withdrawal of 340 mL of mixed liquor and replacement with fresh MSM containing 2 g of glucose (corresponding to an organic load of 0.3 kg COD $m^{-3}\,d^{-1}$). SRTs of 10–30 d are typical in WWTP operated in cold climates to guarantee consistent wastewater treatment efficiencies. Once a steady state was reached again in the AS unit, the EBRT was further decreased to 32 s in both bioreactors. At day 121, the ability of both odour abatement biotechnologies to remove hydrophobic odorants was challenged by the supple-

Table 1
Characterization of the biofilter packing material.

Parameter	Value
Composition	75/25 (% compost/% perlite)
Density (as received)	0.22 $g\,mL^{-1}$
Porosity	64.4%
Water holding capacity	41% (volume basis)
pH	5.60

Table 2
Steady state REs and confidence interval ($p = 0.05$) in the biofilter and the AS system at the studied EBRT.

EBRT (s)	Biofilter			AS system		
	Butanone	Toluene	α-Pinene	Butanone	Toluene	α-Pinene
94.5	98.9 ± 1.4	ND		ND	98.4 ± 2.8	
73.5	97.9 ± 0.4	ND		98.4 ± 0.6	97.9 ± 0.3	
55.0	98.9 ± 0.2	ND		99.3 ± 0.1	96.6 ± 1.0	
48.8	99.4 ± 0.1	99.9 ± 0.2		99.3 ± 0.3	95.0 ± 1.4	
32.0	99.3 ± 0.4	99.9 ± 0.2	7.3 ± 1.9	99.7 ± 0.1	96.2 ± 1.2	6.8 ± 1.9

ND: odorant not detected in the outlet stream.

mentation to the above mentioned synthetic odorous stream with α-pinene. Each operational condition was maintained for at least 3 wk in order to ensure stable steady states.

The pH in the AS system was maintained constant at approximately 6.3 ± 0.3 via daily addition of a 50 g L^{-1} NaOH and 15 g L^{-1} Na_2CO_3 solution (in order to supply inorganic carbon for the autotrophic H_2S oxidising bacteria). In addition, 1 L of AS mixed liquor was periodically removed, centrifuged (10 min, 10,000 rpm) and resuspended in MSM in order to maintain sulphate concentration below 3500 mg L^{-1} (corresponding to a conservative salt concentration of 0.9 wt% [5]). Likewise, 250 mL of MSM were periodically irrigated at a frequency inversely proportional to the EBRT via a spray nozzle located at the top of the biofilter. This irrigation avoided media drying and promoted the wash-out of inhibitory sulphate concentrations.

The concentration of CO_2, H_2S, and VOCs was periodically monitored at both inlet and outlet gas sampling ports. H_2S and VOC concentration was also measured at sampling ports located at 0, 40, 80, and 120 cm from the biofilter inlet in order to determine the biodegradation profiles. pH and biomass, SO_4^{2-}, dissolved total organic carbon (DOC), dissolved inorganic carbon (DIC), dissolved total nitrogen (DTN) and ATP concentrations were periodically recorded in the AS system by drawing 20 mL of mixed liquor. Likewise, the pressure drop in the packing media, the pH, SO_4^{2-}, DOC, DIC, and DTN concentrations in the leachate resulting from biofilter irrigation were also periodically measured. In addition, the inlet moisture content of the synthetic odorous emission was also continuously monitored. Finally, the volumetric mass transfer coefficients (k_La) in the AS system were determined according to Quijano et al. [16].

2.4. AS microbial activity monitoring

Batch tests were conducted periodically to monitor microbial acclimation in the AS system. Seven serological bottles of 120 mL were initially filled with 5 mL of MSM and 5 mL of sludge from the AS reactor, closed with butyl septa and sealed with aluminum caps. Butanone and toluene were added to the headspace at 3.0 ± 0.7 and 0.6 ± 0.1 mg m^{-3}, respectively. The microbial assays were incubated at 20 °C in a thermostatic bath under magnetic agitation at 300 rpm and the concentration of butanone and toluene was measured periodically (by removing a test bottle due to the destructive nature of the solid phase microextraction (SPME)–GC–MS analysis) until complete VOC depletion.

2.5. Analytical procedures

H_2S was analysed using an electrochemical sensor (Dräger X-am 5000) calibrated in the 0–40 ppm range. Gas samples for VOC analysis were collected in 250 mL calibrated glass bulbs (SUPELCO) and pre-concentrated by SPME. VOC concentrations were then determined by GC–MS according to Lebrero et al. [13]. External standards prepared in calibrated glass bulbs with humidified air, and sampled under similar conditions as those used during bioreactor sampling, were used for VOC quantification.

Carbon dioxide was analysed in a GC-TCD (Varian CP-3800) according to Hernandez et al. [17].

Biomass concentration in the AS unit was estimated via culture absorbance measurements (optical density at 600 nm) in a Hitachi U-2000 spectrophotometer (Hitachi, Tokyo, Japan) and as total solids concentration according to Standard Methods [18]. ATP was measured using a Microbial ATP kit HS (Biothema, Stockholm, Sweden) and a Microtox 500 luminometer (Azur Environmental, Carlsbad, Germany).

DOC, DIC and DTN concentration was determined in liquid samples from both reactors (mixed liquor in the AS and leachate in the biofilter) according to Hernandez et al. [17]. Sulphate concentration was determined by HPLC-IC using an IC-Pak Anion HC (150 mm × 4.6 mm) column. Liquid samples of 1.5 mL were filtrated through 0.22 μm filters before analysis. The pH was also measured using a pH/mV/°C meter (pH 510 Eutech Instruments, Nijkerk, The Netherlands).

The pressure drop in the biofilter was determined using a homemade differential pressure meter (a clear glass U-tube filled with water and connected directly to the gas inlet and outlet). The moisture content in the influent odorous streams was measured using a Testo 605-H1 thermohygrometer (Testo AG, Germany).

Liquid samples from the AS unit were also drawn and frozen immediately to monitor the population dynamics of the bacterial communities by denaturing gradient gel electrophoresis (DGGE) profiling as described in Lebrero et al. [13]. DGGE was also used to identify the members of the mixed microbial communities detected by DGGE fingerprinting. For this purpose, individual bands were excised from the DGGE gel with a sterile blade, resuspended in 50 μL of ultrapure water and maintained at 60 °C for 1 h to allow DNA extraction from the gel. A volume of 5 μL of the supernatant was used for reamplification with the original primer set. Before sequencing, PCR products were purified with the GenElute PCR DNA Purification Kit (Sigma-Aldrich, St. Louis, MO, USA). The sequences from the excised bands were analysed and compared with sequences in GenBank by BLAST search tool at the NCBI (National Centre for Biotechnology Information) [19]. The sequences were imported into the MEGA program and aligned using the automatic aligner function. The alignment was further corrected manually and phylogenetic trees were constructed by 1000-fold bootstrap analysis using neighbor-joining methods. Trees were edited using MEGA 3.

The sequences were registered in the GenBank Data Library under accession numbers HQ147605-HQ147612.

2.6. Data treatment

Unless otherwise specified, the REs and concentrations recorded during the steady states achieved were presented as the average value with its corresponding error at 95% confidence interval ($p = 0.05$). The Excel statistical package (Microsoft Corporation, USA) was used for data treatment.

3. Results and discussion

No significant differences were recorded between inlet and outlet concentrations of H_2S, butanone or toluene during the 94 h abiotic test, with maximum standard deviations of 1%, 13% and 5% in the biofilter and 0%, 11% and 4% in the AS system, respectively. These results confirmed that neither odorant adsorption nor photolysis occurred in the experimental set-up.

3.1. AS system performance before glucose addition: H_2S and VOC removal

H_2S outlet concentration was below the detection limits of the electrochemical sensor used for H_2S measurement (1 ppm or 1.4 mg m^{-3}) in the AS system regardless of the EBRT employed, even at 32 s. Thus, H_2S REs ranged from 96% to 100% (Fig. 2a). These high REs have been widely reported for AS systems treating varying H_2S concentrations. For example, Barbosa et al. [9] found H_2S REs higher than 98% in the aeration tank of a wastewater treatment pilot plant treating H_2S at 5–25 ppmv (7–35 mg m^{-3}) and up to 99.4% when operating at inlet H_2S concentrations ranging from 30 to 105 ppmv (42–146 mg m^{-3}) [10]. Similarly, Burgess et al. [3] also recorded REs higher than 99% in an AS system treating inlet H_2S concentrations from 77 to 100 ppm (107–140 mg m^{-3}).

A steady increase of sulphate concentration up to 3500 ppm was observed during the first 70 d of operation, remaining stable afterwards (as a result of periodic MSM exchanges). The experimental and theoretical sulphate concentrations exhibited a good correlation ($[SO_4^{2-}]_{theoretical} = 1.011\ [SO_4^{2-}]_{experimental}$; $R^2 = 0.96$), which suggest a complete oxidation of H_2S to sulphate as a result of the high dissolved oxygen concentrations present in the mixed liquor.

Despite H_2S elimination performance has been widely monitored in AS systems due to its ease of measurement, scarce information exists about VOC treatment at trace level concentrations. In our particular study, process start-up was characterized by high initial butanone and toluene REs due to VOC absorption into the mixed liquor followed by a gradual decrease in VOC removal performance. Butanone and toluene REs increased from 45% and 11% at day 2 up to REs > 99% by day 8 and 20, respectively (Fig. 2b and c). Butanone REs in the AS system ranged from 98 to REs > 99% regardless of the EBRT. In the case of toluene, REs of 98.4 ± 2.8% at 94 and 74 s, 95 ± 1.4% at 49 s and 96 ± 1.2% at 32 s were recorded (Fig. 2b and c). These values represent, to the best of our knowledge, the highest REs ever reported for VOCs at such low inlet concentrations and residence times in AS systems. The capacity of AS processes to remove dissolved VOCs was previously studied by Barbosa et al. [10], who recorded high efficiencies for the treatment of a wide range of VOCs and volatile sulphur compounds coming with the wastewater. However, these authors only supplied H_2S to the system with the odorous stream.

The high VOC abatement performance was probably due to the high k_La values driving odorant transfer from the emission and to the efficient microbial pollutant uptake in the mixed liquor (no VOC diffusion limitations). Mass transfer coefficients increased exponentially when decreasing the EBRT from 241 ± 38 h^{-1} at 94 s to 697 ± 40 h^{-1} at 32 s. These k_La values are quite high compared to those reported by Dorado et al. [20] in a biotrickling filter packed with polyurethane foam at 35 s of EBRT ($k_La \approx 43.2\ h^{-1}$). However, the k_La increased up to 700 h^{-1} when the EBRT of the biotrickling filter decreased to 10 s.

The performance of the AS unit for the removal of hydrophobic VOCs was challenged by the addition of α-pinene to the synthetic odorous stream at day 121. While this odorant was initially removed at approximately 21%, its RE decreased to 6.8 ± 1.9% after two days of operation and remained constant for the following 40 d. Despite lower REs compared to toluene and butanone were expected due to mass transfer limitations (low concentration gradient available for mass transfer as a result of its higher partition coefficient), this poor abatement performance was likely due to the lack of a specialized α-pinene degrading community. In this context, the addition of 250 mL of a *Pseudomonas fluorescens* culture (α-pinene degrading species [14]) after 11 days of α-pinene feeding did not result in significant enhancements in the removal of this terpene. The absence of an active microbial community capable of degrading α-pinene at the low concentrations present in the mixed liquor (0.10-0.13 μg l^{-1}) was confirmed by the fact that operation at low pH values triggered the development of an α-pinene degrading community able to degrade up to 50% of this terpene at an EBRT of 50 s (data shown in Lebrero et al. [13]).

Batch VOC biodegradation tests showed a gradual enrichment of bacterial communities specialized in the degradation of trace level concentrations of VOC (2.6 ± 0.4 mg m^{-3} and 0.6 ± 0.1 mg m^{-3} of butanone and toluene, respectively) in the AS. Figure 3 represents the degradation kinetics for butanone and toluene of sludge samples drawn at different operation times. Hence, while microorganisms were initially not able to completely degrade butanone and toluene in 80 min, less than 60 min were required for its complete depletion after 58 and 27 d, respectively.

3.2. AS system performance after glucose addition: biomass growth

After three months of operation with butanone, toluene and H_2S, the AS system collapsed due to an unexpected biomass aggregation followed by compaction and sedimentation at the bottom of the bioreactor. Hence, biomass concentration rapidly decreased from 1 to 0.12 g L^{-1} (Fig. 4) concomitant with a reduction in butanone and toluene REs (63% and 34%, respectively) as a result of the reduction in the specific interfacial area biomass-cultivation broth due to biomass compaction (Fig. 2b and c). The microbiological reasons underlying this phenomenon are however unknown. Glucose was then supplied at a rate of 0.3 kg COD m^{-3} d^{-1} along with a daily biomass withdrawal (sludge retention time of 25 d) in order to maintain the suspended biomass culture at 2.5 g L^{-1} throughout the rest of the experiment. The addition of an easily biodegradable carbon source, simulating wastewater input during real WWTP operation, resulted in a rapid re-suspension of the compacted biomass and in the recovery of the preceding steady state REs. Therefore, the hypothesis of a reduced VOC-degrading activity in the presence of more easily degradable carbon sources (such as organic matter from wastewater) can be ruled-out since the supply of such a source appears to be a requirement for a successful odour removal in AS systems. Moreover, the presence of an easily biodegradable carbon source resulted in a more active biomass as shown by the higher specific ATP contents, which increased from an initial value of $5.0 \pm 0.5 \times 10^{-9}$ mol ATP (g biomass)$^{-1}$ up to constant values of $3.7 \pm 0.2 \times 10^{-8}$ mol ATP (g biomass)$^{-1}$ (Fig. 4). This increase in the energetic level of the cells, herein quantified as specific ATP content, was in agreement with the experimental findings of Bordel et al. [21], who observed an increase in the specific ATP content of *P. putida* F1 at increasing toluene concentrations.

3.3. AS microbial analysis

Despite the acclimation and specialization of the VOC and H_2S degrading community and the addition of glucose (an easily biodegradable carbon source) from day 94, the biodiversity of the microbial community present in the AS unit remained surprisingly constant over time, as shown by the DGGE (Fig. 5). Therefore, the addition of glucose allowed the recovery of the system due to an increase of microbial activity as shown by the increase in RE (Section 3.2), but it did not affect the biodiversity (letter A, Fig. 5).

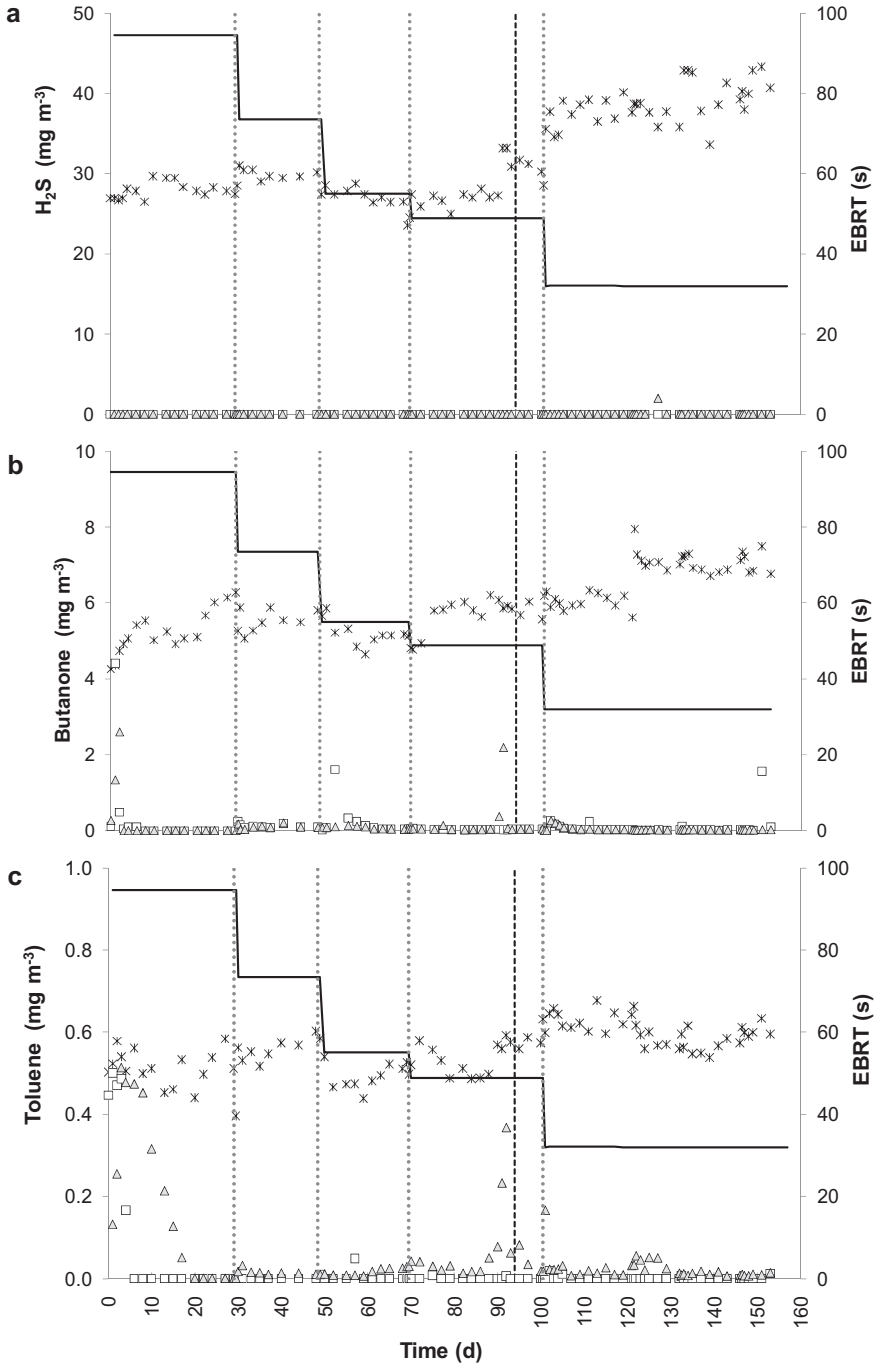

Fig. 2. Time course of H_2S (a), butanone (b) and toluene (c) concentrations in the influent stream (*), biofilter effluent (□) and the AS effluent (△) at different EBRTs (continuous line). Vertical dotted lines represent the operation at different EBRT and the vertical dashed line represents the beginning of AS operation at 25 d of sludge retention time.

Similar results were obtained by Bayle et al. [22] operating two AS reactors supplied with a complex VOC mixture at high and low concentrations. These authors observed a reduction in the bacterial diversity of the bioreactor supplied with the highest VOC loading and the maintenance of a large bacterial diversity in the bioreactor operated at low VOC concentrations. These results support the gradual enrichment of the bacterial communities specialized in the degradation of VOCs and H_2S at trace level concentrations herein obtained and highlight the broad catabolic potential of the AS unit from WWTP. The degree of similarity of DGGE bands to known sequences ranged from 97% to 99%, except for bands 5 and 1, which ranged between 93% and 95%.

DGGE sequencing in our study showed members of four bacterial divisions (Fig. 6). Three out of the eight DGGE bands identified (bands 2, 3 and 6) clustered within the *Proteobacteria* division (γ-*Proteobacteria* and β-*Proteobacteria*). Two DGGE bands (bands 4 and 5) were identified as representatives of *Actinobacteria* while DGGE bands 7 and 8 clustered within *Nitrospirae* and *Chloroflexi,* respectively. The few 16S rRNA-based studies available in literature showed that members of *Proteobacteria*, *Bacteroidetes*, *Chloroflexi* and *Planctomycetes* divisions are abundant in this type of systems [22]. In spite of the addition of a *P. fluorescens* culture on day 132 (letter C in Fig. 5), none of the DGGE bands sequenced were affiliated to the *Pseudomonas* genus, which agreed with the

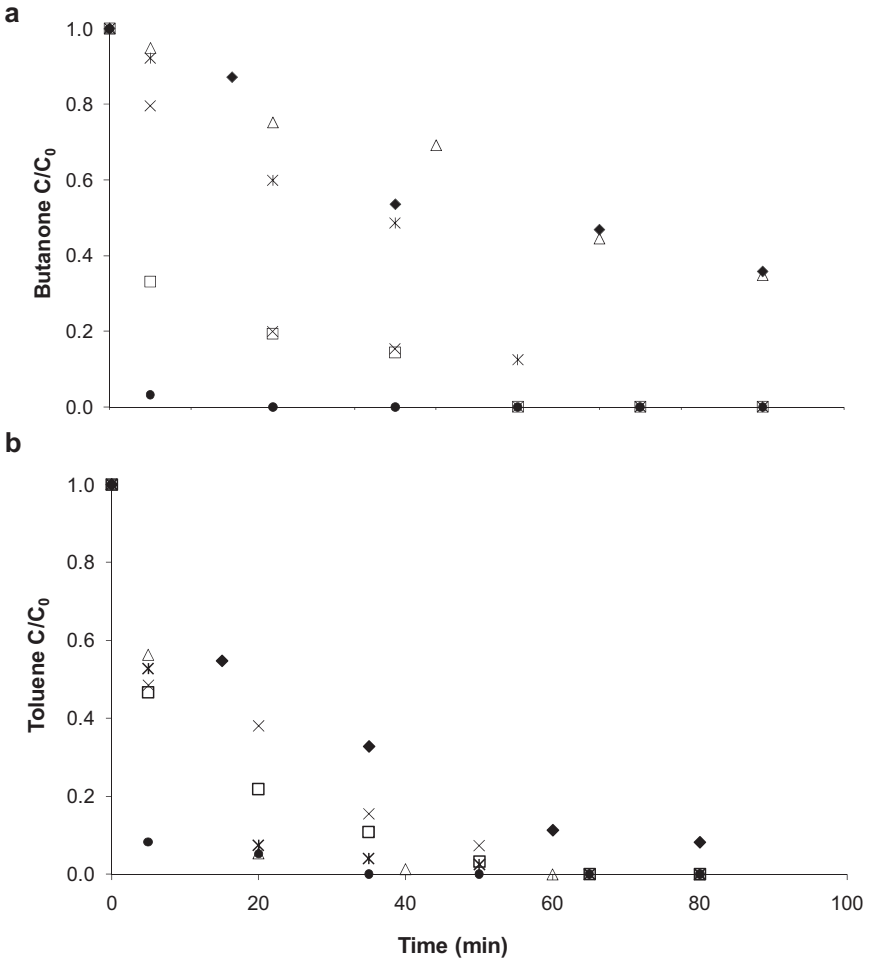

Fig. 3. Time course of butanone (a) and toluene (b) normalized outlet concentrations in the AS mixed liquor at days 9 (♦), 27 (△), 58 (*), 66 (×), 98 (□) and 156 (●).

low REs observed for α-pinene biodegradation. Finally, it must be highlighted that fungi were observed under microscopic analysis, suggesting the presence of a mixed bacterial-fungal population in the AS system. The characterization of these fungal communities is being carried out.

3.4. Biofilter performance: H_2S and VOC removal

H_2S abatement performance in the biofilter was comparable to that recorded in the AS system. Thus, H_2S was also removed at RE > 99% in the biofilter regardless of the EBRT applied (Fig. 2a). High

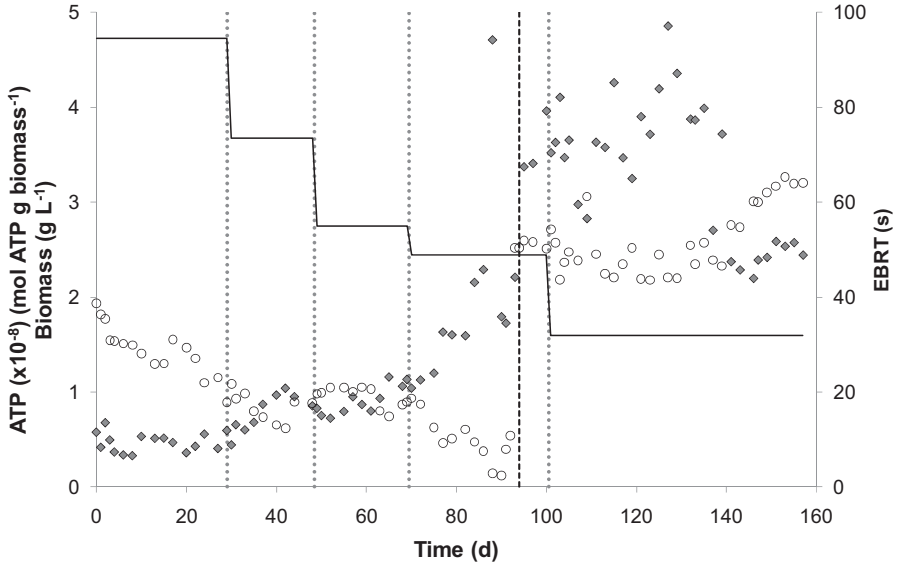

Fig. 4. Time course of biomass concentration (o), specific ATP content (◊) and EBRT in the AS system. The specific ATP values are divided by 10^8. Vertical dotted lines represent the operation at different EBRTs while the vertical dashed line represents the beginning of AS unit operation at 25 d of sludge retention.

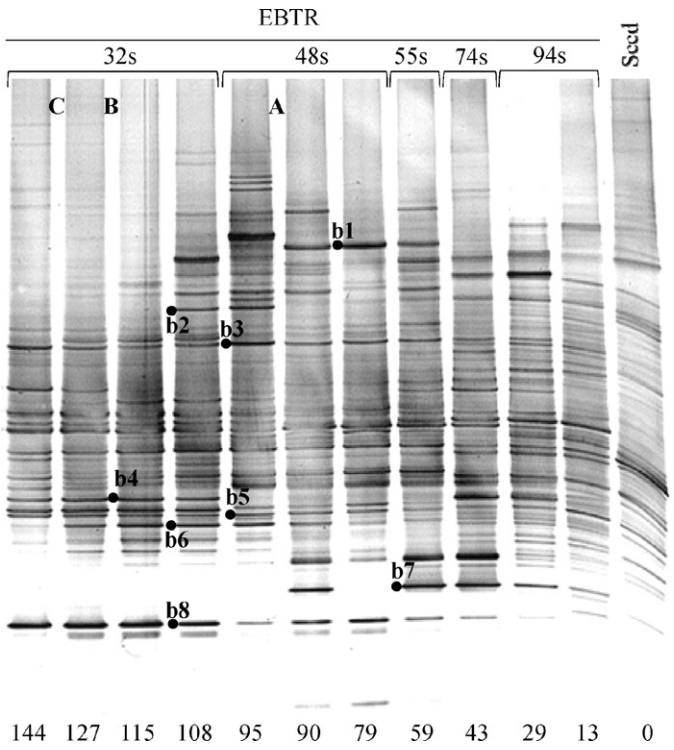

Fig. 5. Bacterial DGGE patterns in the AS system. "DGGE bands are indicated with "B" and the corresponding number of each band. The letters A, B and C indicates the addition of glucose, α-pinene and a *P. fluorescence* culture, respectively. The sampling time and its corresponding EBRT are shown in the lower and upper lane numbers, respectively".

H_2S REs are commonly found in lab and full scale biofilters due to its rapid transfer from the gas phase to the microbial biofilm and the readily biodegradable nature of this volatile inorganic compound (VIC). For example, Morgan-Sagastume and Noyola [23] recorded REs ≈100% in a compost biofilter fed with 100 ppm (140 mg m^{-3}) of H_2S at an EBRT of 50 s. Similarly, Iranpour et al. [24] reported typical H_2S REs ranging from 90% to 100% in full scale biofilters operated in WWTPs in USA. Sulphate, the main byproduct from H_2S oxidation, was periodically washed out from the packed bed as shown by the high concentrations recorded in the leachate after irrigation (maximum value of 18,700 ppm), which prevented from microbial inhibition.

Butanone REs in the biofilter ranged from 98% to 99.5% at the tested EBRTs, which were comparable to those observed in the AS system. Conversely, the biofilter performed better for toluene removal, with REs over 99.9% regardless of the EBRT (Fig. 2b and c). These toluene elimination efficiencies were noticeably higher than those reported in literature. For instance, Liu et al. [25] achieved a maximum RE for toluene in a compost biofilter of 82% at inlet toluene concentration of 0.07–0.73 mg m^{-3} at an EBRT of 65 s. Likewise, Iranpour et al. [24] reported average REs lower than 90% during VOC treatment depending on their hydrophobicity, with hydrophilic VOCs exhibiting highest mass transport gradients due to their lower partition coefficient and slightly soluble compounds presenting poor REs due to a limited mass transfer. Surprisingly, in our particular biofiltration study, toluene REs were slightly higher than those recorded for butanone despite its higher hydrophobicity. This suggests that no mass transfer limitations occurred for these compounds in the biofilter and that other mass transfer mechanism different from the conventional air–water–biofilm (for instance direct air–biofilm transfer) might be present in the biofilm. The biofilter performance for α-pinene treatment was similar to that of the AS system. The REs remained at 7.3 ± 1.9%. Similarly to the AS system, this low terpene removal performance was likely due to the lack of a specialized α-pinene degrading community. The addition of *P. fluorescens* after 11 days of α-pinene feeding did not result in any significant increase in the removal of this terpene. The absence of an active microbial community was further confirmed by the fact that an α-pinene degrading community developed under stress conditions (no irrigation) and α-pinene removal increased up to 65% at EBRT of 50 s (data shown in Lebrero et al. [13]). The low EBRT tested in this study (32 s) might have also mediated the low terpene abatement here recorded. REs of 65% were in the range of those reported for this terpene in conventional biofilters. For example, Jin et al. [26] achieved REs up to 89% in a fungal biofilter treating α-pinene at 2.47 g m^{-3} at EBRTs greater than 1.2 min. A summary of the steady state REs in

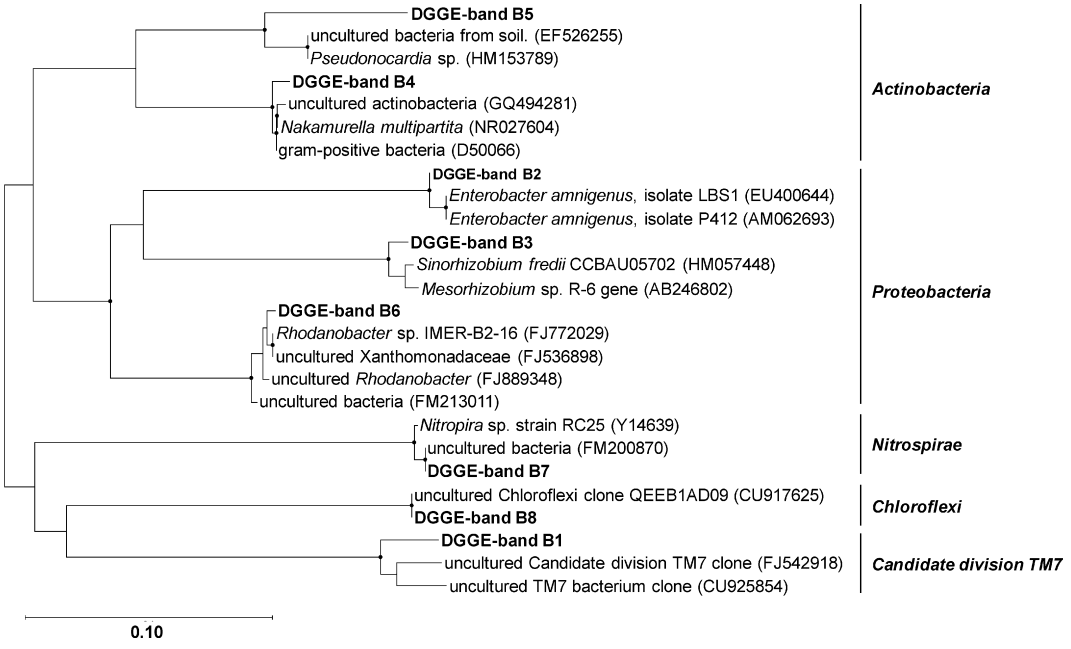

Fig. 6. Phylogenetic tree based on the bacterial 16S rRNA gene sequences obtained from the DGGE bands. Sequences determined in this study are in boldface. Black dots on the nodes indicate bootstrap values of 90% or higher (1000 replicates). The scale bar indicates 10% sequence difference.

the biofilter and the AS system at the studied EBRT is shown in Table 2.

The high REs values recorded in the biofilter were however obtained at the expense of an important pressure drop across the filter bed. The pressure drop in biofilters constitutes the main parameter determining operational costs [7] and 10 cm of water column is often considered as the maximum tolerable pressure drop in biofiltration [27]. This critical value was considerably exceeded in our biofiltration system, where the average pressure drop increased from 6 to 33 cm of water column when decreasing the EBRT from 94 to 32 s, respectively. The gradual deterioration of the packing material used (4.5 cm of bed compaction after 5 months of operation), together with the low diameter of the packing material (1–5 mm for perlite, 0–20 mm for compost) could have contributed to the high pressure drop herein recorded [7].

The packing material lost its buffer capacity at day 80 as shown by the rapid decrease on the pH of the leachate from 4.8 ± 0.2 to 1.3 ± 0.1. Nevertheless, despite the low pH of the biofilter leachate, confirmed by independent measurements of the pH of the packing material (pH of 1.8 at day 157 regardless of the biofilter height), the REs for the tested odorants remained unchanged, which suggests an acclimation of the microbial population to the acidic environment. In this context, a fungal community was observed by microscopy analysis of a packing material sample. Low pH values in biofilters have been previously reported in literature, particularly in fungal biofilters treating sulphur compounds or VOCs and in biofilters inoculated with acidophilic bacterial species [6,28,29].

3.5. Biodegradation profiles of the biofilter

The analysis of the time course of the biodegradation profile along with the biofilter height revealed that most of the H_2S and VOC degradation occurred in the first 40 cm of the bed column, which corresponded to approximately 30% of the bed volume. From the first day of operation, H_2S was totally depleted at the sampling point located 40 cm from the biofilter inlet, whereas butanone and toluene REs at this point increased throughout the experimentation period, being higher than 98.8% for butanone and 95% for toluene from day 29 of operation. Complete removal of VOCs and VICs in the first part of the biofilter column has been already reported in literature. For instance, 40% of a pine bark woodchips biofilter bed was required to completely eliminate oxygenated compounds, while this percentage increased up to 60% when total sulphur and nitrogen compounds removal was evaluated [30].

3.6. CO_2, DOC, DIC and DTN

No significant differences were found between inlet and outlet CO_2 concentrations in the AS system (0.73 ± 0.02 g CO_2 m^{-3} and 0.81 ± 0.03 g CO_2 m^{-3}, respectively) due to the low concentrations of VOCs present in the artificial odour emission treated (data not shown). Slightly higher values (0.87 ± 0.03 g m^{-3}) were recorded in the biofilter during the first three months of operation, probably due to the additional biodegradation of the organic matter present in the compost. However, CO_2 production in the biofilter decreased to 0.76 ± 0.02 g m^{-3} within the last two months of operation, which suggests the complete compost stabilization.

The DOC in the AS system remained stable at 11.1 ± 0.7 mg L^{-1} during the first 72 days of experiment, steadily increasing to a maximum value of 34.8 mg L^{-1} when glucose was added to the system. However, the DOC gradually decreased to the preceding steady value from day 105. In the biofilter, the DOC of the leachate remained approximately constant at 132 ± 10 mg L^{-1} due to the leaching of organic matter from the compost. The concentration of DIC remained constant at 1.6 ± 0.2 and 0.43 ± 0.03 mg L^{-1} in the AS system and the biofilter leachate, respectively. On the other hand, the DTN underwent a sharp decrease in the AS system after glucose addition at day 93 from 162 ± 7 down to 2.3 mg L^{-1} due to the increase in biomass concentration, increasing afterwards to a steady value of 91 ± 5 mg L^{-1}. High DTN values of up to 718 ± 21 mg L^{-1} were initially recorded in the biofilter leachate as a result of N-compounds leaching from the compost. The DTN concentration in the leachate steadily decreased but no limitation was observed throughout the experiment.

3.7. Cost evaluation

AS system is a low cost alternative to biofiltration for odour treatment in WWTP [31]. The investment costs are very limited in AS diffusion since this biotechnology employs the equipment already present in the WWTP. Minimal capital costs are due to ductwork, installation of moisture traps, dust and grease aerosol filters and the replacement of certain equipments with anticorrosion materials (i.e. blowers) [31,32]. In addition, the operating costs are limited to the maintenance of filters and moisture traps, since no packing material is present and the energy cost of air diffusion into the aeration basin is included in the operating cost of the water line. However, if the system needs a dedicated blower or diffuser, or if a long gas pipeline is required, a cost-effectiveness analysis is required [31]. In this context, AS systems are recommended for treating the gaseous emissions of the primary sedimentation tank and the sludge treatment units, the closer unit operations. According to a recent study [32], biofiltration capital costs range from 5 to 28 € $(m^3/h)^{-1}$, while operation costs are about 0.21 € $(1000\ m^3\ treated)^{-1}$.

4. Conclusions

The results here obtained confirmed the potential of AS systems as a robust and efficient biotechnology for odour treatment in WWTPs, with comparable steady state REs (>95%) for H_2S, butanone and toluene to those recorded in the biofilter regardless of the EBRTs (94–32 s). High k_La values were recorded in the AS, which increased exponentially with decreasing EBRT. The supply of wastewater to the AS unit contributed to an enhanced process stability by preventing biomass compaction. Therefore, a complete VOC removal can be expected in aerated tanks with fine bubble diffusers in WWTPs under the typical operational conditions.

Acknowledgements

This research was supported by the Spanish Ministry of Education and Science (RYC-2007-01667 and AP2008-01574 contracts and projects CTQ2009-07601, CTC2007-64324 and CONSOLIDER-CSD 2007-00055). The Regional Government of Castilla y Leon (Ref. GR76) is gratefully acknowledged for its financial support. Finally, Dr. Valérie Desauziers is also thanked for her technical support in the SPME–GC–MS analysis.

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Lebrero R, Rodriguez E, Martín M, García-Encina PA, Muñoz R (2010). Water Research 44: 3905-3914

Chapter 4

H_2S and VOCs abatement robustness in biofilters and air diffusion bioreactors: A comparative study

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H_2S and VOCs abatement robustness in biofilters and air diffusion bioreactors: A comparative study

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ARTICLE INFO

Article history:
Received 22 February 2010
Received in revised form
30 April 2010
Accepted 7 May 2010
Available online 19 May 2010

Keywords:
Air diffusion bioreactor
Biofiltration
Odour treatment
Robustness
VOC biodegradation

ABSTRACT

The robustness of a conventional biofilter and an air diffusion bioreactor (ADB) was comparatively evaluated in laboratory-scale plants treating a mixture of H_2S, butanone, toluene and alpha-pinene at gas residence times of 50 s. Under steady state conditions, H_2S, butanone and toluene were almost completely degraded, while alpha-pinene removal did not exhibit removal efficiencies (REs) higher than 11.0 ± 2.3%. Fluctuations in temperature from 8 °C to 30 °C did not impact significantly process performance in any of the biotechnologies tested. However, while the ADB unit was able to cope with three and six fold step increases in pollutant loadings, volatile organic compounds (VOCs) REs noticeably decreased in the biofilter when subjected to a six fold step change (i.e. 90% reduction for butanone and 30% for toluene). A process shutdown of five days resulted in the temporary loss of butanone and toluene RE in the ADB system. A lack of irrigation during five days caused a slight decrease in the biofilter REs, while a failure in the pH control system drastically affected the ADB performance. Finally, process robustness was quantified. The calculated overall risks showed that both biotechnologies were reliable for H_2S and VOCs treatment in wastewater treatment plants, ADB diffusion exhibiting a higher robustness towards fluctuations commonly found under routine operation. This robustness was further confirmed by the high stability of the DGGE profiles.

1. Introduction

A cost-efficient odour treatment is crucial for correct odour management in wastewater treatment plants (WWTPs). Traditionally, odour abatement in WWTPs has been based on physical/chemical methods such as chemical scrubbing, activated carbon adsorption or incineration. However, despite the high removal efficiencies achieved by these techniques, their large operational and maintenance costs often limit their implementation to nuisance sensitive scenarios. Besides, the generation of toxic secondary products and their large CO_2 footprint render them ever less attractive in a world increasingly concerned about environmental sustainability (Kennes and Thalasso, 1998; Van Groenestijn and Hesselink, 1993; Burgess et al., 2001).

In the last few decades, biological air treatment has emerged as a cost-efficient and environmentally friendly alternative to physical/chemical methods. Biotechnologies are based on the conversion of gaseous contaminants into CO_2, SO_4^{2-}, NO_3, H_2O and biomass under ambient pressure and temperature, which results in low energy requirements when treating large emission flows with gaseous pollutants at low

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0043-1354/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.watres.2010.05.008

concentrations (Revah and Morgan Sagastume, 2005). Of these, biofiltration is certainly the most popular technology adopted in odour emission treatment in WWTPs (Hansen and Rindel, 2000a). It consists of an organic (peat, compost, soil, etc.) or organic/inorganic packed bed where the microorganisms responsible for pollutant removal grow attached as biofilm. Compared to other media-based biotechniques (such as biotrickling filters or bioscrubbers), biofiltration offers the highest removal efficiencies for moderately hydrophobic pollutants (Iranpour et al., 2005). However, due to the long gas residence times and the structural weakness of most conventional packing materials, biofilters require large land areas and a frequent replacement of the packing material. Their effectiveness is also limited by operational parameters such as moisture content or the accumulation of toxic metabolites, which are difficult to control as there is no continuous aqueous phase involved in the deodorization process.

Activated sludge (AS) diffusion constitutes a cost-effective alternative for treating WWTPs foul emissions due to their low operational costs and minimal footprint. In this process, malodorous air is collected from its source, transferred to the aeration tank and sparged into the mixed liquor (Hardy et al., 2001; Moussavi et al., 2007). Odorants diffuse into the liquid media where wastewater-treating microorganisms carry out their biodegradation. Problems due to corrosion or tar-like deposits may arise when polluted air is transferred to the aeration tank, but they can be easily overcome by a proper installation design (filtration systems, corrosion resistant materials, etc.) (Barbosa et al., 2002). Thus, activated sludge diffusion avoids the problems associated to media-based odour control systems since any potential toxic end products are rapidly washed-out and there is no need for humidity or temperature control. However, despite its obvious advantages, WWTP operators are still reticent to implement activated sludge diffusion mainly due to the lack of full-scale data concerning its performance during the treatment of odorous volatile organic compounds (VOCs) (Burgess et al., 2001; Bowker, 2000). Moreover, the few studies that are available are focused on steady state performances and there is a lack of knowledge on the ability of AS systems to cope with process fluctuations and operational failures.

This work aims to comparatively assess the impact of process fluctuations (such as temperature and inlet H_2S and VOCs concentration) and operational failures (process shutdown, failures in pH and irrigation control) on the performance of a conventional biofilter and an air diffusion bioreactor (ADB). Hydrogen sulfide, butanone, toluene and alpha-pinene were selected as model sulfur and VOCs, representative of a wide range of volatilities. These compounds are commonly found at trace levels (from 0.1 mg m^{-3} to 5 mg m^{-3}) in WWTPs emissions as reported by Zarra et al. (2008).

2. Materials and methods

2.1. Chemicals

Butanone, toluene and alpha-pinene were purchased from Sigma–Aldrich with purity higher than 99.9%. All other chemicals and reagents were purchased from PANREAC with a purity of +99% (Barcelona, Spain).

2.2. Pilot plant description

The experimental setup consisted of a biofilter and an air diffusion bioreactor operating in parallel (Fig. 1). Both bioreactors were jacketed PVC cylinders of 120 cm of height and 10 cm of inner diameter with a working volume of 8.5 L operated at 20 °C (Huber water bath, Offenburg, Germany). The ADB was filled with 7.5 L of mineral salt medium (Muñoz et al., 2008) and the biofilter with 8.5 L of a mixture of compost and perlite. Both bioreactors were inoculated with 1 L of return activated sludge (17 g l^{-1}) from Valladolid WWTP and supplied with a synthetic H_2S and VOCs mixture at an empty bed residence time of 50 s. The contaminated air stream was obtained by mixing a primary stream of humidified H_2S and VOCs-free air (activated carbon filter + air sparging through an 8.5 L water column) and a secondary synthetic stream from a calibration mixture bottle containing H_2S, butanone, toluene and alpha-pinene (Abello Linde S.A., Spain). This resulted in inlet concentrations for these compounds of 44.8 ± 1.3 mg m^{-3}, 6.78 ± 0.25 mg m^{-3}, 0.56 ± 0.02 mg m^{-3} and 0.123 ± 0.005 mg m^{-3}, respectively. Hence, the inlet loading rates for H_2S, butanone, toluene and alpha-pinene were 3.2 g m^{-3} h^{-1}, 0.48 g m^{-3} h^{-1}, 0.04 g m^{-3} h^{-1} and 0.01 g m^{-3} h^{-1}, respectively. The biofilter was operated in a downflow configuration, while the ADB was aerated from the bottom of the bioreactor using three porous spargers. Volumetric bulbs of 250 ml (Sigma–Aldrich) were connected to the inlet and outlet ports of each bioreactor for gaseous sampling by SPME.

The packing material of the biofilter consisted of a mixture of 75% compost (Pindstrup Mosebrug SAE, Spain) and 25% perlite (World Minerals, Spain) (v/v). This packing material was characterized according to standard methods (TMECC, 2002): pH = 5.60, void volume = 64.4%, density (as received) = 0.22 g ml^{-1} and water holding capacity = 41% (on a volume basis). Pressure drop in the biofilter was measured using a home-made differential pressure meter with water as manometric fluid. The biofilter was irrigated every two days (250 ml of mineral salt medium/irrigation) via a spray nozzle located at the top of the column to prevent biofilter bed drying and liquid samples from the leachate were collected periodically.

The ADB was operated at a sludge retention time of 25 days by daily replacement of 340 ml of culture medium with fresh mineral salt medium containing 2 g l^{-1} of glucose to represent organic matter in wastewater. Biomass concentration in the ADB was thus maintained constant at approx. 3.5 g dry weight l^{-1}. The pH was maintained at 6.1 ± 0.2 by daily manual addition of a 50 g l^{-1} NaOH and 15 g l^{-1} Na_2CO_3 solution (the latter to supply a carbon source for autotrophic H_2S oxidizing bacteria). In addition, 1 L of the ADB culture broth was periodically removed, centrifuged (10 min, 9200 g) and resuspended in fresh mineral salt medium in order to maintain SO_4^{2-} concentration below 3000 mg l^{-1} (Hansen and Rindel, 2000b). Liquid samples of 40 ml were periodically drawn to record pH, biomass, ATP, sulfate, total organic carbon (TOC), inorganic carbon (IC), total nitrogen (TN) concentrations and the dynamics of bacterial population. Finally, the volumetric mass transfer coefficients (k_La) were determined according to Quijano et al. (2009).

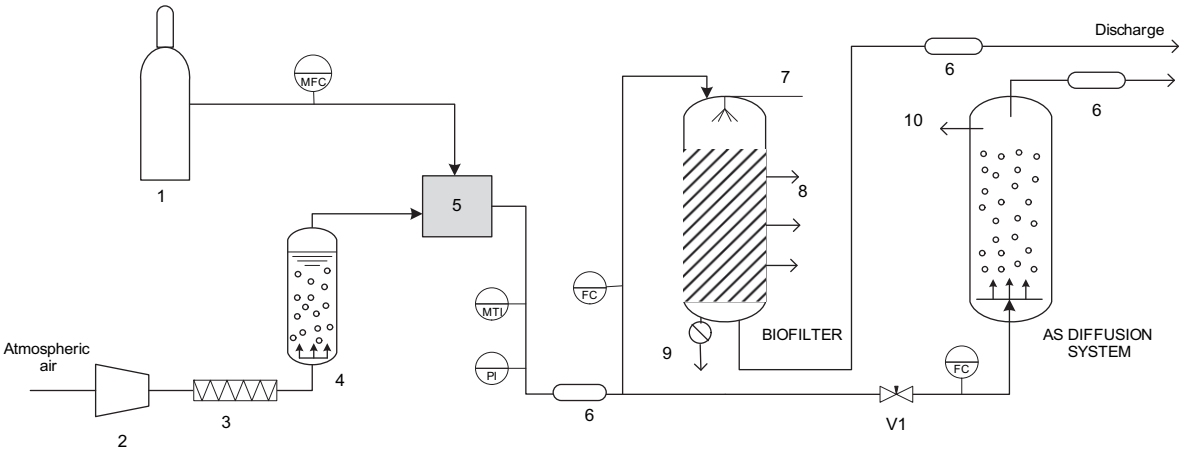

Fig. 1 – Schematic representation of the experimental setup. 1 H_2S and VOC reservoir, 2 Compressor, 3 Activated carbon filter, 4 Humidifier, 5 Mixing chamber, 6 Gas sampling bulb, 7 Irrigation system, 8 Gas sampling ports, 9 Leachate port, 10 Liquid sampling port, MFC: Mass flow controller; FC: Flow controller MTI: Temperature and moisture indicator PI: Pressure indicator VI: Needle valve.

Both bioreactors were operated under identical conditions for five months prior to the beginning of the experimentation. Each series of operational failures or fluctuations was preceded by a steady state of at least two days. The experiments were carried out in duplicate to assess the reproducibility of the process response. Five series of experiments were performed to compare process robustness.

2.3. *Influence of temperature*

The influence of temperature on process performance was evaluated by allowing the bioreactors to stabilize at 8, 10, 15, 20, 25 and 30 °C for 16 h prior to sampling (three consecutive samples at 30 min intervals). Process temperature was then restored to 20 °C and the systems allowed to stabilize again (while monitored) before the next temperature change. The specific cell ATP content at each temperature in the ADB culture broth was also determined.

2.4. *Fluctuations in odorant loading*

Process response to surges in inlet H_2S and VOCs concentrations was evaluated by inducing two sequential 3-h step increases. Following a three fold step increase, inlet concentrations were decreased to their steady state levels and the systems were allowed to recover for 20 h prior to a six fold increase. Process monitoring was carried out by sampling every 30 min.

2.5. *Process starvation*

The robustness of both biological systems to a three day starvation period (only humidified air and glucose were supplied) was investigated by monitoring the transient process performance following H_2S and VOCs supply restoration. The process was allowed to equilibrate for 1 day before the next pollutants starvation period.

2.6. *Process shutdown*

The ability of both systems to recover from a five day shutdown (neither polluted air supply nor biofilter irrigation and glucose addition) was investigated by monitoring H_2S and VOCs removal efficiencies following the restoration of steady state operational conditions. A steady state was maintained for 48 h before the same shutdown protocol was applied again.

2.7. *Failure of biofilter irrigation and pH control in the air diffusion bioreactor*

The effect of biofilter drying on H_2S and VOCs removal was assessed by monitoring process performance after a five day interruption of biofilter irrigation (humidification and supply of the contaminated inlet stream was however maintained). Mineral salt medium irrigation was then resumed and the biofilter was allowed to recover for ten days before the next irrigation interruption. Likewise, the robustness of the ADB to a three day interruption of pH control (pH decreased to 2.8) was assessed.

2.8. *Analytical Procedures*

The concentration of H_2S was measured using a Dräger X-am 5000 electrochemical sensor (detection limit 0.5 ppm). Butanone, toluene and α-pinene were collected in 250 ml glass bulbs (Sigma–Aldrich) and pre-concentrated for 10 min using 85 μm PDMS/Carboxen SPME fibers (Supelco, Bellefonte, PA, USA) according to Larroque et al. (2006). The SPME fibers were injected in a GC-FID (HP 6890 Series, Hewlett Packard, USA) equipped with a SupelcoWax (15 m × 0.25 mm × 0.25 μm) capillary column. Oven, injector and detector temperatures were maintained at 60, 280 and 250 °C, respectively. Helium was used as carrier gas at 2 ml min^{-1} while H_2 and air were fixed at 30 and 300 ml min^{-1}. Dinitrogen was used as make up gas at 28 ml min^{-1}. External standards prepared in

volumetric bulbs with humidified air and sampled under similar conditions as those used during bioreactor sampling were used for VOCs quantification.

Sulfate was analyzed by HPLC using a Waters 515 HPLC pump coupled with a Waters 432 conductivity detector and equipped with an IC-Pak Anion HC (150 mm × 4.6 mm) Waters column. Total organic carbon, IC and TN were analyzed using a TOC-V_{CSH} analyzer (Shimadzu, Japan) coupled with a TN module (Shimadzu TNM-1, Tokyo, Japan) based on chemiluminesce detection. The pH was measured using a pH/mV/°C meter (pH 510 Eutech Instruments, The Netherlands).

Biomass concentration in the ADB was estimated via culture absorbance measurements (optical density at 600 nm) in a Hitachi U-2000 spectrophotometer (Hitachi, Japan). A correlation between absorbance at 600 nm and biomass dry weight was performed. Cell ATP was measured using a Microbial ATP kit HS (Biothema, Stockholm, Sweden) and a Microtox 500 luminometer (Azur Environmental, Carlsbad, Germany).

Liquid samples from the ADB unit were drawn and frozen immediately. Biomass DNA was extracted with the FastDNA Spin Kit for Soil (MP Biomedicals) and stored at −20 °C. Bacterial 16S RNA genes were amplified by polymerase chain reaction (PCR) for DGGE using universal bacterial primers 968 GC-f and 1401r (Sigma–Aldrich, St. Louis, MO, USA) (Nubel et al., 1996), performed in an iCycler Thermal Cycler (Bio Rad Laboratories). Size and yield of PCR products were estimated using a 2000 bp DNA ladder, Hyperladder II (Bioline, USA inc.) in 1.8% agarose gel (w/v) electrophoresis and SYBR Green I staining. The DGGE analysis of the amplicons was performed on 8% (w/v) polyacrilamide gels using urea/formamide denaturant gradient of 42–67% (Ben-Amor et al., 2005; Roest et al., 2005). Electrophoresis was performed with a D-Code Universal Mutation Detection System (Bio Rad Laboratories) in 0.5-X TAE buffer at 60 °C and 85 V for 16 h for bacterial amplicons. The gels were stained with SYBR Green I nucleic acid gel stain (1:10 000 dilution) (Sigma–Aldrich, St. Louis, MO, USA) for 1 h.

2.9. Robustness analysis

Robustness was quantified by determining the risk of negative effects on the biological system associated to process fluctuations or operational failures according to Kraakman (2003). The risk is expressed as the percentage of loss removal per year, which is calculated by multiplying the frequency of the upset by its negative effect on the system and summing over all possible failures:

$$\text{Risk} = \sum(\text{probability} \times \text{negative effect})$$

Where the probability is expressed as the number of episodes of fluctuations or operation failures per year. This negative effect can be calculated for each upset period as the difference between the maximum yearly removal capacity (no upset) of the system and the real removal capacity decreased by the impact of the fluctuation or operation failure (kg of pollutant/year). Most of the probability values were taken from Kraakman (2003), which were based on field experience.

3. Results

Unless otherwise specified, the removal efficiencies (REs) and concentrations recorded during the experiments are presented as the average value with the corresponding error at 95% confidence interval ($\alpha = 0.05$). The Excel statistical package (Microsoft Corporation, USA) was used for data treatment.

3.1. Influence of temperature

When the biofilter was operated at 20 °C, steady state REs for butanone, toluene and alpha-pinene remained stable at 99.7 ± 0.1%, 98.3 ± 0.9% and 11.0 ± 2.3%, respectively. Similarly, steady state REs of 99.7 ± 0.1, 97.5 ± 0.3 and 8.0 ± 1.2% were recorded in the ADB for the VOCs above mentioned.

Butanone removal was not significantly influenced by temperature neither in the biofilter nor in the ADB, remaining constant at 99.7 ± 0.1%. However, toluene REs decreased with decreasing temperatures (88.7 ± 0.3% and 93.3 ± 1.3% at 8 °C in the biofilter and ADB, respectively). A slight decrease in toluene RE was recorded at 30 °C (95.4 ± 0.2% in the ADB and 96.6 ± 0.4% in the biofilter). Alpha-pinene RE slightly decreased by 3% in the biofilter at 10 °C, showing no variation in the ADB. On the other hand, no H_2S was detected in the effluent streams regardless of the bioreactor configuration and the temperature tested, with H_2S-REs ranging from 96% to 100%.

The specific ATP concentration of the microbial community present in the ADB gradually increased with increasing temperatures from $2.21 \pm 0.16 \times 10^{-8}$ mol ATP g DW^{-1} at 8 °C to $2.83 \pm 0.30 \times 10^{-8}$ mol ATP g DW^{-1} at 30 °C.

3.2. Fluctuations in odorant loading

The REs for H_2S, butanone and toluene in the ADB remained stable at 98 ± 2%, 99.7 ± 0.03% and 98.4 ± 0.13%, respectively, regardless of the odorant inlet concentration (Fig. 2). However, alpha-pinene REs increased from steady state values of 5.7 ± 0.8% to 27.1 ± 1.4% and 25.1 ± 0.8% during the three and six fold VOCs steps, respectively.

Biofilter performance was not affected by a three fold increase in inlet H_2S and VOCs concentrations. However, when pollutant loading was increased by a factor of 6, the REs gradually dropped to 91.9% for H_2S and 69.5% for toluene during the step increase, rapidly increasing to steady state values when the original loadings were restored. On the other hand, the REs for butanone in the biofilter remained similar to steady state values during the 3-h six-fold loading step but gradually decreased to 6.3% immediately after the restoration of steady state loadings (Fig. 2a). Alpha-pinene exhibited negative REs immediately after the resumption of steady state loadings (Fig. 2c).

3.3. Process starvation

Both systems recovered steady state REs for H_2S and all target VOCs within the first 30 min following the resumption of pollutant supply after the three day starvation period (results provided as supplementary material).

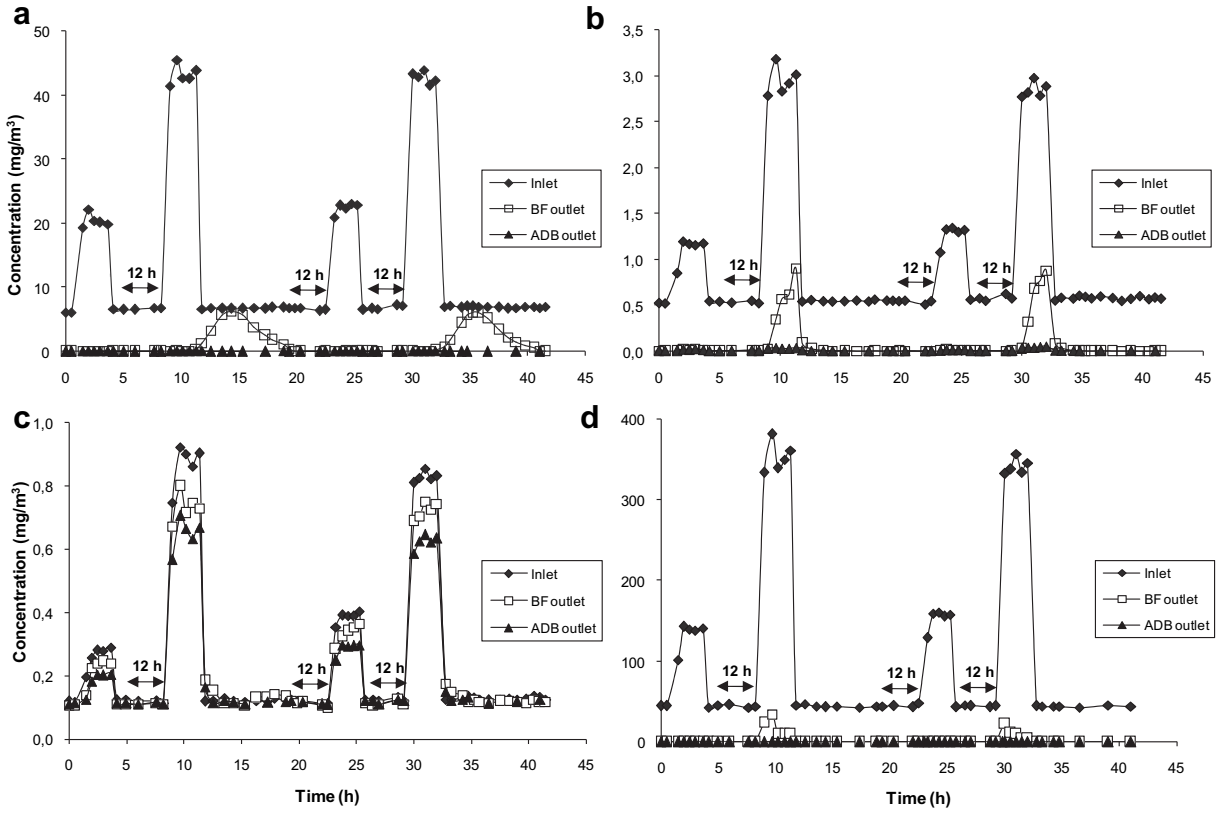

Fig. 2 – Effluent concentrations of butanone (a), toluene (b), alpha-pinene (c) and H_2S (d) in biofilter (□) and ADB (▲) during three and six fold odorants inlet concentration (◆) steps.

3.4. *Process shutdown*

Prior to process shutdown, process performance was characterized by butanone and toluene REs of 99.7 ± 0.0% and 98.9 ± 0.2% in the biofilter, respectively. Likewise, REs in the ADB were 99.8 ± 0.0% for butanone and 98.5 ± 0.2% for toluene. Biofilter performance was not influenced by a five day process shutdown, recovering steady state REs immediately after process start-up (Fig. 3). On the other hand, the REs for butanone and toluene in the ADB decreased to 83.0% and 14.0%, respectively. However, process performance was rapidly restored within 2.5 h after process start-up. Alpha-pinene biodegradation increased immediately after the process start-up (maximum RE of 26.3% in the biofilter and 19.9% in the ADB), followed by a return to the steady values (8.8 ± 1.9% and 5.7 ± 2.0%) after 3 h.

3.5. *Failure on biofilter irrigation and pH control in the air diffusion bioreactor*

VOCs biodegradation in the biofilter remained within steady state values despite the lack of irrigation during the first 4 days. However, toluene and butanone REs decreased by 10% and 20% at day 5 (Fig. 4). Steady state REs for both VOCs were restored following 5 h from irrigation. Pressure drop across the biofilter bed steadily decreased from 18.5 to 15.5 cm of H_2O while packing material dried up (Fig. 4). On the other hand, alpha-pinene biodegradation gradually increased during biofilter drying up to 38% prior to the restoration of irrigation. Biofilter irrigation resulted in a decrease in alpha-pinene REs followed by another progressive improvement in RE, which reached 63.3 ± 1.4% by day 19.

The pH in the ADB severely impacted toluene removal efficiencies, which decreased to 38% at pH of 2.8. The ADB was not able to recover the previous steady state REs (98.6 ± 0.2%), reaching a new steady state at 95.5 ± 0.1% seven days after pH restoration. However, a decrease in the cultivation pH did not affect butanone RE, which remained constant at steady state values (99.8 ± 0.0%). Alpha-pinene REs increased up to 48.8 ± 1.1% 12 days after the first induced failure in pH control (Fig. 5).

Finally, it must be highlighted that despite the failures in biofilter irrigation and ADB pH control, H_2S removal remained unaffected (96% – 100%).

3.6. *Bacterial population dynamics*

No significant variation in bacterial community profiles was observed in the ADB following process operation at fluctuating inlet loading, episodes of pollutant starvation or process shutdowns as shown by the continuity of the bands in the gel (Fig. 6). However, a decrease in the diversity of the population was recorded after a prolonged exposure to low pH values.

3.7. *Volumetric mass transfer coefficients*

K_La values of 325 ± 32 h^{-1} were measured in the ADB tank at EBRTs of 50 s.

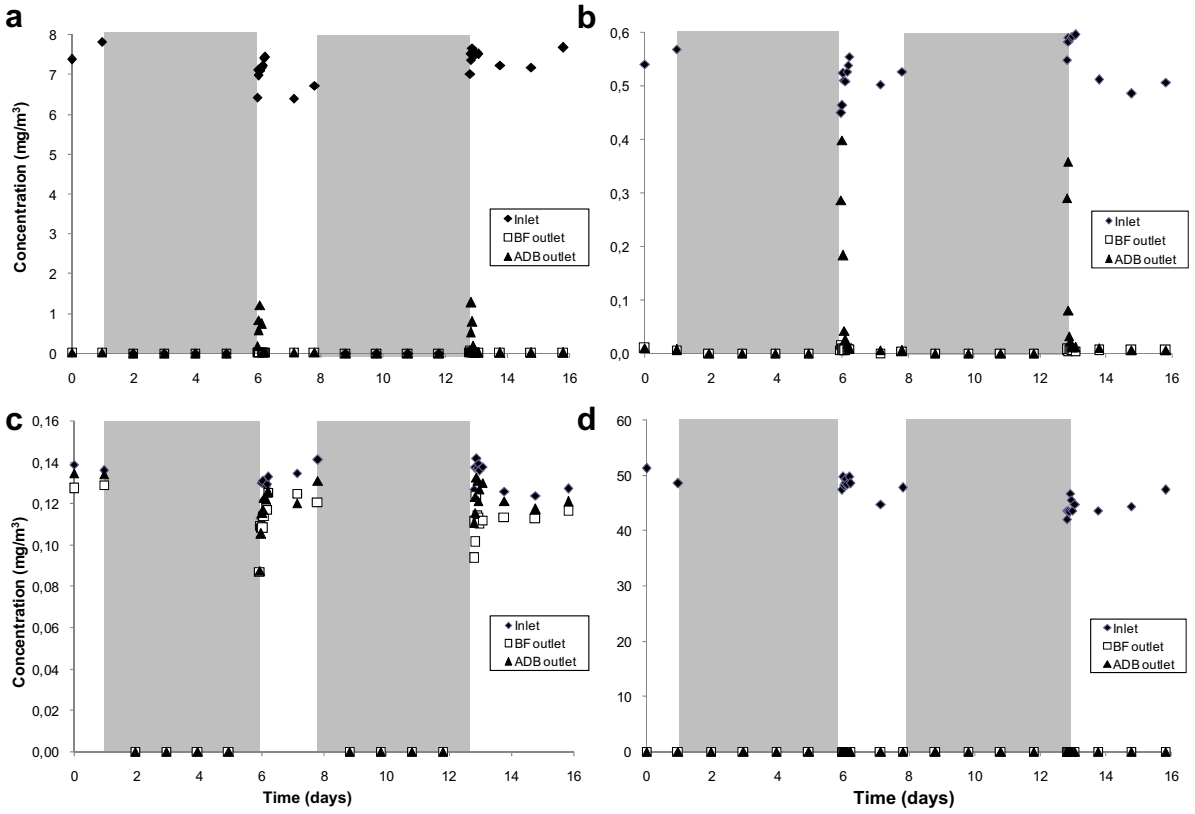

Fig. 3 – Time course of butanone (a), toluene (b), alpha-pinene (c) and H_2S (d) concentrations in the influent stream (◆), biofilter effluent (□) and ADB effluent (▲) after process shutdowns. Shaded areas represent the five day pollutant starvation period.

4. Discussion

Process robustness is one of the key parameters evaluated when selecting the appropriate treatment technology for a specific odour problem. Detractors of biological treatment technologies have always pointed out process robustness as their main drawback, although recent studies suggest that state-of-the-art biotechnologies can be as robust as their physical/chemical counterparts (Kraakman, 2003; Muñoz et al., 2008).

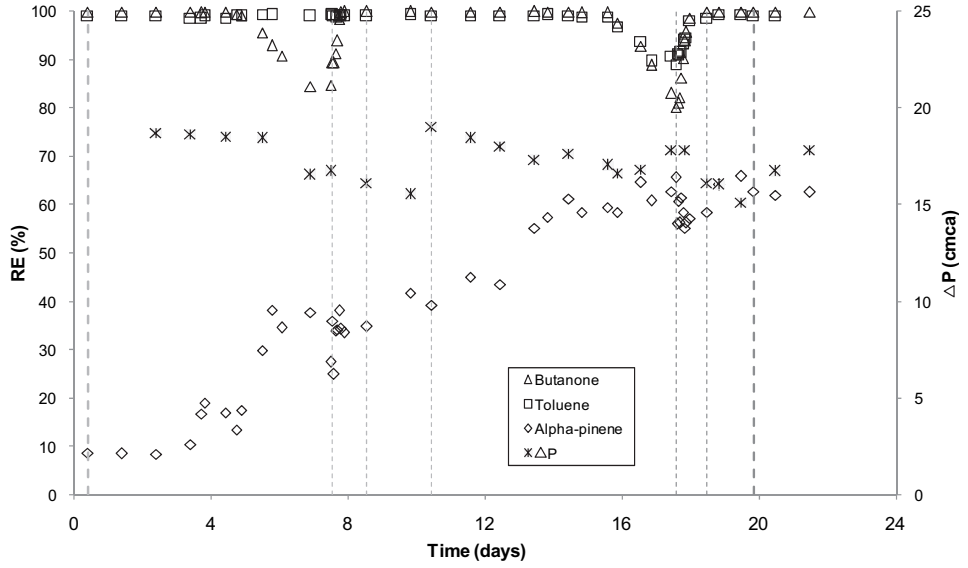

Fig. 4 – Time course of butanone (triangle), toluene (squares) and alpha-pinene (diamond) removal efficiencies and pressure drop (*) in the biofilter. Dashed lines represent the period irrigation in the biofilter.

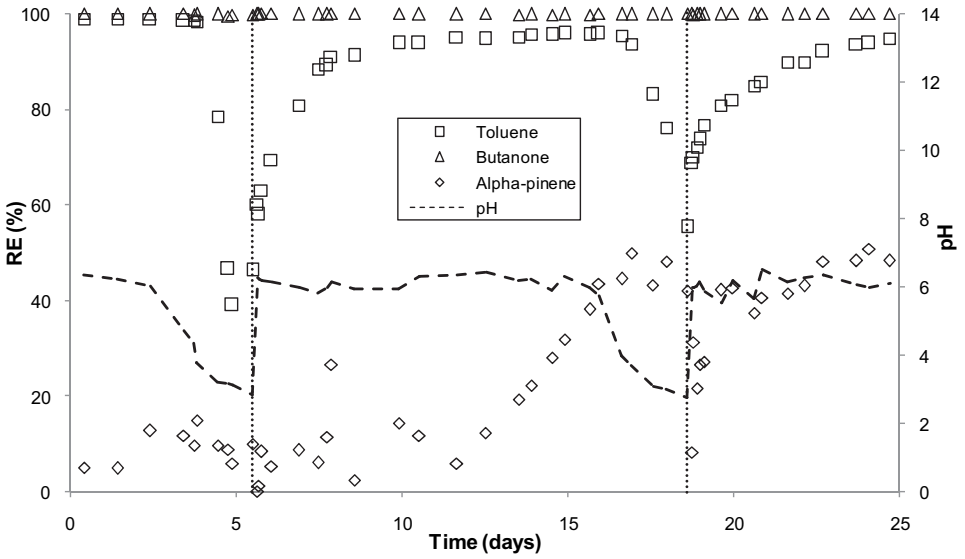

Fig. 5 – Time course of butanone (triangle), toluene (squares) and alpha-pinene (diamond) removal efficiencies and pH (dashed line) during a failure in pH control in the ADB. "Vertical dotted lines represent the restoration of pH control".

Fluctuations in temperature ranging from 15 to 25 °C, commonly found under daily routine operation, did not affect process performance. Even a decrease to 8 °C did not cause a relevant deterioration in biofilter and ADB removal capacity, which highlights the high stability of the microbial population present in both systems. These results correlated with the specific cell ATP content, which showed a decrease in the energetic level at decreasing temperatures. When temperature increased to 30 °C, toluene and alpha-pinene removal efficiencies slightly decreased. This phenomenon could be attributed to the less favorable mass transfer associated to high temperatures (lower VOCs solubility) since the highest specific ATP levels were recorded at 30 °C. Hence, while the hydrophilic nature of butanone and H_2S ensured a high mass transfer to the biofilm regardless of the operational temperature, the transfer of the more hydrophobic VOCs might have been negatively affected by the increase in temperature. A similar behavior was reported by Vergara-Fernández et al. (2007), who observed enhancements in toluene RE when temperature decreased in a compost-shells biofilter.

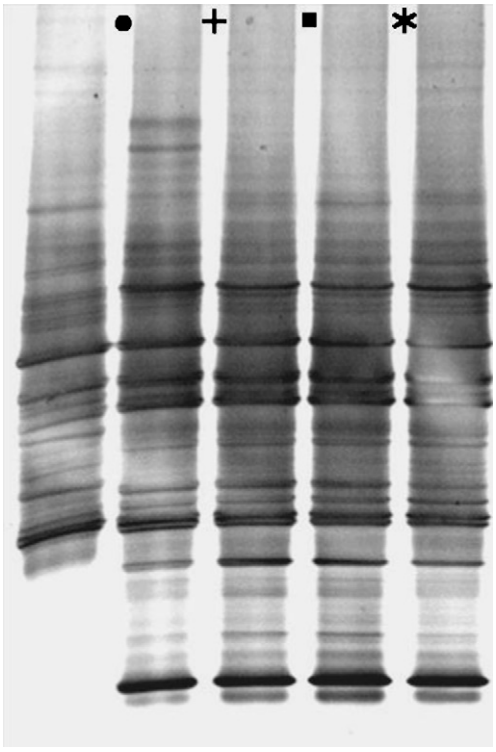

Fig. 6 – DGGE profile of 16S rRNA amplicons of the bacterial community in the ADB after the step changes in pollutant loading (*), process starvation (■), process shutdown (+) and failure in pH control system (●).

The air diffusion bioreactor showed a higher robustness to surges in inlet pollutant concentrations compared to the biofilter. This bioreactor was capable to cope with step concentration peaks of three and six fold probably due to its high k_La values and to the efficient pollutant uptake in the suspended culture (no diffusion limitations). These K_La values are quite high compared to those reported in literature. For example, Dorado et al. (2009) reported k_La values of 43.2 h^{-1} in a biotrickling filter packed with polyurethane foam, treating 6 g/m^3 of toluene and operated at an EBRT of 35 s. Besides, Kim and Deshusses (2008) recorded abiotic mass transfer coefficients (based on the gaseous phase) up to 600–900 h^{-1} in a biofilter using compost and woodchip mixtures as packed bed.

On the other hand, biofilter performance was severely affected during the six-fold step increase as shown by the decrease in toluene and H_2S-REs during the step change and by the poor REs for butanone and alpha-pinene immediately after the resumption of steady state operation. Toluene outlet concentration started to increase 30 min after inducing the concentration peak, while the increase in H_2S outlet concentration was immediately recorded. Butanone and alpha-pinene were initially adsorbed onto the packing material and gradually desorbed after restoration of steady state loadings.

These experimental findings suggest that process performance was limited by microbial activity rather than by pollutants mass transport (the VOCs adsorbed were biodegraded at lower rates than their maximum mass transfer from the gas phase), which resulted in a VOC build-up in the packed bed. This poor biodegradation performance during pollutant overloading in biofilters has been widely reported in literature for biofilters operated at high VOCs concentrations (approx. three orders of magnitude higher). For instance, Vergara-Fernández et al. (2007) observed a reduction in toluene RE from 98% to 83% when inlet loading was doubled in a compost-shell biofilter. On the other hand, alpha-pinene removal, which was always limited by its mass transfer, increased during the transient load due to the higher concentration gradient available for transport and to the capacity of the packing material to buffer surges in VOCs concentrations, the latter shown by alpha-pinene desorption following the restoration of steady state concentrations. This high buffer capacity of the packing material has already been observed by several authors and it is assumed to be responsible for the negative REs recorded after episodes of VOCs surges (Marek et al., 2000; Wani et al., 1998). However, less than 10 h were needed for the biofilter to recover steady state REs. In this context, the results reported in literature regarding the recovery of process performance after shock loads are diverse. Thus, while Barona et al. (2004) observed an instantaneous H_2S RE recovery after an eight fold step increase in pollutant concentration, Wani et al. (1998) recorded recovery times of few hours after a two fold step increase maintained for half an hour.

Another useful way to analyze the results obtained is to calculate the cumulative elimination capacity (CEC) (Choi et al., 2004), since it is not affected by the retardation of the pollutant in the bioreactor, thus permitting the assessment of the long-term effect of the perturbation. The CECs were calculated for butanone and toluene during the first six fold step in both bioreactors (Fig. 7). The pattern was similar for both VOCs, with a sharp initial increase (despite the decrease of REs) followed by a gradual and steady increase when the inlet concentration was restored.

Both bioreactors handled starvation periods of up to 3 days without any loss in removal efficiency or long-term damage. Steady state removal efficiencies were rapidly reached within the first 30 min after the resumption of pollutants supply. Likewise, a process shutdown of five day did not affect pollutant biodegradation performance in the biofilter during process restart probably due to a sustained cell maintenance on additional carbon sources such as the organic matter from compost in the absence of VOCs supply. Conversely, butanone, toluene and alpha-pinene REs in the ADB gradually dropped after process re-start and recovered within the next 3 h. This phenomenon, which was not observed during the three day starvation period, could be explained by a pollutant initial absorption and a deteriorated microbial activity following the five day process shutdown. In this context, the absence of additional carbon sources available for cell maintenance in the cultivation medium might have induced the low biocatalytic activities observed. The H_2S degrading community exhibited a remarkable robustness as shown by the absence of removal deterioration following starvation or process shutdown periods.

The biofilter was capable of supporting steady state REs for butanone and toluene during 4 days after a failure in the irrigation control system. At day 5, butanone and toluene removal efficiencies decreased by approx 20% and 10%, respectively. A low water activity in biofiltration systems is known to reduce microbial activity and the sorption of the more hydrophilic gaseous pollutants. The fact that steady state REs were recovered in less than 8 h together with the larger extent of RE deterioration for butanone support these

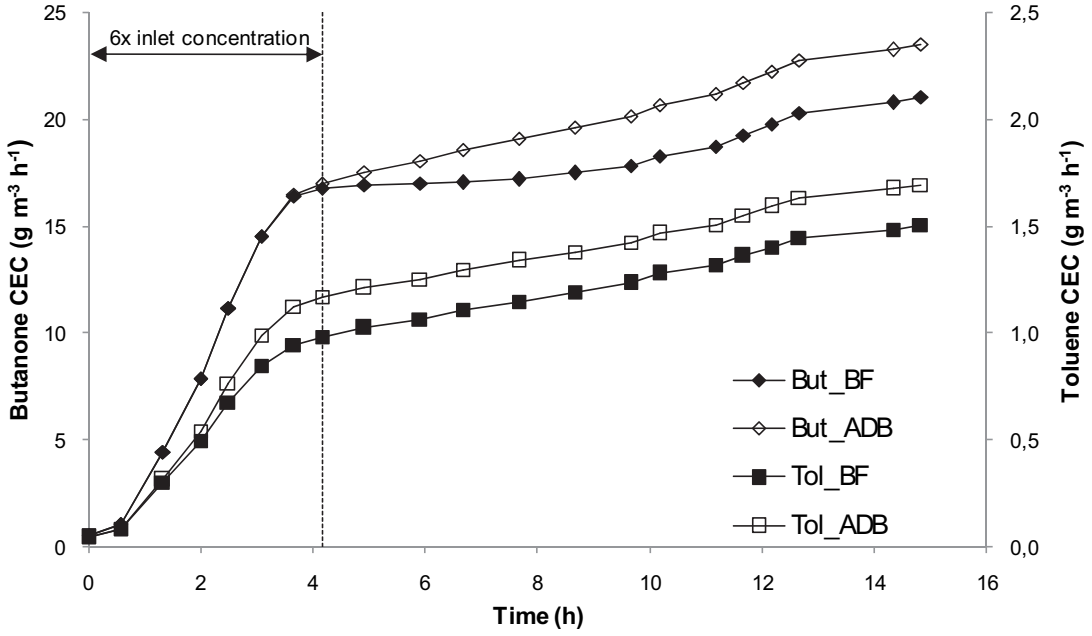

Fig. 7 – Effect of a six-fold inlet concentration step on the cumulative butanone (diamond) and toluene (square) elimination capacities in the biofilter (closed symbols) and the ADB (open symbols). Vertical dotted line represents the restoration of steady state inlet concentrations.

Table 1 – Estimated overall risks (R) of the BF and the AS systems treating butanone and toluene.

Upset	Probability of occurrence (p)	BF Risk (R)			AS Risk (R)		
		Butanone	Toluene	H_2S	Butanone	Toluene	H_2S
Fluctuations of temperature							
+10 °C	1 per day	−0.002%	0.097%	0	−0.002%	0.106%	0
−10 °C		0.006%	0.450%	0	0.002%	0.192%	0
Fluctuations of inlet odorants concentration[a]	1 per day (365 per year)	16.19%	2.67%	0.18%	0.013%	0.040%	0
No inlet pollutants	–	0	0	0	0	0	0
Process shutdown	1 per year	0	0	0	0.004%	0.021%	0
No pH control	1 per year	–	–	–	0	0.425%	0
No water	1 per year	0.010%	0.002%	0	–	–	–
TOTAL RISK		*16.2%*	*3.2%*	*0.18%*	*0.02%*	*0.78%*	*0*

a Odorants load increased by a factor of 6.

hypotheses. These results are in agreement with previous studies conducted at higher VOCs concentrations. For example, Sun et al. (2002) observed a decrease in the degradation rate from 100% to 60% when the initial biofilter moisture decreased from 60–70% to 30–40% in a compost-perlite biofilter. Morales et al. (2003) also recorded a gradual decrease in the elimination capacity of toluene, which decreased to a value close to zero when the water content was below the minimum required for microbial activity. Finally, the gradual biofilter dry-up was correlated with a decrease in the pressure drop through the biofilter bed. In the ADB, a sharp decrease in toluene RE occurred at pH < 3. Unlike previous tests, the restoration of pH to 6 did not result in a total recovery of toluene steady state performance, which remained 3% lower than the original REs even after seven days at pH 6. This damage in toluene microbial population by the low pH values was supported by the decrease in the number of bands (biodiversity) observed in leftmost lane of Fig. 6. In addition, acclimation to alpha-pinene was probably the mechanism underlying the increase in alpha-pinene removal in the biofilter and in the ADB since this increase occurred at approximately the same time and under totally different conditions (water activity stress and low pH stress, respectively).

The robustness of both systems towards process failures and operational fluctuations was calculated assuming that both bioreactors operate during 364 days per year, 24 h per day, and the risk was expressed as percentage of loss removal (the loss in total removal due to the upset divided by the total removal per year) (Table 1). The overall risk for operating the biofilter is 16.2% and 3.2% loss of total butanone and toluene removal per year, respectively, daily loading fluctuations being the main contribution to these risks. The total risk for the ADB was noticeably lower (0.02 and 0.78% loss of total butanone and toluene removal per year, respectively), mainly due to the higher robustness of ADB towards the daily step increases in pollutant concentration. However, despite a failure in the pH control system resulted in a significant deterioration in toluene RE, the risk associated to this upset was low due to its low probability of occurrence. Finally, the risk analysis herein conducted indicates that the overall risk for H_2S treatment in both biotechnologies was negligible, which confirmed that VOCs rather than H_2S removal is the key parameter to optimize in biotreatment designs (Iranpour et al., 2005).

5. Conclusions

The results herein presented indicated that both conventional biofilters and air diffusion bioreactors are reliable and robust biotechnologies for sustained H_2S and VOCs treatment, being able to rapidly recover from the negative impact of most common process fluctuations or operational failures. Both systems maintained high elimination efficiencies when temperature varied within the range 8 °C–30 °C. Likewise, pollutants starvation caused no deterioration in the abatement performance of both biofilter and ADB. Step increases in inlet concentration, commonly present under daily routine operation, severely affected process performance in the biofiltration unit. On the other hand, process shutdowns for five days caused a temporary decrease in the ADB REs whereas biofilter performance was not affected. Biofilter dry-up due to a five day interruption in process irrigation reduced butanone and toluene REs, although alpha-pinene biodegradation underwent a gradual enhancement, increasing from steady state value of 8.5 ± 0.2% to 63.3 ± 1.4%. A decrease in cultivation pH to 2.8 in the ADB due to a failure in the pH control system resulted in a significant deterioration in toluene RE and in a severe damage of the microbial community present in the bioreactor, which was not able to fully recover the initial steady state removal of this VOC. Finally, H_2S biodegradation was never affected by any of the process fluctuations or operational failures tested, with outlet concentrations in both bioreactors always below the detection limit of the electrochemical sensor.

Acknowledgments

This research was supported by the Spanish Ministry of Education and Science (RYC-2007-01667 and AP2008-01574

contracts and projects CTQ2009-07601, CTC2007-64324 and CONSOLIDER-CSD 2007-00055). The Regional Government of Castilla y Leon (Ref. GR76) is also gratefully acknowledged.

Appendix. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.watres.2010.05.008.

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Chapter 5

Characterization and Biofiltration of a Real Odorous Emission from Wastewater Treatment Plant Sludge

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Abstract

Biofilters have been widely employed for the treatment of malodorous emissions from sludge handling activities in wastewater treatment plants (WWTPs), although their optimized design is usually hindered by the lack of information on the dynamics of odorant formation. Besides, the odour abatement efficiency of biofilters has been rarely assessed on an individual odorant elimination basis. The characterization of odours from WWTP sludge in this study revealed the occurrence of a wide range of chemicals, including reduced sulfur compounds and volatile organic compounds (VOCs), and their dynamic concentration profile. The abatement of these odorants was evaluated in a compost-based biofilter at different empty bed residence times (EBRTs). Removal efficiencies (REs) higher than 99% were recorded for limonene, ketones and benzene, while toluene and DMTS REs exceeded 80% at an EBRT of 60 s. A stable biofilter performance was recorded despite the inlet odorant concentration fluctuations. Conversely, DMS and acetic acid were poorly removed due to their likely formation within the biofilter packing material. No correlation of the odorant elimination efficiency and their individual partition coefficients was herein observed.

Keywords: Biofiltration; Odour treatment; Sludge handling and treatment; Solid Phase Microextraction; Volatile Organic Compounds

INTRODUCTION

The treatment and handling of the residual sludge is one of the major sources of odorous emissions in wastewater treatment plants (WWTPs). These emissions contain not only H_2S and organic reduced sulfur compounds but also volatile organic compounds (VOCs) responsible for odour nuisance (Vincent, 2001). The negative effects of these malodorous emissions on human health and welfare have been consistently reported in the past years, with the subsequent increase in the number of public complaints and the enforcement of stricter environmental legislations (Lebrero et al. 2011a). Therefore, the abatement of these odorous emissions is nowadays a major concern for WWTPs operators.

A large number of technologies, based on both physical-chemical and biological mechanisms, have been applied to date for odour treatment. Biological technologies have been marketed as low-cost and environmentally friendly odour abatement methods, and constitute nowadays a robust and reliable alternative to physical-chemical technologies (Estrada et al. 2011). Off them, biofilters have been widely implemented for odour treatment since the early 50s due to their easier operation and maintenance, their lower operating costs and the extensive experience in their design and operation compared to their biological counterparts. Biofilters consist of a packed bed where the odorant-degrading microorganisms grow attached as a biofilm and where the air to be treated, previously humidified, is passed through. The pollutants are thus transferred from the malodorous air emission to the biofilm and then biodegraded by the microbial community to innocuous and odorless end-products (CO_2, H_2O, SO_4^{2-}, and biomass). The selection of the packing material during biofilter design is of key importance since it

constitutes the major contribution to the operating costs in biofiltration (Estrada et al. 2011). In this context, compost is still one of the preferred materials due to its high microbial richness and biodiversity, large contact areas, high air and water permeability, good buffering capacity and low cost (Easter et al. 2005; Prado et al. 2009).

Despite the above mentioned advantages, the performance of full scale biofilters is often limited by biofilter design, which itself is hindered by i) the lack of information about the dynamics of odorant generation and ii) lack of data of biofilter performance treating real odorous emissions, since most studies reported in literature are often carried out using synthetic odorous streams of either one or few odorants under constant operating conditions and at concentrations much higher than those typically found in WWTPs emissions (in the order of µg m^{-3}) (Iranpour et al. 2005). Besides, most of these studies focused on the abatement of H_2S and sulfur species as major odour surrogates, but the removal of individual VOCs was rarely investigated despite their toxicity, potential carcinogenicity and synergistic effects in terms of odour impact, which makes mandatory their elimination from malodorous emissions (Laing et al. 1994; Iranpour et al. 2005).

The purpose of this work was to elucidate the dynamics of the chemical composition of the odorous emissions produced during sludge handling. Furthermore, this study evaluated the VOC abatement performance of a compost-based biofilter treating a real odorous emission from sludge under different gas residence times.

MATERIALS AND METHODS

Sludge

The mixed activated sludge (primary and secondary) used throughout the experimentation was obtained from Valladolid WWTP (Spain). All samples were stored upon received at 5ºC before utilization.

Evaluation of odorant generation from mixed sludge

Glass bottles of 2 L were filled with 1.8 L of mixed sludge and maintained at 30ºC for 6 days. A sludge aliquot of 100 mL was periodically withdrawn from the incubation bottle and equally distributed into two 120 mL serological bottles, which were closed with butyl septa and sealed with 20 mm aluminum caps (Supelco, Bellefonte, PA, USA). These 50 ml sludge samples were magnetically agitated at 150 rpm in a water bath at 30ºC for 30 min before the analysis of the headspace composition by solid phase microextraction coupled with gas chromatography mass-spectrometry (SPME-GC-MS). This monitoring experiment was performed with mixed sludge samples retrieved from the WWTP in May, June and July (experiments 1, 2 and 3, respectively) in order to evaluate the temporal variability of the sludge odour emission potential.

Experimental set-up

The lab-scale plant was composed of an odour generation line and the odour treatment biofilter (Fig 1). The odour generation line consisted of a 7.5 L cylindrical PVC tank (25 cm of height and 20 cm of internal diameter), mechanically agitated by means of two paddle stirrers at 50 rpm and filled with 5.5 L of sludge. The sludge was previously sieved to remove the large solids and incubated for 2 days at 35ºC before being pumped to the tank at a concentration of 50 g L^{-1} and a flow rate of 1.5 L d^{-1}, which resulted in a solid retention time of 3.5 days. The wasted sludge was removed from the reactor once a day. The odorous emission was generated by aeration of the sludge through a ceramic diffuser located at the bottom of the tank at a flow rate imposed by the empty bed residence time (EBRT) set in the biofilter and controlled by a rotameter (Aalborg, Orangeburg, NY, USA).

The odorous stream was treated in a 2.8 L PVC biofilter (0.53 m of height and 0.083 m of internal diameter) operated at ambient temperature (22±3 ºC) and packed with 2 L of a mixture of compost (Pindstrup Mosebrug SAE, Spain) and perlite (World Minerals, Spain) at a ratio of 75%/25% v/v. The packing material, characterized according to standard methods (Lebrero et al., 2011b), exhibited a pH of 5.6, a porosity of 64.4%, a density of 0.22 g mL^{-1} and a water holding capacity of 41% (v/v). The humidity and temperature of the odorous emission were measured by a thermohygrometer (Testo 605-H1, Testo AG, Germany).

The odour generation line was operated for one week before being coupled to the biofilter. At day 8, the biofilter was inoculated with 1 L of aerobic activated sludge from Valladolid WWTP (Spain) and fed with the odorous emission. The biofilter was operated continuously for 36 days in order to test 3 different EBRTs: 120 s, 60 s and 40 s (corresponding to gas flow rates of 1, 2 and 3 L min^{-1}, respectively). The biofilter was irrigated every 4 days with 100 mL of tap water to avoid packing drying and to prevent the accumulation of secondary byproducts and/or metabolites. The inlet and outlet composition of the odorous stream was daily measured by SPME-GC-MS. Leachate samples were periodically collected to determine the pH, total organic carbon (TOC), inorganic carbon (IC) and total nitrogen (TN) concentrations. The dissolved oxygen (DO) was daily measured in the odour stripping tank. The pH of the biofilter packing material was analyzed at the end of the experimental period.

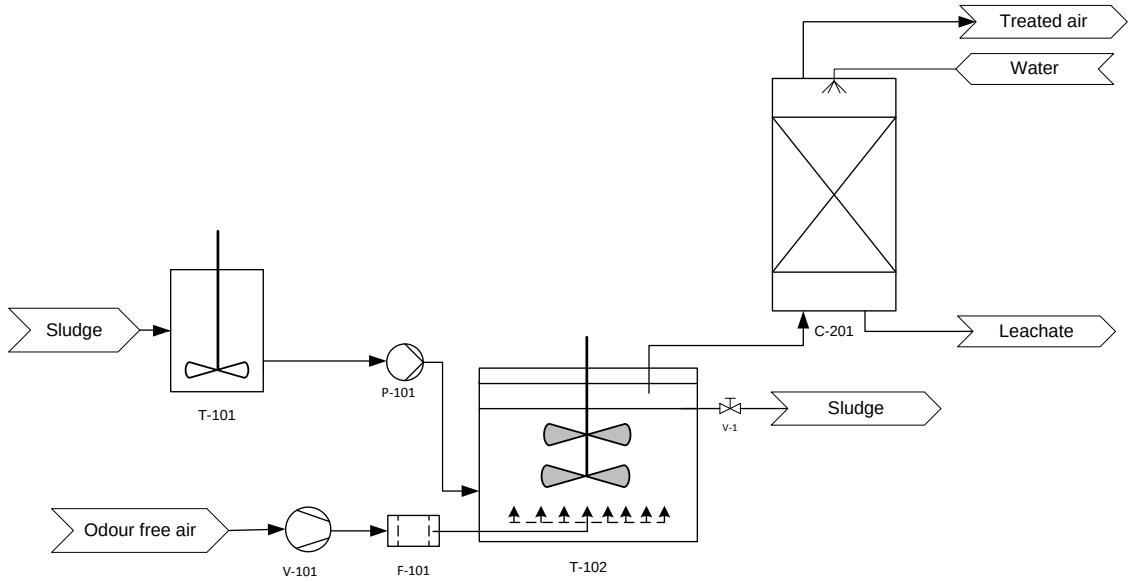

Fig. 1 Schematic representation of the experimental set-up. T-101: sludge storage tank, P-101: sludge dosing pump; V-101: air compressor, F-101: air flow controller, T-102: odour stripping tank, C-201: compost-based biofilter

Analytical procedures

Gas samples of the inlet and outlet odorous emission were collected in 500 mL glass bulbs (Supelco, Bellefonte, PA, USA) and pre-concentrated by SPME using 85 µm PDMS/Carboxen Stable Flex^{TM} fibers (Supelco, Bellefonte, PA, USA), which exhibit a high VOC and VSC extraction capacity (Kleeberg et al. 2005). These SPME fibers were initially conditioned for 5 hours at 280ºC and desorbed for 1 min prior to each analysis to ensure the absence of compounds adsorbed from previous determinations. The analysis of the odorants was performed in a gas chromatograph (6890, Hewlett-Packard, Palo Alto, CA) coupled with a mass spectrometer detector (5973 MSD, Hewlett-Packard) and equipped with a DB-WAX (30 m × 0. 25 mm × 0.25 µm, JW Scientific) capillary column. Adsorption and desorption times were 15 and 1 min, respectively. The temperature and split ratio of the injector were 320ºC and 1:10, respectively. The oven temperature was maintained at 40ºC for the first 15 min and increased at 8 ºC min^{-1} up to 120ºC. Helium was used as the carrier gas at 1.8 mL min^{-1}.

The DO in the odour stripping tank was periodically measured using a Multiline P4 Oxical-SL Universal Meter with an accuracy of ±0.04 mg O_2 L^{-1} (WTW, Germany). The pH analysis was conducted in a pH 510 pH/mC/°C meter (Eutech Instruments, The Netherlands). The determination of the TOC, IC and TN concentrations was carried out in a VCSH analyzer coupled with a TNM1 module (Shimadzu, Tokyo, Japan) in samples previously centrifuged at 10000 rpm for 10 min. The pH of the packing material was determined by standard methods (TMECC 2002) by mixing the packing media and water in a ratio 1:5 v/v. The sample was stirred for 20 minutes at ambient temperature and the pH was then measured.

RESULTS AND DISCUSSION

Evaluation of odorant generation from mixed sludge

Eight compounds from different chemical families (sulfur derived compounds, aromatics, terpenes, aldehydes and volatile fatty acids) were identified in the headspace of the bottles with a quality match > 98% (Fig. 2, in order of GC-MS retention time): methanethiol, toluene, limonene, benzene, dimetyl trisulfide (DMTS), acetic acid, benzaldehyde and propionic acid. These compounds were previously detected in WWTPs off-gases (Zarra et al. 2008) as well as in composting facilities treating WWTP sludge (Smet et al. 1999). Some of them are characterized by odour thresholds below 1 mg m^{-3}: methanethiol (4×10^{-5} mg m^{-3}), benzaldehyde (8×10^{-4} mg m^{-3}), DMTS (6.2×10^{-3} mg m^{-3}), propionic acid (8.4×10^{-2} mg m^{-3}) or acetic acid (9×10^{-2} mg m^{-3}). Conversely, benzene, limonene and toluene odour thresholds are 1.5, 1.7 and 3.8 mg m^{-3}, respectively (Ruth 1986, Suffet et al. 2004, Gallego et al. 2012).

Organic sulfides are often found in malodorous emissions from sludge thickening, dewatering and

storing (Vincent 2001), and are responsible for the characteristic rotten cabbage/garlic odour. The concentration of methanethiol and DMTS increased at the beginning of the tests under anaerobic conditions (Fig. 3a and 3b, respectively). However, while methanethiol concentration remained approximately constant after this initial increase and slightly decreased after 100 h of experiment, DMTS concentration gradually decreased from 50 h onwards, regardless of the sludge sample evaluated. During sludge fermentation under anaerobic conditions, the hydrolysis of proteinaceous materials and organic sulfur-containing compounds results in the production of mercaptans and sulfides (Vincent 2001). The emission pattern of these odorants could be correlated to the metabolic activity of the hydrolytic bacteria (anaerobic or facultative anaerobic species), while the final decrease in methanethiol and DMTS concentrations might be due to their late biodegradation by sulfide-degrading microorganisms in the sludge sample.

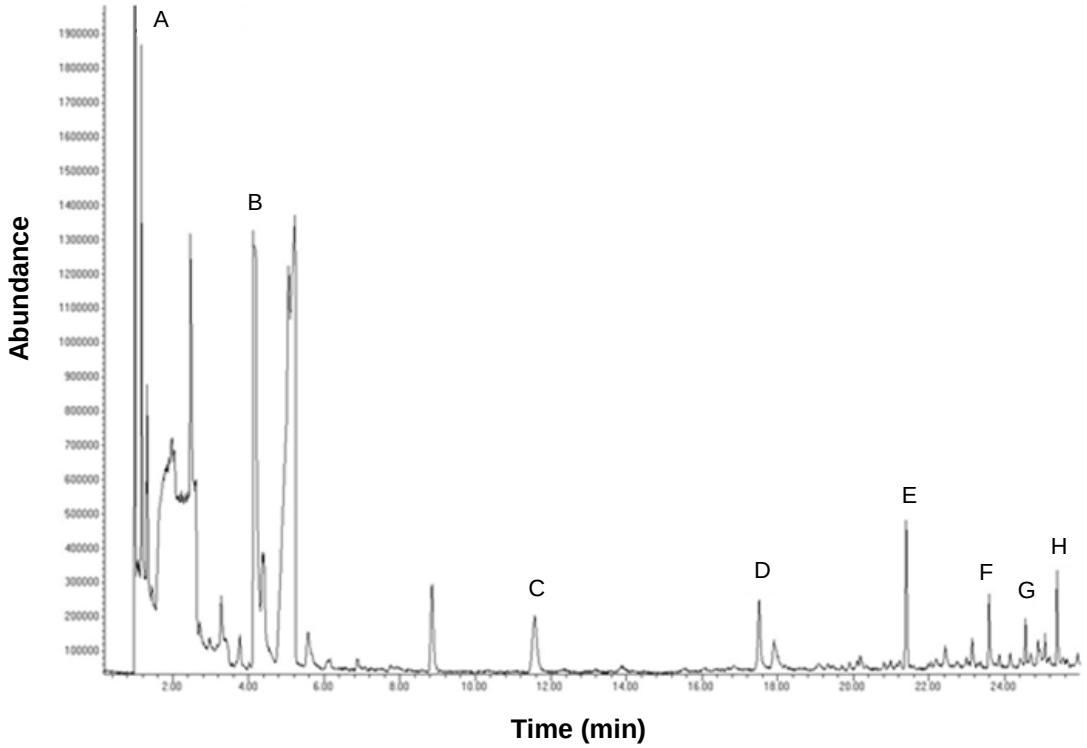

Fig. 2. Characterization by SPME-GC-MS (quality match > 98 %) analysis of the odorants generated from the sludge: A: methanethiol (1,161s), B: toluene (4,160 s), C: limonene (11,552 s), D: benzene (17,542 s), E: dimethyl trisulfide (21,390 s), F: acetic acid (23,586 s), G: benzaldehyde (25,072 s), H: propionic Acid (25,544 s).

Toluene and benzene are aromatic hydrocarbons usually discharged to municipal treatment plants with industrial wastewater. Previous studies demonstrated the negative effect of these pollutants on the performance of biological nutrients removal processes and on human health (Mrowiec and Sushka, 2010). In our particular study, the toluene concentration emitted from the mixed sludge increased constantly throughout the experiment (Fig. 3c). In this context, the release of toluene from oxygen-depleted sludge has been previously reported in literature and suggests that toluene is formed by natural processes instead of being simply desorbed and stripped under anaerobic conditions. For instance, Devoldere et al. (2001) observed an increase of two orders of magnitude in toluene emissions from anaerobic sludge while other investigations recorded formation of toluene during the acidogenic phase of sludge digestion (Mrowiec et al. 2005). However, the mechanisms underlying this phenomenon are still unknown and deserve further investigation due to the toxic nature of toluene. On the other hand, benzene concentration also increased for the first 50 hours, although a steady decrease in benzene emission likely due to biodegradation was observed afterwards (Fig. 3d) (Mrowriec and Sushka, 2010).

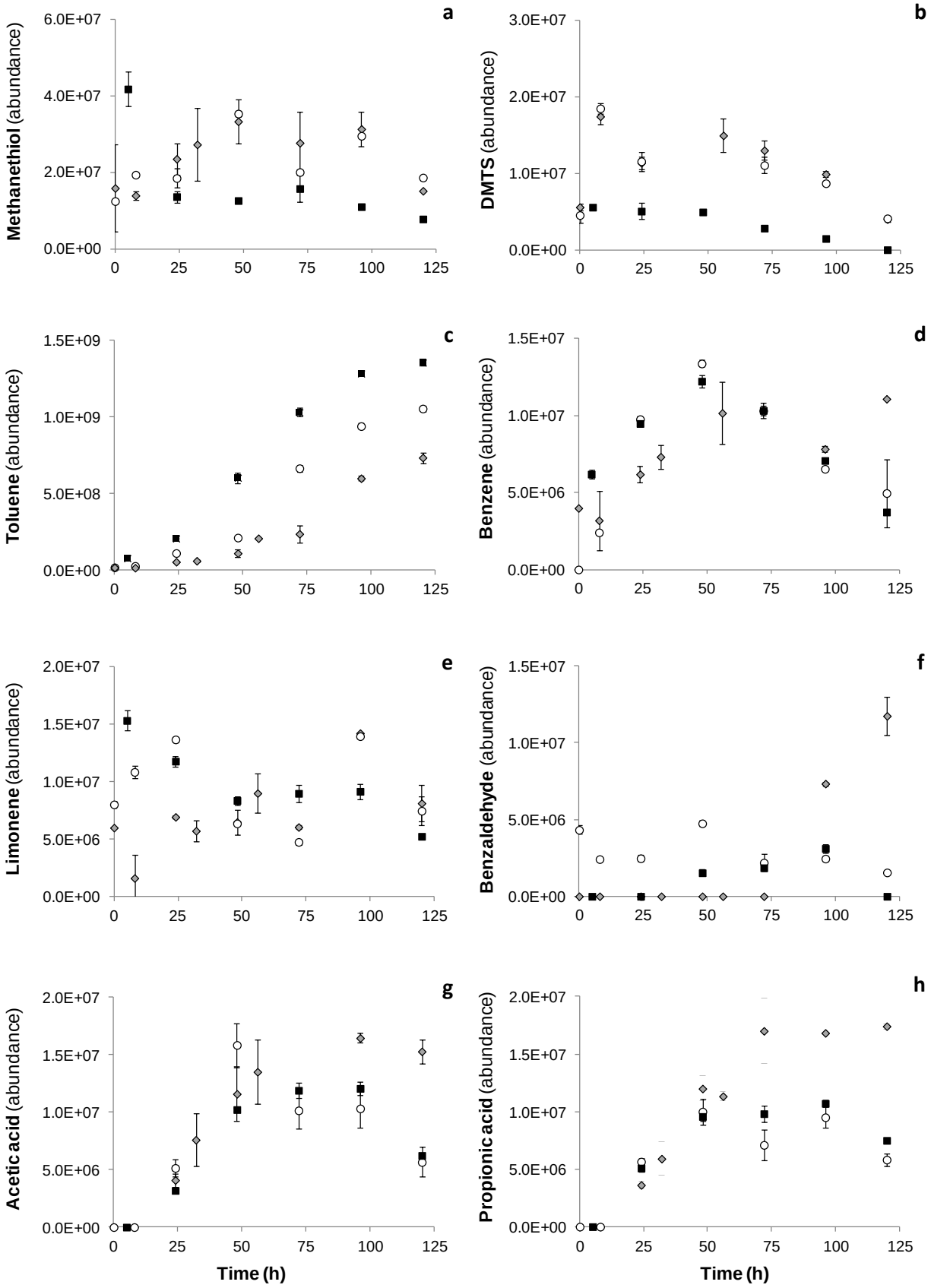

Fig. 3. Time course of the headspace concentration, as abundance, of (a) methanethiol, (b) dimethyl trisulfide, (c) toluene, (d) benzene, (e) limonene, (f) benzaldehyde, (g) acetic acid and (h) propionic acid from the sludge in May (♦), June (○) and July (■). Vertical bars represent the standard deviation from two independent measurements.

Limonene concentration in the sludge headspace fluctuated over the monitored period with no particular trend (Fig. 3e). No formation, biodegradation or effect of oxygen limitation in the limonene concentration was clearly observed during the odour monitoring tests. This terpene is commonly discharged to wastewaters from industrial operations (mainly from terpene-based cleaners) and subsequently released during sludge handling activities (Alvarez et al. 1999; Smet et al. 1999).

Benzaldehyde (Fig. 3f), acetic acid (Fig. 3g) and propionic acid (Fig. 3h) are typical intermediates of fermentation processes. The breakdown of complex organic matter in the hydrolysis step (polysaccharides, proteins and lipids are transformed into monosaccharides, amino acids and fatty acids, respectively) is followed by a fermentation step, where primary and secondary alcohols, carboxylic acids and aldehydes are formed. In our particular test, the concentration of these compounds remained low and nearly constant at the beginning of the tests (hydrolysis phase). Benzaldehyde slightly increased after 75 hours of sludge incubation (this increase being more noticeable in the sludge from May), whereas both acetic and propionic acid emissions gradually increased after the first 10 hours. Surprisingly, while a considerable decrease was observed after 75-100 hours of incubation in the sludge samples retrieved in June and July due to their anaerobic oxidation, the concentration of these odorants increased or remained constant at the end of the test in the sludge from May.

Due to the qualitative nature of this study, only a comparison of the emission profile dynamics for each odorant was feasible. A quantitative analysis requires multi-component standards including all the compounds present in the emission (and therefore the identification of all the compounds) due to the competitive adsorption mechanisms occurring in the fiber, especially among low molecular weight compounds (Kleeberg et al. 2005). In brief, the emission pattern of the identified odorants in the three sludge samples was reasonably similar, whereas the recorded areas differed as expected due to the inherent variation of the mixed sludge characteristics throughout the year.

Treatment of the odorous air emission

The stripping tank remained under anaerobic conditions during the entire experimentation period despite the aeration, as shown by the low DO concentrations measured in the stripping chamber (average value of 0.3 ± 0.06 mg L^{-1}). Similar compounds to those observed in the batch odorant generation tests were identified by SPME-GC-MS in the odorous stream exiting the stripping tank: dimethyl sulphide (DMS), dimethyl trisulphide (DMTS), toluene, benzene, acetone, butanone, limonene and acetic acid. While anaerobic conditions due to insufficient aeration may induce the formation sulfur derived compounds, incomplete aerobic degradation often results in the generation of alcohols, ketones or organic acids (Smet et al. 1999). Despite being an important contributor to malodors produced from the degradation of organic matter in the absence of oxygen and nitrate, H_2S was not detected in the stripped air (Dräger X-am 5000, lower detection limit = 0.5 ppm_v). The odorant emission rate depends upon the concentration of the VOCs dissolved in the sludge, their volatility, the surface area exposed to the gas phase, the degree of turbulence and aeration and the residence time of the sludge in the tank. In our particular study, the highest odorant concentrations were recorded at the highest aeration rate (corresponding to an EBRT in the biofilter of 40 s) likely due the rapid stripping of volatile substrates, except for DMS, toluene and limonene. At this point it must be stressed that the conditions prevailing in the stripping tank (anaerobic conditions in the presence of an air atmosphere) are similar to those existing during sludge thickening and handling in WWTP.

The average removals observed in the biofilter were very high (>99%) for limonene and acetone, regardless of the EBRT and despite acetone concentration increased by a factor of 4 at an EBRT of 40 s (Fig. 4a and 4b, respectively). Previous studies on waste gas biofiltration also reported efficient removals for these compounds (Prenafeta-Boldú et al. 2012). However, while acetone is a water-soluble, easily biodegradable pollutant, limonene is a hydrophobic terpene relatively difficult to transport from the gas phase to the aqueous biofilm. Therefore, the biofilter elimination capacity was not correlated to the VOC biodegradability and/or water solubility. Besides, the high abatement performance recorded for odorants with such varied mass transport concentration gradients confirmed the high transfer capacity of compost-based biofilters. Butanone and benzene removal was also high at 60 and 120 s of EBRT (>99%), although an unstable abatement performance was observed in the biofilter when the EBRT was decreased to 40 s (Fig. 4c and 4d, respectively). Likewise, the biofilter supported

DMTS RE > 80% at EBRTs higher than 60 s after an initial acclimation period of 4 days (Fig. 4f), but a fluctuating abatement following the decrease in EBRT, with REs as low as 28%. However, it is not possible to elucidate whether biodegradation or mass transfer limited odorant elimination at the lowest residence time due to the significant increase in their concentration. Similarly, toluene REs ranging from 32% to 97% were recorded at an EBRT of 40 s, while higher contact times not only mediated an increase in the RE but also a more stable abatement performance (RE > 87%) (Fig. 4e).

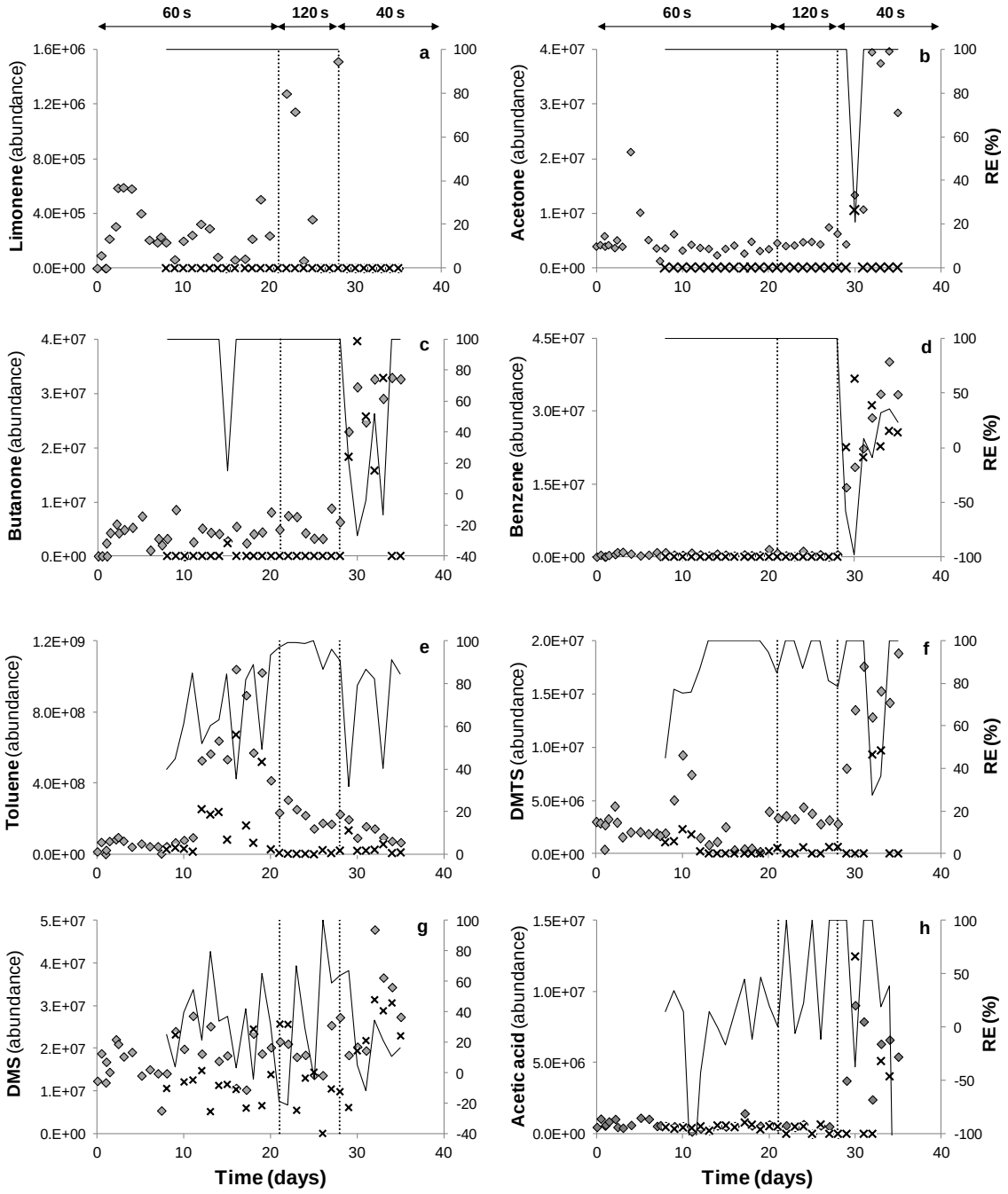

Fig. 4. Time course of the odorant inlet (◊) and outlet (×) concentrations in the biofilter as abundance of (a) limonene, (b) acetone, (c) butanone, (d) benzene, (e) toluene, (f) dimethyl trisulfide, (g) dimethyl sulfide and (h) acetic acid). The continuous line represents the biofilter removal efficiency (secondary axis) and the vertical dashed lines represent the changes in EBRT.

The biofilter was not capable of efficiently removing DMS and acetic acid, with REs fluctuating between 99% and negative values (Fig. 4g and 4h, respectively). These results suggested that both odorants were likely formed within the biofilter, probably due to the presence of anaerobic zones in the compost packing since the generation of sulfur derived compounds and short chain acids from oxygen-depleted sludge was already observed during the odour monitoring tests.

The TOC and TN concentrations present in the biofilter leachate initially increased from 76 and 109 mg L^{-1} to maximum values of 323 and 197 mg L^{-1}, respectively, after 7 days of operation, which was likely due to the leaching of organic matter and N-compounds present in the compost. Both parameters steadily decreased afterwards and finally achieved steady values of 100±1 mg TOC L^{-1} and 73±2 mg TN L^{-1}. Therefore, no nitrogen limitation occurred throughout the biofilter operation although the high rates of nitrogen leaching might derived in N limiting scenarios under long term biofilter operation. The pH of the leachate gradually decreased from 6.5 to 3.02 at day 27 due to the production of acidic by-products during odorant biodegradation. The acidification of the media was also confirmed at the end of the experiment by measuring the pH of the packing medium, which exhibited values of 6.44, 4.97 and 4.15 at the top, middle and bottom sections. Although a neutral pH is optimal for microbial activity, and therefore odorant biodegradation (Mudliar et al. 2010), no deterioration of the biofilter performance was attributed to this decrease in pH since microbial communities can rapidly adapt to mild acidic environments. Indeed, Lebrero et al. (2011b) previously reported the acclimation capacity of activated sludge and indigenous compost microorganisms to pH as low as 1.3 without an important loss in the VOC abatement capacity of the biofilter

A significant variability in the individual VOC removal performance of biofilters treating odorous emissions can be found in the literature. For instance, Iranpour et al. (2005) found VOC REs ranging from 20% to 90% (even for easily biodegradable compounds such as acetone or toluene) in a study comparing 40 pilot-plant and full-scale biofilters treating the emissions from WWTPs and other facilities. BTEX compounds such as benzene and toluene were generally efficiently removed, with RE > 80% at EBRTs higher than 60 s (Ergas 1995, Liu 2009, Lebrero et al. 2011b). On the other hand, the typical REs reported for terpenes such as α-pinene or limonene varied between 30% and > 99% (Ergas et al. 1995, Liu et al. 2009, Prenafeta-Boldú et al. 2012). Overall, low REs are recorded for sulfur derived compounds such as DMS or DMDS (Iranpour et al. 2005, Prenafeta-Boldú et al. 2012), although some authors have reported REs > 99% for organic sulfides and disulfides (Liu et al. 2009). In our particular case, while satisfactory removals were observed for DMTS at EBRT > 60 s, a low DMS abatement efficiency was obtained at the EBRTs tested. The formation of sulfur derived compounds within the biofilter packing material as a result of the development of anaerobic zones could have mediated the low removals observed for DMS.

The correlation between the removal of a particular VOC and its mass transfer properties and biodegradability is still a hot topic for discussion in the biofiltration field. For instance, Prenafeta-Boldú et al. (2012) found positive and negative correlations between the biofilter RE values and the air/octanol (Kao) and water/air (Kwa) partition coefficients, respectively, while no significant influence of the octanol/water (Kow) or the VOC intrinsic biodegradability was found. In our particular case, no correlation between the VOC elimination and Kow (R^2=0.17, Fig. 5a), Kao (R^2=0.28, Fig. 5b) or Kwa (R^2=0.38, Fig. 5c) was recorded, since odorant removal was the result of the combination of complex interdependent processes such as adsorption, volatilization, biodegradation and odorant formation.

CONCLUSIONS

This study confirmed the complex composition of gas emissions from sludge handling activities, containing not only sulfur reduced compounds but also aromatics, terpenes, aldehydes and volatile fatty acids. The dynamics of odorant formation was mainly governed by the sludge anaerobic fermentation process, and more specifically by the hydrolysis and acidogenesis. The compost-based biofilter demonstrated high and stable removal efficiencies for most of the odorants emitted from WWTPs sludge under real operating conditions in spite of the fluctuating odorant concentrations, with REs > 99% for limonene, acetone, butanone and benzene, and higher than 80% for toluene and DMTS at an EBRT of 60 s. However, the formation of DMS and acetic acid within the biofilter packing media likely resulted in periodical negative removals. No correlation between the

biodegradability/Kow/Kao/Kwa of the odorants and the biofilter removal capacity was found.

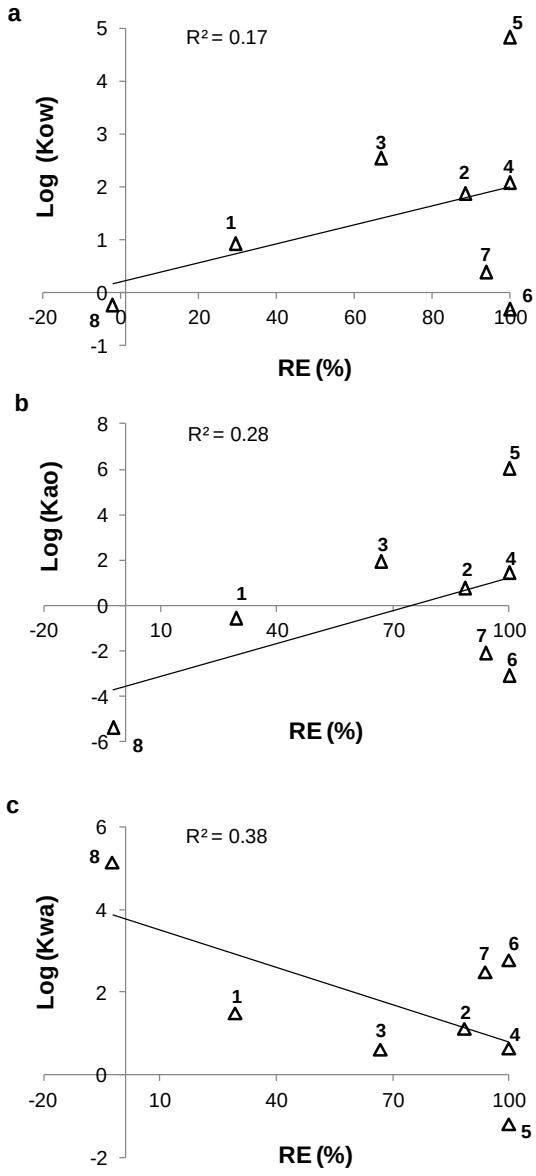

Fig. 5. Correlation of the average removal efficiency for dimethyl sulfide (1), dimethyl trisulfide (2), toluene (3), benzene (4), limonene (5), acetone (6), butanone (7) and acetic acid (8) at an EBRT of 60 s and their octanol-water partition coefficient (a, Kow), air-octanol partition coefficient (b, Kao) and water-air partition coefficient (c, Kwa). Data collected from Sangster, 1989 and Sander, 1999.

ACKNOWLEDGEMENTS

This research was supported by the Spanish Ministry of Economy and Competitiveness (RYC-2007-01667 contracts and projects CTQ2009-07601 and CONSOLIDER-CSD 2007-00055). The Regional Government of Castilla y León is also gratefully acknowledged for its funding (Project VA004A11-2).

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(2012). Bioresource Technology 109: 38-45

Chapter 6

Odor abatement in biotrickling filters: Effect of the EBRT on methyl mercaptan and hydrophobic VOCs removal

Bioresource Technology 109 (2012) 38–45

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Odor abatement in biotrickling filters: Effect of the EBRT on methyl mercaptan and hydrophobic VOCs removal

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ARTICLE INFO

Article history:
Received 21 November 2011
Received in revised form 10 January 2012
Accepted 13 January 2012
Available online 21 January 2012

Keywords:
Odor treatment
Biotrickling filter
Methyl mercaptan
VOC biodegradation
Microbial diversity

ABSTRACT

The performance and microbiology of a biotrickling filter (BTF) treating methyl mercaptan, toluene, alpha-pinene and hexane at the mg m^{-3} level was studied at empty bed residence times (EBRT) of 50, 30, 11 and 7 s. Removal efficiencies (REs) higher than 95% were observed for MeSH, toluene and alpha-pinene even at 11 s, while hexane REs exceeded 70%. At 7 s, an irreversible damage of the microbial activity due to the accumulation of toxic metabolites resulted in a decrease of REs. The addition of silicone stabilized process performance but only re-inoculation allowed achieving a complete removal of MeSH, toluene and alpha-pinene, and hexane REs of 80%. The high K_La values (ranging from 38 ± 4 to 90 ± 11 h^{-1}) explained the good BTF performance at such low EBRTs. A high bacterial diversity, along with a vertical distribution of the bacterial communities was observed, the main phyla being *Proteobacteria*, *Actinobacteria*, *Nitrospira*, *Chloroflexi* and *Gemmatimonadertes*.

1. Introduction

Biotrickling filters (BTFs) are becoming one of the most promising treatment technologies for odor control (Cox and Deshusses, 2002). This technology relies on odorant degrading microorganisms growing in a biofilm attached to an inert packing material (usually random or structured plastic rings, open pore foam or lava rock). The odorous stream passes through the packed bed transferring the odorants to the biofilm while an aqueous solution is trickled over the packing media to provide water and essential nutrients to the microbial community. BTFs exhibit all the advantages of biotechnologies when compared to conventional physical/chemical methods: lower operating costs, lower environmental impact, high efficiency and robustness, etc. (Estrada et al., 2011). However, BTFs are superior to biofilters (the most commonly implemented biotechnology) due to their lower operating costs, lower empty bed residence time required (EBRT) (thus lower footprint), lower head loss even at higher gas velocities and better control of the operating parameters (pH, nutrients, temperature and accumulation of toxic metabolites) (Estrada et al., 2011).

Biotrickling filters show a high abatement performance when treating H_2S, a highly soluble odorant usually present in wastewater treatment plants (WWTPs) odorous emissions, with removal efficiencies (REs) higher than 99% at EBRT ranging from 2 to 10 s (Cox and Deshusses, 2002). However, WWTP odorous emissions also contain a wide range of moderately and highly hydrophobic volatile organic compounds (VOCs) such as terpenes, aromatic and aliphatic hydrocarbons, sulfur organic odorants, etc., whose removal is mandatory for an efficient odor abatement. In this context, biotrickling filtration performance can be limited by the low transfer rates of these hydrophobic pollutants from the gaseous phase to the microorganisms present in the aqueous phase as a result of the low concentration gradient available for mass transport (low driving force). Despite the performance of BTFs for the removal of H_2S or industrial VOCs at high concentrations has been investigated (Cox and Deshusses, 2002; Kennes et al., 2009), there is a lack of studies on the treatment of off-gases containing mixtures of hydrophobic VOCs and sulfur compounds at the concentrations typically found in WWTPs, which range from μg m^{-3} to mg m^{-3} according to Zarra et al. (2008).

The microbial populations established in BTFs also play a key role in the malodour abatement performance. In this regard, the systematic study of the spatial and temporal dynamics of microscopic ecosystem components, in combination with macroscopic components, provides clues to disentangle the potential relationships between ecosystem function and community structure (Cabrol and Malhautier, 2011). Despite molecular techniques allow addressing this issue, their application in biological systems for odor control is scarce compared to other biotechnologies such as anaerobic or aerobic wastewater treatment (Cabrol and Malhautier, 2011).

In this work, the biodegradation of a mixture of methyl mercaptan (MeSH) and three hydrophobic VOCs (toluene, alpha-pinene and hexane) at trace level concentrations was investigated in a

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doi:10.1016/j.biortech.2012.01.052

BTF packed with polyurethane foam (PUF). The pollutants were selected within a wide range of solubility (Henry's law constants (H) = 3.8×10^{-1}, 1.6×10^{-1}, 4.9×10^{-2} and 7.3×10^{-4} M atm^{-1} for MeSH, toluene, alpha-pinene and hexane, respectively) (Sander, 1999). Low H values impair the mass transfer of the VOCs from the gaseous phase to the aqueous phase as a result of a reduced concentration gradient (driving force). The abatement performance and the volumetric mass transfer coefficients of the BTF (K_La) were evaluated at different EBRTs (50, 30, 11 and 7 s). The profiles, diversity and composition of the bacterial communities at different stages of the bioreactor operation were studied by the PCR–DGGE molecular technique in order to get more insights into the mechanisms underlying odor removal in BTFs.

2. Methods

2.1. Chemicals

Toluene and silicone oil with a kinematic viscosity of 20 centistokes were purchased from Sigma–Aldrich with a purity higher than 99.9%. All other chemicals and reagents were purchased from PANREAC with a purity higher than 99% (Barcelona, Spain).

2.2. Experimental set-up

The BTF consisted of a cylindrical jacketed PVC column with a working packed bed volume of 4 L (8 cm internal diameter and 100 cm height) (Fig. 1). The packing material of the BTF was 1 cm^3 PUF cubes (Filtren TM 25280, Recticel Iberica S.L.) with a net density of 20–24 kg m^{-3} and a specific surface area of 1000 m^2 m^{-3}. PUF was selected as the packing material due to its large specific surface area, high porosity, resistance to compaction and relatively low price (Dorado et al., 2009). The bioreactor was operated in a countercurrent configuration. Two perforated plates, one at the bottom and one at the top of the bioreactor, acted as distributors of the odorous emission and the recycling liquid mineral medium, respectively.

The odorous stream was obtained by mixing a MeSH, toluene, alpha-pinene and hexane mixture from a calibration bottle (Abello Linde S.A., Spain) with an air stream previously humidified and filtered through an activated carbon bed. The odorant concentrations were accurately controlled by a mass flow controller (Aalborg, Denmark) at 22.2 ± 1.4 mg m^{-3} for MeSH, 0.22 ± 0.03 mg m^{-3} for toluene, 0.23 ± 0.03 mg m^{-3} for alpha-pinene and 0.28 ± 0.02 mg m^{-3} for hexane.

The reactor was operated at a constant temperature of 20 °C. One liter of sulfate-free mineral salt medium (MSM) prepared according to Muñoz et al. (2008) was recycled at 0.63 m h^{-1}. The trickling solution was continuously agitated (200 rpm) in an external 1 L tank with automatic pH control at 7.0 ± 0.1 (BL-7916-2 Black Stone pH controller) by addition of a 9.2 g NaOH L^{-1} and 12.2 g Na_2CO_3 L^{-1} solution. An abiotic test was initially performed to assess the abiotic removal (adsorption and photolysis) of the target odorants. The BTF was operated for 52 h at an EBRT of 30 s in absence of biocatalytic activity.

2.3. Microbial acclimation and BTF operation

The BTF was inoculated with 2 L of PUF containing an acclimated activated sludge and 2 L of new PUF. The activated sludge was acclimated during 20 days to MeSH and the target VOCs in a similar BTF at the same concentrations until REs >98% were achieved for all the target compounds. After inoculation, fresh MSM was continuously pumped to the BTF to supply the nutrients required for microbial growth and to compensate for water evaporation and sampling losses.

The influence of decreasing EBRTs (50, 30, 11 and 7 s) on odorant removal performance was evaluated. An increasing fraction of the trickling solution (60 mL at 50 s, 100 mL at 30 s, 150 mL at 11 s and 200 mL at 7 s) was daily replaced by fresh MSM to prevent for sulfate accumulation. At day 40 (corresponding to the operation at an EBRT of 7 s) a gradual decrease on odorant REs started to occur and 200 mL of the trickling MSM were replaced by silicone oil at day 48 (corresponding to 20% on a volume basis) in order absorb any potential accumulation of toxic metabolites and stabilize the biodegradation process. Silicone oil was then gradually wasted from the BTF to restore operation with an aqueous trickling solution and by day 59 the EBRT was increased back to 11 s. Once steady state REs were achieved again at day 89, the BTF was re-inoculated with 1 L of fresh activated sludge re-suspended in MSM. The EBRT was decreased again to 7 s by day 123 with a daily

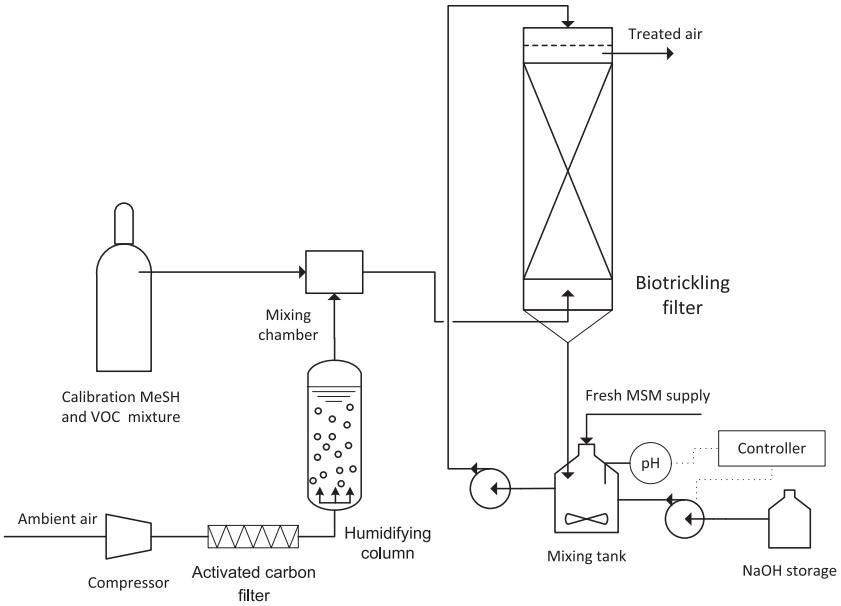

Fig. 1. Schematic representation of the experimental set-up.

MSM exchange of 300 mL. Each steady state was maintained for at least 1 week in order to ensure stable process operation.

The concentrations of MeSH and the target VOCs were periodically monitored at the influent and effluent gas sampling ports. The pH and the concentration of SO_4^{2-} were also periodically recorded in the trickling solution. Likewise, the moisture content of the inlet odorous emission was continuously monitored. Finally, silicone oil biodegradation by the microbial consortium present in the BTF was assessed for 121 days. Biodegradability tests were conducted in 120 mL glass flasks supplied with 10 mL of silicone oil and 10 mL of the microbial consortium. The flasks were closed with butyl septa, sealed with aluminum caps and incubated under magnetic agitation (300 rpm) in a water bath at 30 °C. Control flasks were prepared and incubated under similar conditions with 10 mL of MSM instead of silicone oil in order to account for bacterial endogenous respiration. The headspace CO_2 concentration of the flasks was periodically monitored.

2.4. Experimental evaluation of K_La

The K_La values in the BTF were determined according to Dorado et al. (2009) at each EBRT tested (7, 11, 30 and 50 s). The BTF was filled with 4 L of PUF and operated at 20 °C. A pre-humidified 5 g toluene m^{-3} gas stream was supplied to the bottom of the reactor, while 1.4 L of MSM were recycled at 0.63 m h^{-1} counterflow-wise. Inlet and outlet toluene concentrations were periodically measured in the gas stream up to saturation ($C_{g,out}/C_{g,in} \geqslant 0.97$). Five milliliters of liquid samples at the inlet and outlet of the filtration bed were also periodically drawn and injected in 10 mL gas-tight serological bottles (closed with butyl septa and sealed with aluminum caps). Toluene in the gas-tight bottles was allowed to equilibrate prior to headspace analysis and estimation of the toluene aqueous concentration by mass balance calculations. These K_La determinations were carried out in duplicate. The individual film coefficients (K_L) for the other VOCs were estimated from their corresponding diffusivities using the Van Krevelen & Hoftijzer equation, assuming that the main resistance for the mass transfer of the compounds lies in the water film (Lebrero et al., 2012). A good correlation between the experimental data and the values obtained by this empirical correlation was recently demonstrated (Lebrero et al., 2012).

$$K_L = 0.015\left(\frac{D_L}{\left[\mu_L^2/(\rho_L^2 g)\right]^{1/3}}\right)\left(\frac{\rho_L \mu_L}{\mu_L a_e}\right)^{2/3}\left(\frac{\mu_L}{\rho_L D_L}\right)^{1/3}$$

where μ_L and ρ_L are the solvent viscosity (kg m^{-1} s^{-1}) and density (kg m^{-3}), respectively, u_L is the liquid superficial velocity (m s^{-1}), g is the gravitational constant (m s^{-2}), and a_e is the effective specific interfacial area (m^{-1}).

The Wilke–Chang equation was used to calculate the diffusion coefficients (D_L).

$$D_L = \frac{7.4 \times 10^{-8}(\alpha M_B)^{1/2} T}{\mu_B V_A^{0.6}}$$

where α is the association factor of the solvent (2.6 for water), M_B is the molecular weight of the solvent (g mol^{-1}), T is the temperature in K, μ_B is the viscosity of the solvent (cP), and V_A is the molar volume of the solute A at its normal boiling temperature (cm^3 mol^{-1}).

2.5. Analytical procedures

MeSH was analyzed using an electrochemical sensor (*Dräger X-am 7000*) calibrated in the 0–20 ppm range. VOCs analyses were carried out by SPME-GC-FID according to Lebrero et al. (2010). Carbon dioxide was analyzed in a GC–TCD (Lebrero et al., 2010). Toluene gas concentration in the K_La tests was determined in a GC-FID (HP 6890 Series, Hewlett Packard, USA) equipped with a SupelcoWax (15 m × 0.25 mm × 0.25 μm) capillary column. Oven, injector and detector temperatures were maintained at 140 °C, 150 °C and 200 °C, respectively. Helium was used as the carrier gas at 2 mL min^{-1} while H_2 and air were fixed at 30 and 300 mL min^{-1}, respectively. N_2 was used as the make up gas at 28 mL min^{-1}.

Liquid samples were filtered and the supernatant was used to measure the pH using a pH/mV/°C meter (pH 510 Eutech Instruments, Nijkerk, The Netherlands). Sulfate concentration was determined by HPLC-IC using an IC-Pak Anion HC (150 mm × 4.6 mm) column in 1 mL liquid samples previously filtered through a 0.22 μm filter.

The moisture content in the influent odorous stream was measured using a Testo 605-H1 thermohygrometer (Testo AG, Germany).

2.6. Microbiological procedures

Samples for microbial analysis were collected and stored at −20 °C. Samples of the inoculum (sample A) and day 0 of operation (sample B) were collected in order to determine the effect of the 20 days acclimation period over the bacterial communities. Sample C was collected at day 89 just prior to the re-inoculation of the system. A sample of the activated sludge used as inoculum at day 89 was also collected (sample D). All samples extracted from the biotrickling system were taken from its upper part. Finally, samples from the upper (sample E1), medium (sample E2) and lower (sample E3) parts of the reactor were collected at the end of the experiment in order to evaluate the changes in microbial communities as a function of the BTF height. Samples collected from the reactor were vortexed and centrifuged to separate the biofilm from the PUF packing material and concentrate the biomass.

Genomic DNA was extracted using the protocol described in the Fast® DNA Spin Kit for Soil (MP Biomedicals, LLC) handbook, but adjusting the time of binding of DNA to a silica matrix to 1 h. The PCR mixture (50 μL) contained 5 U of *Taq* polymerase (Ecogen), Taq polymerase buffer (10 mM), $MgCl_2$ (50 μM), deoxynucleotide triphosphates (10 mM), l or 2 μL of the extracted DNA, PCR primers 968-F-GC and 1401-R (10 μM) (Sigma–Aldrich, St. Louis, MO, USA) for bacterial 16S rRNA gene amplification, and Milli-Q water up to a final volume of 50 μL. The PCR thermo-cycling program consisted of 2 min of pre-denaturation at 95 °C, 35 cycles of denaturation at 95 °C for 30 s, annealing at 56 °C for 45 s, and elongation at 72 °C for 1 min, with a final 5 min elongation at 72 °C.

DGGE analysis of the amplicons was performed on 8% (w/v) polyacrylamide gels using an urea/formamide denaturant gradient of 45–65%. Electrophoresis conditions applied and gel staining were previously described in Lebrero et al. (2010).

DGGE profiles were compared using the GelCompar IITM software (Applied Maths BVBA, Sint-Martens-Latem, Belgium). After image normalization, bands were defined for each sample using the bands search algorithm within the program. Similarity indices of the compared profiles (sample A was compared with sample B, and samples E1, E2 and E3 were compared between them) were calculated from the densitometric curves of the scanned DGGE profiles by using the Pearson product–moment correlation coefficient (Hane et al., 1993). The peak heights in the densitometric curves were also used to determine the diversity indices based on the Shannon–Wiener diversity index (H), calculated as follows:

$$H = -\sum [P_i \ln (P_i)]$$

where H is the diversity index and P_i is the importance probability of the bands in a lane ($P_i = n_i/n$, where n_i is the height of an individual peak and n is the sum of all peak heights in the densitometric curves).

Some bands were excised from the DGGE gel in order to identify the microorganisms present into the reactor. The procedure was previously described in Lebrero et al. (2011). The taxonomic position of the sequenced DGGE bands was obtained using the RDP classifier tool (50% confidence level) (Wang et al., 2007). The closest cultured and uncultured relatives to each band were obtained using the BLAST search tool at the NCBI (National Centre for Biotechnology Information) (McGinnis and Madden, 2004). The sequences generated from this work are deposited in GenBank under accession numbers JQ038779 to JQ038793.

2.7. Data treatment

Unless otherwise specified, the REs and concentrations recorded during the steady states are presented as the average value with its corresponding standard deviation. The Excel statistical package (Microsoft Corporation, USA) was used for data treatment.

3. Results and discussion

This study showed the potential of BTFs to effectively remove moderately and highly hydrophobic VOCs and sulfur organic odorants. No significant MeSH and VOC removal was recorded in the abiotic test, with maximum deviations between the inlet and outlet concentrations of 3% for MeSH, alpha-pinene and hexane and 4% for toluene. These results confirmed that pollutants were neither removed by absorption, adsorption nor photolysis, thus microbial degradation was the only mechanism responsible for pollutants removal during the entire experiment. The relative humidity of the inlet stream remained between 75% and 91%.

No MeSH, toluene and alpha-pinene was detected in the effluent 2 days after the BTF start-up at an EBRT of 50 s (Fig. 2a,b and c, respectively). However, 8 days were necessary to achieve steady state hexane removals of 96.4 ± 0.4% (Fig. 2d). Start-up periods from few days to several weeks have been widely reported in BTFs (Cox and Deshusses, 2002; Yang et al., 2011). However, the inoculation of the system with already acclimated microorganisms allowed a faster system start-up of few days. For example, Chen et al. (2010) reported start-up periods ranging from 4 to 12 days for a BTF treating a mixture of benzene, toluene and *o*-xylene.

When the EBRT was decreased to 30 s, REs of 99% were rapidly achieved for MeSH, toluene and alpha-pinene, while hexane REs reached a steady state within 5 days at 91.5 ± 5% (Fig. 2). When the EBRT was decreased to 11 s at day 25, the process was characterized by a complete removal of MeSH and steady state REs of 96.4 ± 0.5% and 95.5 ± 1.8% for toluene and alpha-pinene, respectively. Steady REs of 70.2 ± 3.5% were recorded for hexane after 6 days from the step EBRT decrease (Fig. 2a). A further decrease in the EBRT to 7 s resulted in a gradual decrease in the REs to minimum values of 47.8%, 39.9%, 14.2% and 25.1% at day 48 for MeSH, toluene, alpha-pinene and hexane, respectively. This deterioration in process performance was likely mediated by the accumulation of toxic metabolites derived from MeSH or VOC biodegradation, which was totally unexpected based upon the low concentration and the high Henry's law constants of the treated odorants. Sulfate concentration was maintained below 1800 mg L^{-1} during the first 30 days of operation and increased afterwards up to 2500 mg L^{-1} due to insufficient trickling solution replacement. Therefore, process deterioration due to an inhibitory sulfate accumulation can be ruled out since this phenomenon is expected to occur above 12000 mg L^{-1} (Ramirez et al., 2009).

Silicone oil was added to the BTF at day 48 to prevent a further decrease in odorant abatement performance based on its ability to absorb toxic organic metabolites. The presence of a non-aqueous phase can reduce any potential inhibition mediated by the occurrence of high concentrations of toxic by-products by lowering their aqueous concentration. It has been consistently shown that the addition of an organic phase into BTFs improves the mass transfer of hydrophobic compounds from the gaseous phase to the microorganisms and acts as a metabolite reservoir (Kennes et al., 2009; Muñoz et al., 2007). For example, Rene et al. (2011) observed a

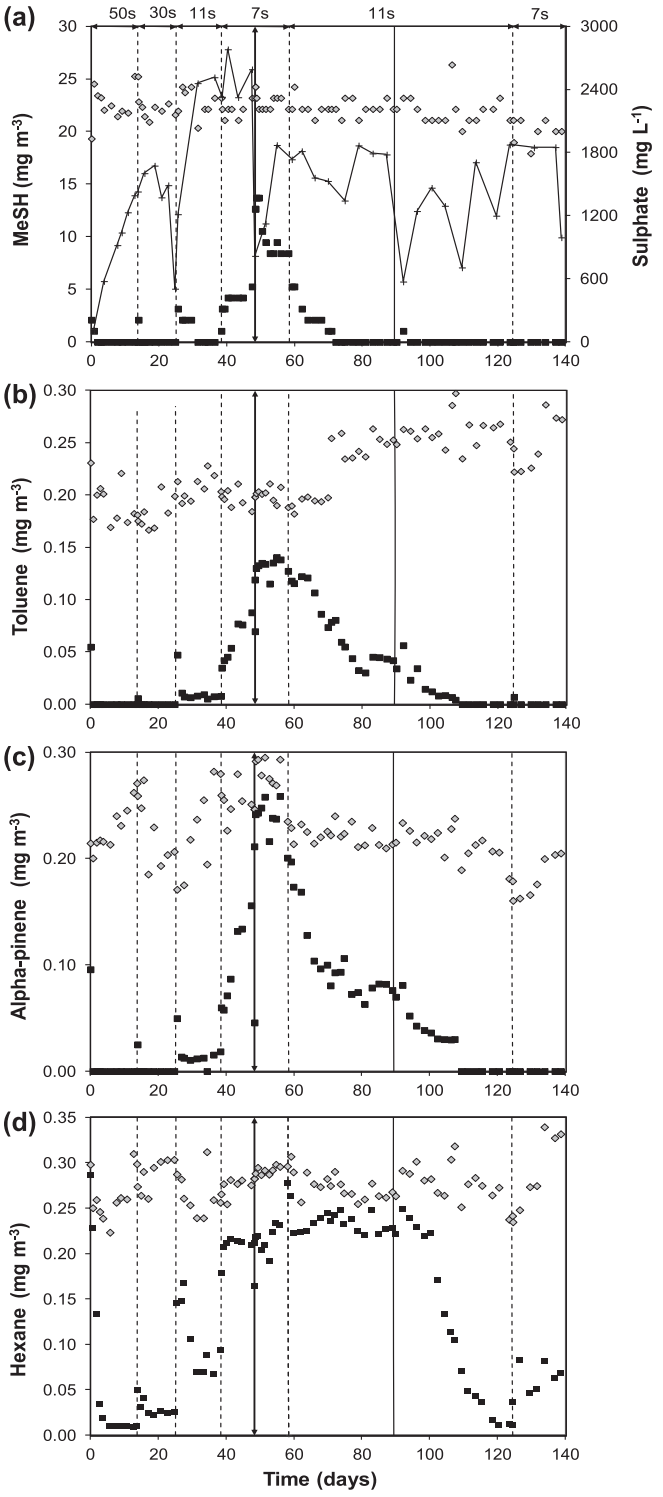

Fig. 2. Time course of the inlet (diamonds) and outlet (squares) concentrations of MeSH (a), toluene (b), alpha-pinene (c) and hexane (d). The continuous line in figure (a) represents the sulfate concentration. The clear dashed vertical lines represent the changes in EBRT, the vertical arrow on day 48 represents the addition of silicone oil and the vertical continuous line on day 90 the re-inoculation of the system.

fourfold improvement in the steady removal of styrene in a BTF after silicone oil addition, and the two-phase system also performed better during shock-load experiments. A temporary increase in process performance for toluene, alpha-pinene, and hexane was observed during the first hours following the addition of the organic phase due to physical VOC absorption into the silicone oil, while their REs decreased afterwards to steady values of 33.3 ± 1.2% for toluene, 12.3 ± 1.0% for alpha-pinene and 24.8 ± 2.1% for hexane. However, the removal of MeSH did not increase after silicone oil addition, reaching a steady RE of 62 ± 1.6% at day 53. This behavior was not expected since previous studies demonstrated the high affinity of silicone oil for the organic sulfur compounds (Dumont et al., 2010). Although the mass transport mechanisms are different for hydrophobic and soluble compounds, where the transfer will occur mainly through the organic and aqueous phase, respectively, the addition of an organic phase increases the interfacial area gas phase-aqueous phase (Quijano et al., 2010), therefore improving the mass transfer of the soluble compounds. The surprising effect here obtained might be attributed to a detrimental interaction between the organic phase and the microorganisms. Hence, since the microbiology seems to be the limiting factor of this system, an improvement on the mass transport could have been hindered by a lower microbial activity. However, and despite the steady REs obtained after silicone oil addition were slightly lower than those recorded before, the addition of this organic phase rapidly stabilize process performance. Finally, the similar CO_2 concentrations recorded in the control and the silicon oil-containing bottles during the silicone oil degradation tests (Fig. 3) demonstrated that the bacterial community was not able to mineralize and therefore degrade the organic phase. This result ruled out the possibility of a process stabilization mediated by a co-metabolic effect.

Once a stable process performance was recovered by day 58, the EBRT was increased to 11 s to allow for microbial recovery. Silicone oil was gradually removed from the trickling solution and the amount of trickling solution wastage was increased to maintain sulfate concentration again below 1800 mg L^{-1} and to avoid another episode of toxic metabolite accumulation. Despite the system was able to completely recover the RE of MeSH, lower steady REs were obtained for the VOCs (83.8 ± 1.7% for toluene, 62.5 ± 2.6% for alpha-pinene and 13.6 ± 1.7% for hexane) from day 79 onwards. This lower performance of the BTF compared to the previous steady state at 11 s suggested an irreversible and permanent damage on the microbial population. To confirm whether the low REs achieved at 11 s were due to an irreversible inhibition of the microbial activity or to mass transfer limitations derived from the deterioration of the packing PUF distribution as a result of the increase in the air flow rate at EBRTs of 7 s, the system was re-inoculated at day 89 with fresh activated sludge. A gradual RE recovery was observed for all the odorants. Nineteen days were needed for complete toluene and alpha-pinene removal, while 25 days were necessary to achieve a hexane RE of 94.9 ± 0.9%. Finally, the EBRT was decreased back to 7 s with REs of 99% for MeSH, toluene and alpha-pinene. Hexane was only removed at 80.1 ± 2.3% as expected from its higher hydrophobicity. These REs were higher than those obtained during the operation at 11 s from days 26 to 38 and at 7 s from day 38 to 48, which suggests an irreversible deterioration of the catabolic capacity of the system probably caused by the accumulation of toxic biodegradation metabolites. In this context, the new microbial community re-inoculated supported a robust VOC removal and was either more resistant or metabolically more versatile (and therefore capable of degrading the above mentioned toxic metabolites).

The TOC and IC in the BTF recycling liquid remained stable at 9.0 ± 2.8 mg L^{-1} and 3.4 ± 1.0, respectively. TN values of 193 ± 31 mg L^{-1} were recorded throughout the experiment, thus no nitrogen limitation occurred.

High REs (98–99.9%) have been reported for H_2S and MeSH in both laboratory and field scale BTFs at a wide range of EBRTs (from 5 to 60 s) (Patria et al., 2001; Ramirez et al., 2009). However, the REs for the VOCs here achieved were higher than those reported in literature, even at higher EBRTs. Chen et al. (2010) reported REs of 91.3% for benzene and toluene and 82.8% for *o*-xylene in a BTF packed with pelletized PUF at EBRTs ranging from 90 to 30 s. Higher REs (~98.8%) were observed by Yang et al. (2011) in a BTF also packed with polyurethane sponges treating toluene at an EBRT of 30 s, while the removal decreased to 74.2% when the system was operated at 5 s of EBRT. Nevertheless, a fair comparison with literature data is difficult since most laboratory studies in BTFs have been performed at inlet concentrations in the range of g m^{-3} (3–4 orders of magnitude higher than those treated in this study). In the field scale, Easter et al. (2005) collected odor removal performance data from several BTFs operating in WWTPs. The results indicated that this biotechnology is capable of achieving high REs for H_2S, but the REs of VOCs and hazardous air pollutants are typically lower (average VOCs REs of 39% at 19 s of EBRT). The unprecedented high performance of this BTF, even at an EBRT as

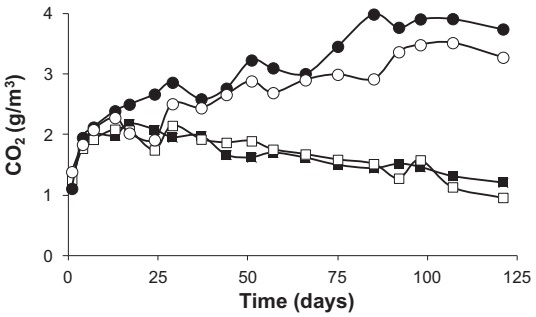

Fig. 3. Time course of the CO_2 headspace concentration during the silicone oil biodegradation assay in batch tests: -□- control 1, -■- control 2, -○- test 1, -●- test 2.

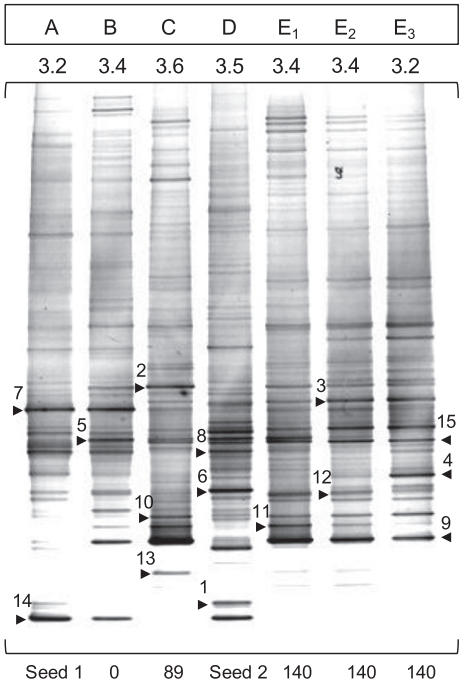

Fig. 4. Bacterial DGGE profiles in the BTF. Sample names and Shannon diversity indices are indicated in the upper part of the gel. The days of operation at which samples were collected are shown in the lower part of the gel. The DGGE bands that were sequenced are indicated by "▶" and the corresponding number of each band.

low as 7 s, can be attributed to the high K_La values of the system: 35 ± 8, 53 ± 6, 98 ± 5 and 113 ± 2 h^{-1} at 50, 30, 11 and 7 s of EBRT. The K_La values increased exponentially at decreasing EBRTs as a result of the increase in the turbulence within the packed bed and are in accordance to those obtained by other authors at similar trickling solution velocities (Dorado et al., 2009). Individual film coefficients based on the water layer (corresponding to the main resistance for mass transfer according to Lebrero et al., 2012) of 0.0126, 0.0099, 0.0084 and 0.0090 m h^{-1} were calculated for methyl mercaptan, toluene, alpha-pinene and hexane, respectively. The K_L values are comparable for the three VOCs, however, the concentration gradient for alpha-pinene and hexane are one and three orders of magnitude lower than that of toluene, respectively. This explains the lower REs obtained for hexane during the entire experimentation period. The high K_L estimated for methyl mercaptan, together with its relatively high concentration gradient, support the high REs recorded.

Finally, the short time required by the BTF to reach a new steady state also demonstrated the robustness of the BTF to cope with sudden fluctuations in the emission flow rates, although longer times were required for the most hydrophobic compound.

The bacterial DGGE profiles and the bands sequenced in this work are shown in Fig. 4. A high Pearson similarity coefficient of 72% between the inoculum (sample A) and sample B (after acclimation) was obtained, which highlights the broad odorant biocatalytic potential of the activated sludge. The Shannon diversity index takes into account the number of species (richness) and the evenness of the species, and typically its value ranges from 1.5 (low species evenness and richness) to 3.5 (high species evenness and richness) (McDonald, 2003). The low VOC mass loadings applied in this work supported the selection of a highly diverse bacterial community ($H_{sample\text{-}B}$ = 3.4) from the inoculum ($H_{sample\text{-}A}$:3.2), which was previously observed by other authors (Bayle et al., 2009). High Shannon–Wiener diversity indices were also obtained for the rest of the analyzed samples, which is consistent with the fact that systems treating low odorant concentrations are usually characterized by high diversity levels (Friedrich et al., 2002; Lebrero et al., 2010, 2011). Communities with a high species diversity often posses a high degree of functional redundancy, which confers them a higher robustness towards process fluctuations (Girvan et al., 2005). This agrees with the short transient periods required by the BTF to reach a new steady state after each operational change.

In order to elucidate the structure of the bacterial communities inside the BTF, samples from the bottom, middle and top sections (samples E1, E2 and E3, respectively) of the reactor were analyzed using the Pearson similarity correlation coefficient. High similarity coefficients of 87% and 86% between samples E1 and E2 and E2 and E3, respectively, were found, while a relatively lower Pearson similarity of 66% was obtained between samples E1 (top of the reactor) and E3 (bottom of the reactor). These results suggest a gradual vertical stratification of the bacterial communities in the BTF likely due to the differences in the biodegradation extent of the different compounds fed to the system. Unfortunately, no samples were taken to analyze the radial microbial stratification. Recent studies have demonstrated that it is possible to find radial gradients in the microbial population due to heterogeneities in the packing material, that may result from the occurrence of preferential pathways, different pollutants and nutrients concentration, etc. (Gadal-Mawart et al., 2011).

To obtain further information of the microorganisms present in the BTF, 15 bands were excised and sequenced from the DDGE gel.

Table 1
The taxonomic position at the phylum level and the taxonomic classification level achieved using a bootstrap value of 50% (RDP classifier) are shown for each band. The closest relatives for each band obtained by Blast search are shown with their corresponding percentage of identity and the environment from which they were retrieved.

DGGE band	Taxonomic position (RDP-50% bootstrap) Phylum		Closest relatives in GenBank	Identity (%)	Origin source
1	Proteobacteria	fam. Acetobacteraceae	Uncultured bacteria (CR933244)	90	Anaerobic sludge digester
			Uncultured Alphaproteobacteria (CU925661)	88	Anaerobic digester treating municipal wastewater sludge
2		gen. *Thiobacillus*	*Thiobacillus denitrificans* ^{T} (NR025358)	99	
			Thiobacillus thioparus ^{T} (HM173634)	99	
3		class Betaproteobacteria	Uncultured bacteria (AB255098)	99	Corroded concrete sample in sewer systems
			Uncultured bacteria (EF467563)	99	Sulfidic cave stream biofilm
4		fam. Xanthomonadaceae	Uncultured bacteria (AY765997))	99	Stream in acid mine drainage from an abandoned copper mine
			Rhodanobacter thiooxidan s^T (NR041565)	97	Biofilm on sulfur particles used in autrotrophic denitrification
5		gen. *Dokdonella*	*Dokdonella* sp. (FJ455531)	99	Triphenyl methane dye treatment bioreactor
			Dokdonella fugitiva ^{T} (NR042397)	98	Membrane bioreactor
6	Actinobacteria	ord. Actinomycetales	Uncultured bacteria (GU912960)	99	Activated sludge
7		gen. *Iamia*	Uncultured bacteria (CU924521)	100	Anaerobic digester which treats municipal wastewater sludge
			Microthrix parvicella (X89774)	99	Activated sludge
8		fam. Intrasporangiaceae	Uncultured *Tetrasphaera* (GU552258)	98	Full-scale EBPR plant
			Uncultured Intrasporongiaceae (EU639277)	98	Thermophilic microbial fuel cell time zero control
9	Nitrospira	gen. *Nitrospira*	Uncultured bacteria (HQ147611)	100	Activated sludge system treating VOCs
10			*Candidatus* Nitrospira defluvii (EU559167)	99	Wastewater treatment plant
11					
12	Chloroflexi	gen. *Bellilinea*	Uncultured bacteria (GU194184)	98	Deciduous forest soil
13		phyl. Chloroflexi	Uncultured Caldilineaceae (HM438265)	95	Soil contaminated with anthracene
14		gen. *Herpetosiphon*	Uncultured Caldilineaceae (HM438265)	99	Activated sludge
15	Gemmatimonadetes	gen. *Gemmatimonas*	Uncultured Gemmatimonadetes (DQ640655)	94	Activated sludge
			Gemmatimonas aurantica ^{T} (NR027529)	93	Enhanced biological phosphorus removal reactor

The RDP classifier tool (bootstrap value of 50%) classified the obtained sequences into five different phyla: Proteobacteria (5 bands), Actinobacteria (3 bands), Nitrospira (3 bands), Chloroflexi (3 bands) and Gemmatimonadetes (1band). The closest cultured and uncultured relatives of each band were determined by NCBI BLAST analysis and summarized in Table 1 along with the environment from which the closest organisms were retrieved.

Microorganisms potentially involved in the degradation of MeSH were detected in this work. The DGGE fragment 2, which was found in all BTF samples analyzed, showed 99% identity with *Thiobacillus denitrificans* (NR025358) and *Thiobacillus thioparus* (HM173634). Species from the genus *Thiobacillus* have the ability to metabolize MeSH, and these microorganisms have been applied in – and retrieved from – biofilters and BTFs treating MeSH and other sulfur organic odorants (Maestre et al., 2010; Ramirez et al., 2011). Although fragment 3 could only be classified up to the class level (Betaproteobacteria), its closest relatives (AB255098 and EF467563, 99% similarity) have been retrieved from sulfur reducing environments, which suggests its potential role in the degradation of sulfur reduced compounds.

Fragment 5 was affiliated to *Dokdonella* while the closest cultured representative of band 4 was *Rhodanobacter thiooxidans*, NR041565 (97% similarity). *Dokdonella* has not been previously related to VOCs degradation, but it has been detected in bioreactors treating sulfurous compounds or ammonia (Maestre et al., 2010). On the other hand, *Rhodanobacter*-like bacteria are able to degrade aromatic hydrocarbons and chlorinated aliphatic compounds (Kanaly et al., 2002). Actinobacteria, which includes aromatic and aliphatic hydrocarbon-degrading microorganisms, were also found in this study (DGGE fragments 6, 7 and 8) but mostly in the inoculum samples. Fragments 9, 10 and 11 were affiliated to the *Nitrospira* genus and presented a high intensity in all samples analyzed. Despite the ability of *Nitrospira*-related organisms to degrade VOCs has not been confirmed experimentally, nitrifying organisms like *Thauera* have the ability to grow on toluene (Shinoda et al., 2004) while some nitrifying consortia are capable of degrading aromatic and non-aromatic hydrocarbons (Silva et al., 2009). Moreover, *Nitrospira*-like bacteria have been found in other bioreactors treating VOCs at trace level concentrations (Lebrero et al., 2011).

Microorganisms classified into the Chloroflexi phylum correspond to the high intensity bands 12, 13 and 14. These bacteria are commonly retrieved from a wide variety of biological treatment systems, but information about their functional role is scarce. Finally, fragment 15 was present in almost all samples analyzed and was affiliated to the *Gemmatimmonas* genus, a microorganism which is able to grow with benzoate (Zhang et al., 2003), a common intermediate in the aerobic metabolism of toluene.

4. Conclusions

This study confirmed the potential of BTFs to treat moderately hydrophobic VOCs and sulfur compounds at RE = 99%, while supporting an efficient abatement for highly hydrophobic VOCs (>80%), at concentrations of mg m^{-3} and EBRTs as low as 7 s. These results might be explained by the high K_La values recorded for the PUF. The accumulation of toxic metabolites in the BTF, even at such low odorant concentrations, was hypothesized to cause an irreversible damage on the microbial community. Finally, a high microbial diversity was observed over the long term operation of the BTF despite the limited C source spectrum treated.

Acknowledgements

This research was supported by the Spanish Ministry of Education and Science (RYC-2007-01667, AP2008-01574 and BES-2010-030994 contracts and projects CTQ2009-07601 and CONSOLIDER-CSD 2007-00055) and the Regional Government of Castilla y Leon (project VA004A11-2). Ignacio de Godos is greatly acknowledged for his technical assistance.

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Lebrero R, Estrada JM, Muñoz R, Quijano G (2012). Biochemical Engineering Journal 60: 44-49

Chapter 7

Toluene mass transfer characterization in a biotrickling filter

Biochemical Engineering Journal 60 (2012) 44–49

Contents lists available at SciVerse ScienceDirect

Biochemical Engineering Journal

journal homepage: www.elsevier.com/locate/bej

Toluene mass transfer characterization in a biotrickling filter

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ARTICLE INFO

Article history:
Received 29 June 2011
Received in revised form 8 September 2011
Accepted 25 September 2011
Available online 1 October 2011

Keywords:
Biological off-gas treatment
Biotrickling filter
Mass transfer modeling
Toluene
Volatile organic compounds

ABSTRACT

Biotrickling filters (BTFs) devoted to air pollution control often present mass transfer limitations for hydrophobic volatile organic compounds (VOCs). Under such limiting conditions, BTF design and scale-up should be based on mass transfer data. A general and simple model was developed to characterize the VOC transfer by means of the overall mass transfer coefficient (K_La). The K_La values were obtained by fitting the model to experimental data of toluene absorption obtained at empty bed residence times (EBRT) from 7 to 50 s. The model fitted well the experimental data (r^2 = 0.97) and the resulting K_La values ranged from 35 to 113 h^{-1}. These values are similar to those reported in the literature for BTF despite the lower liquid recycling velocity here used (0.6 m h^{-1}). A critical gas-to-liquid flow rate ratio (Q_G/Q_L) of 200, above which K_La was poorly increased, was observed. In addition, the individual film coefficients were estimated from the Van Krevelen and Hoftijzer correlations, which revealed that the main resistance for toluene mass transfer was in the liquid film regardless of the EBRT used. Finally, the high mass transfer potential of BTFs was confirmed by estimating the mass transfer capacity under varied operating conditions.

1. Introduction

Volatile organic compounds (VOCs) are widely used in industrial processes and consequently, large amounts of these compounds are daily emitted to the atmosphere and wastewaters [1,2]. This entails a severe threat to both human health and natural ecosystems. Nowadays, the treatment of VOCs emitted by industries and waste treatment facilities is a key environmental issue due to the stricter environmental regulations [3]. In this context, biological systems for air pollution control are becoming increasingly popular for the removal of VOCs. Recent research has focused on biotrickling filters (BTFs) due to their key advantages over their biological counterparts: (i) effective treatment of acid-producing pollutants and (ii) lower pressure drop during long-term operation [4]. A high removal performance for hydrophilic VOCs such as ethanol and acetone has been recorded in BTFs [5]. However, mass transfer limitations may arise when treating moderately and highly hydrophobic VOCs. In this scenario, the design and scale-up of BTFs should be based on mass transfer data. Unfortunately, VOC mass transfer data in BTFs are scarce in the literature, research being mainly focused on biodegradation kinetics [6].

The mathematical modeling approaches used so far to characterize the mass transfer of hydrophobic VOCs are either complex or can be applied only under particular experimental conditions. For instance, Pedersen and Arvin [7] developed a model to characterize the toluene mass transfer using an overall mass transfer coefficient (K_La). However, the model was valid only for high recirculation velocities of the liquid phase. Later, Heymes et al. [8] developed a steady-state model to characterize the toluene mass transfer in a BTF operated with a viscous recycling liquid (di(2-ethylhexyl)adipate). Kim and Deshusses [9] recently developed a simple and robust method to characterize mass transfer in BTFs with several packing materials. Nonetheless, the mass transfer characterization required the addition of high amounts of NaOH in the recycling liquid phase (40 g L^{-1}). Such high salt concentration, much higher than that normally used in culture media, may lead to the overestimation of the mass transfer coefficients due to the high ionic strength established under these conditions [10]. Therefore, there is a lack of simple and general models to assess the VOC mass transfer in BTFs under normal operational conditions.

The aim of this work was to develop a simple and reliable model able to characterize the mass transfer in a BTF operated under typical VOC treatment conditions. The K_La value was obtained from experimental data using toluene as model VOC. Based on the agreement between the experimental K_La values and the Van Krevelen and Hoftijzer correlations, the individual gas and liquid film coefficients (k_G and k_L, respectively) were estimated. Finally, the application of the model in the design and optimization of BTFs was further discussed.

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doi:10.1016/j.bej.2011.09.017

Nomenclature

A_C	Cross sectional area of the packing column (m^2)
a_e	Effective specific interfacial area, defined as $a_p\,\varepsilon^{-1}$ (m^{-1})
a_p	Packing specific surface area (m^{-1})
C	Toluene concentration ($g\,m^{-3}$)
D	VOC diffusion coefficient ($m^2\,s^{-1}$)
d_C	Column inner diameter (m)
g	Gravitational constant ($m\,s^{-2}$)
H	Henry's law constant
k	Individual mass transfer coefficient ($m\,h^{-1}$)
K_La	Overall mass transfer coefficient (h^{-1})
K_L^*a	Theoretical overall mass transfer coefficient (h^{-1})
Q	Flow rate ($m^3\,h^{-1}$)
u	Superficial velocity ($m\,s^{-1}$)
V_C	Packed column volume (m^3)
V_T	Holding tank volume (m^3)

Greek letters

ρ	Density ($kg\,m^{-3}$)
ε	Packed bed porosity
μ	Viscosity ($kg\,m^{-1}\,s^{-1}$)

Subscripts

G	Gas phase
L	Liquid phase
in	Packed column inlet
out	Packed column outlet

2. Materials and methods

2.1. Chemicals

Toluene (99.9% purity) was purchased from Sigma–Aldrich (Madrid, Spain). The liquid phase consisted of a sulphate-free mineral salt medium (MSM) prepared according to Lebrero et al. [11], which is often used as a culture medium. All chemicals for MSM preparation were purchased from PANREAC (Barcelona, Spain) with a purity of at least 99.0%.

2.2. Bioreactor set up

The mass transfer characterization was performed in a laboratory scale BTF at empty bed residence times (EBRTs) of 7, 11, 30 and 50 s. The BTF consisted of a cylindrical jacketed PVC column with a working packed bed volume of 4 L (0.08 m inner diameter, 1 m height). The BTF was packed with 1 cm^3 polyurethane-foam (PUF) cubes (Filtren TM 25280, Recticel Iberica S.L.) with a net density of 20–24 $kg\,m^{-3}$ and a specific surface area of 1000 $m^2\,m^{-3}$. The BTF was operated in countercurrent mode at a constant temperature of 20 °C and an inlet toluene concentration of ~5 $g\,m^{-3}$. The liquid phase volume (1.4 L of MSM) was recycled at a velocity of 0.63 m h^{-1} (corresponding to a flow rate of $4.8 \times 10^{-3}\,m^3\,h^{-1}$) and continuously agitated at 200 rpm in an external 1.5-L holding tank. A detailed diagram of the experimental set-up is shown in Fig. 1.

2.3. Porosity of the packing material

The porosity of the packing material was experimentally determined by introducing a known mass of dry PUF in a test-tube. The PUF cubes were then filled with water and the amount of water was determined by weight difference. The porosity can be expressed as:

$$\varepsilon = \frac{V_L - m_s/\rho_s}{V_L} \quad (1)$$

where V_L represents the water volume added, m_s is the mass of the packing material and ρ_s its density. The effective porosity for the wet packing material was determined using the same experimental procedure.

2.4. Overall mass transfer coefficient

A simple mathematical approach based on mass balances over the whole packed column was here proposed to characterize the toluene mass transfer in the BTF. The mass balance for the gas phase, which considers the overall mass transfer coefficient ($K_La = H \times K_Ga$), can be written as follows:

$$\frac{dC_{G,out}}{dt} = \frac{Q_G}{V_C}(C_{G,in} - C_{G,out}) - K_Ga(C_{G,out} - HC_{L,out}) \quad (2)$$

where H represents the Henry's law constant, $C_{G,out}$ is the VOC concentration at the outlet of the packed column and $C_{G,in}$ is the VOC concentration at the inlet of the column (Fig. 1). On the other hand, the mass balance for the liquid phase at the outlet of the BTF is as follows:

$$\frac{dC_{L,out}}{dt} = K_La\left(\frac{C_{G,out}}{H} - C_{L,out}\right) + \left(\frac{Q_L}{V_C}\right)C_{L,in} \quad (3)$$

A mass balance over the holding tank is also necessary to describe the toluene concentration in the liquid phase at the inlet of the BTF. This balance must consider that the liquid phase is first diluted in the holding tank volume (V_T) and then recycled at a constant rate (Q_L). Thus, the mass balance for the VOC in the liquid phase at the inlet of the BTF can be expressed as:

$$\frac{dC_{L,in}}{dt} = \frac{Q_L}{V_T}(C_{L,out} - C_{L,in}) \quad (4)$$

The model represented by Eqs. (2)–(4) is a simplified description of the system and allows for the characterization of the mass transfer performance in BTFs considering the following assumptions: (i) the liquid phase is perfectly mixed and (ii) conditions are isothermal throughout the experimental system (reactor and holding tank), so H remains constant during all experiments. In order to obtain the overall mass transfer coefficient from experimental data, the kinetics of toluene saturation in the recycling MSM were assessed in duplicate at each EBRT. In brief, $C_{G,in}$ and $C_{G,out}$ were continuously monitored until liquid phase saturation was reached ($C_{G,out}/C_{G,in} \geq 0.97$). At the same time, liquid samples (5 mL) from the inlet and outlet of the packed column were periodically drawn and injected into 10.5 mL gas-tight serum bottles (closed with butyl septa and sealed with aluminum caps). Toluene in the gas-tight bottles was allowed to equilibrate and then analyzed in the headspace to determine the corresponding values of $C_{L,in}$ and $C_{L,out}$ by means of the Henry's law (liquid concentrations at equilibrium).

The experimental measurements of C_G and C_L were used to determine the mass transfer coefficients by solving simultaneously Eqs. (2)–(4), K_La being the fitting parameter. The 4th order Runge–Kutta method was used to solve the differential equations, whereas the Levenberg–Marquardt method was used for parameter fitting according to Jia et al. [12]. ModelMaker® software (Cherwell Scientific, UK) was used to solve the model.

2.5. Analytical methods

Toluene gas concentration was determined in a GC-FID (HP 6890 Series, Hewlett Packard, USA) equipped with a SupelcoWax

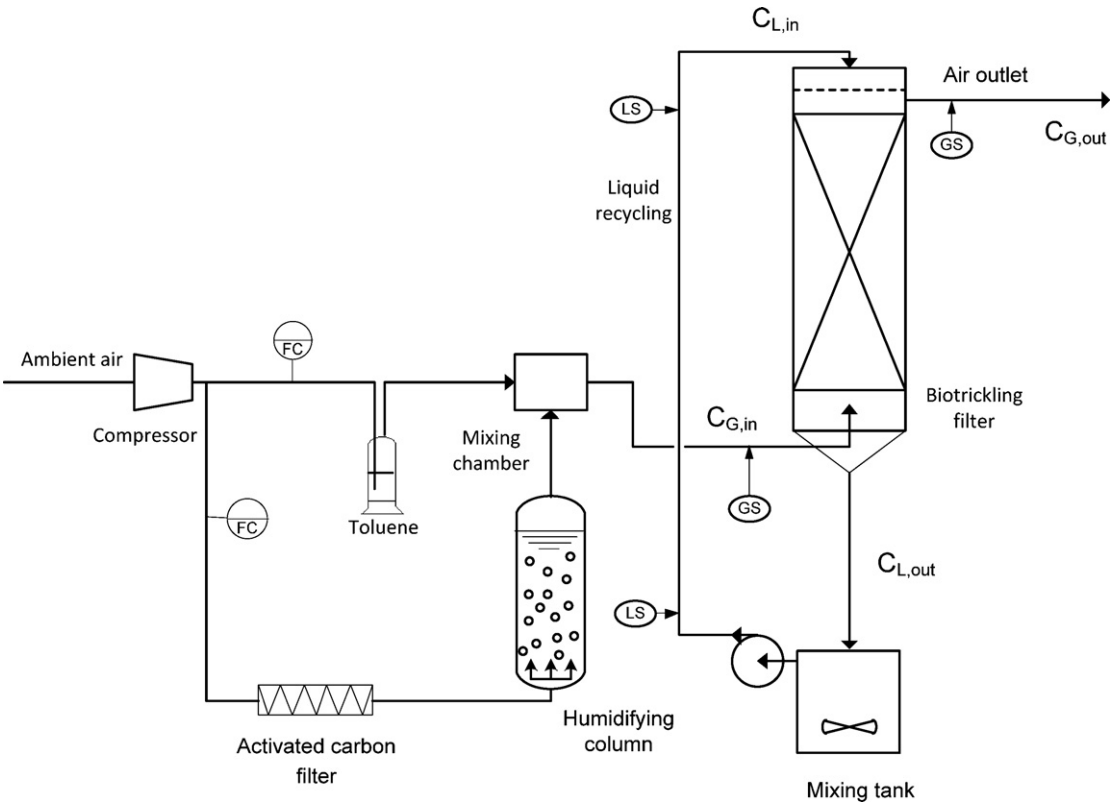

Fig. 1. Schematic representation of the experimental set-up. FC: flow controller; GS: gas sampling port; LS: liquid sampling port.

(15 m × 0.25 mm × 0.25 μm) capillary column. Oven, injector and detector temperatures were maintained at 140, 150 and 200 °C, respectively. Helium was used as the carrier gas at 2 mL min^{-1} while H_2 and air were fixed at 30 and 300 mL min^{-1}, respectively. N_2 was used as the make-up gas at 28 mL min^{-1}.

3. Results and discussion

3.1. Overall mass transfer coefficients

The accurate measurement of toluene concentration in the aqueous phase (based on air-tight serum bottles) resulted in VOC concentration time courses with a characteristic saturation curve shape for $C_{L,out}$. Fig. 2 shows a typical time course of toluene concentration in the gas and liquid phases obtained at an EBRT of 30 s. Liquid phase saturation was reached in 60 min under these conditions, which was faster than that reported by Dorado et al. [6] using a BTF with the same packing material and similar EBRT (35 s). This apparent delay in the saturation of the liquid phase might have been due to the underestimation of toluene aqueous concentrations, since these authors determined the toluene concentration by spectrophotometry (technique subjected to a rapid VOC evaporation).

Eqs. (2)–(4) were fitted to the experimental toluene concentrations in the gas and liquid phases in order to determine K_La. The average correlation coefficient between the model and the experimental data was 0.970 (minimum r^2 0.947; maximum r^2 0.989; standard deviation 0.021). Therefore, the model was able to accurately describe the time course of toluene concentration in both gas and liquid phases regardless of the EBRT. Fig. 3 shows the model fitting to the time course of $C_{G,out}$, $C_{L,in}$ and $C_{L,out}$ obtained at EBRT from 7 to 50 s.

The assessment of the effect of key operational parameters on the VOC mass transfer performance is a crucial step in the optimization of BTF operation. In this regard, both the gas and liquid flow rates are the most important operational parameters affecting the mass transfer performance in BTFs [9]. Thus, the ratio Q_G/Q_L was used to lump the effect of both parameters on K_La. According to

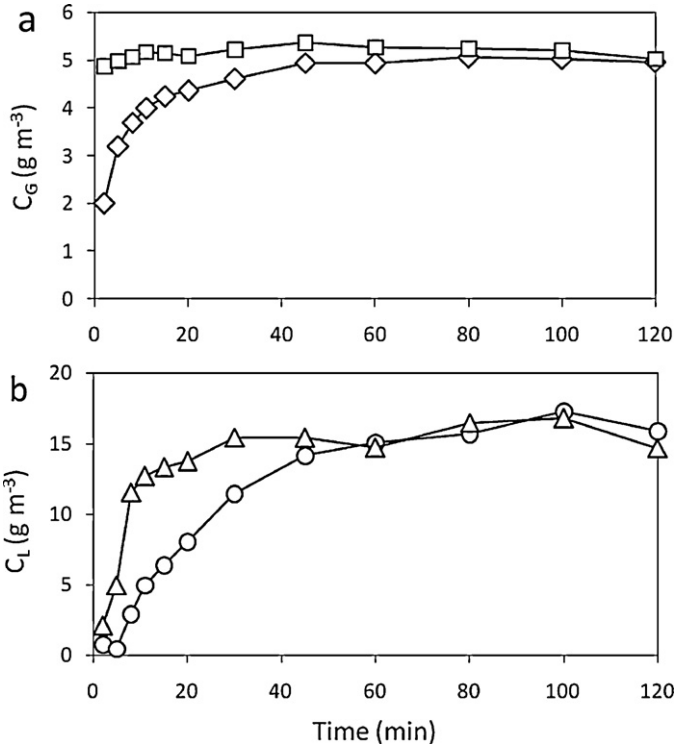

Fig. 2. Time course of toluene concentration in (a) the inlet (□) and the outlet (◊) gas phase and (b) the inlet (○) and the outlet (△) liquid phase at an EBRT of 30 s.

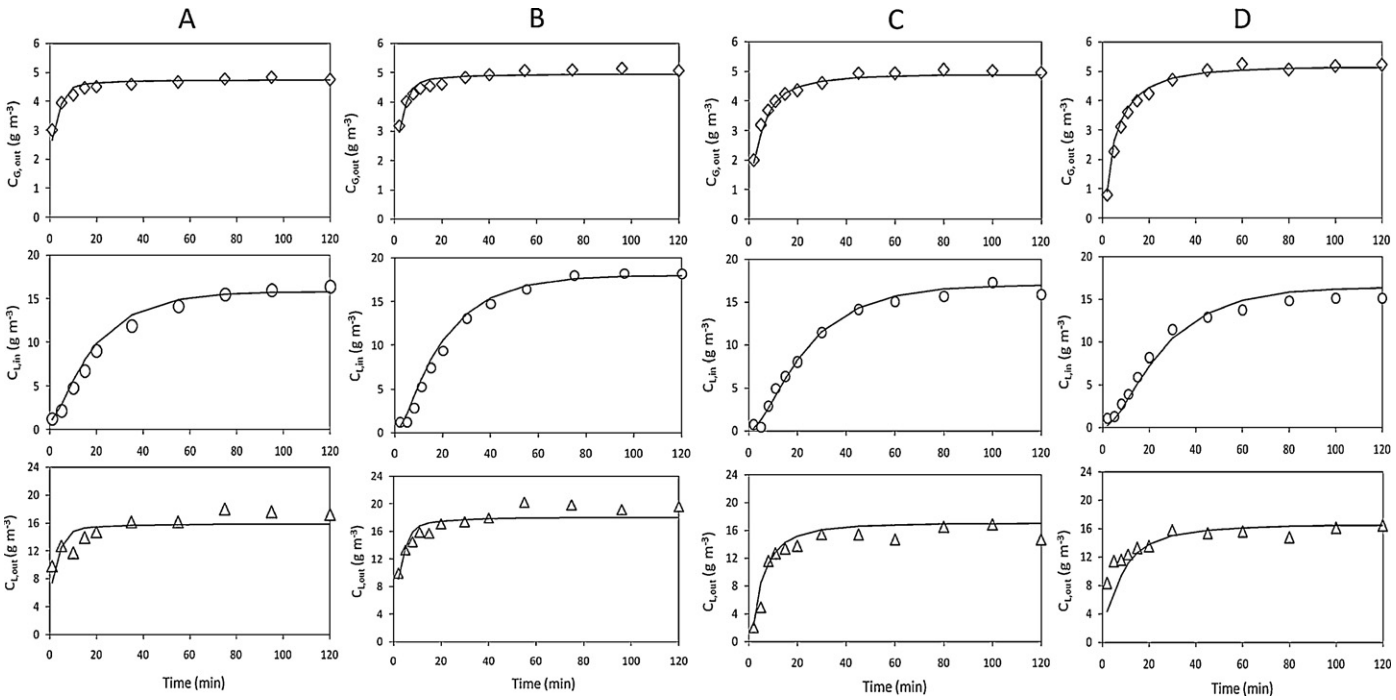

Fig. 3. Typical model fitting performance for $C_{G,out}$, $C_{L,in}$ and $C_{L,out}$ time courses at EBRTs of (A) 7 s, (B) 11 s, (C) 30 s, and (D) 50 s. Continuous lines represent the model solution.

Gabriel et al. [13], typical Q_G/Q_L ratios ranging from 125 to 300 are used in BTFs, although BTFs operated at Q_G/Q_L ratios as low as 10.5 can also be found in the literature [14]. Here, Q_G/Q_L ratios ranging from 60 to 275 (corresponding to EBRT of 50 to 7 s, respectively) were used. Q_G/Q_L ratios lower than 60, derived from EBRTs higher than 50 s, are not interesting from a process optimization point of view.

Fig. 4 depicts K_La as a function of Q_G/Q_L. A linear increase of K_La with the Q_G/Q_L ratio was observed at Q_G/Q_L ratios of 60, 100 and 200, while a further increase of Q_G/Q_L to 275 supported a small increase in K_La. These results confirm the strong effect of both gas and liquid flow rates on the mass transfer performance of BTFs, as previously showed by Kim and Deshusses [9]. These results suggest that there is a critical Q_G/Q_L ratio, above which further increases do not result in the corresponding linear increase of K_La. Therefore, the assessment of this critical Q_G/Q_L ratio constitutes a useful tool to identify the best operational conditions in BTFs for a particular VOC biodegradation process.

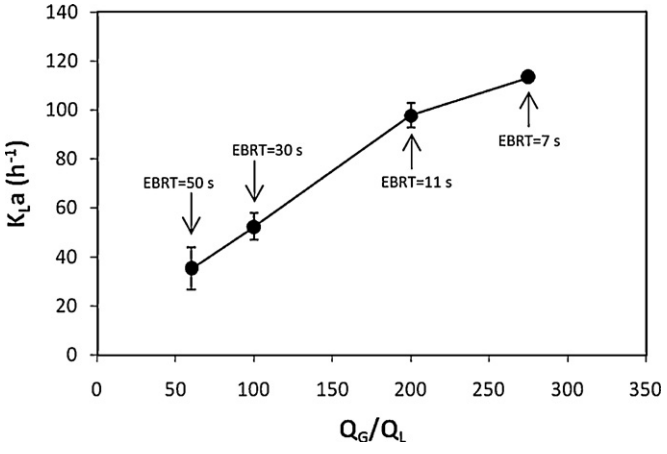

Fig. 4. Influence of the Q_G/Q_L ratio on the overall mass transfer coefficient (K_La).

The K_La values here obtained are in accordance to those recorded by other authors at similar EBRTs. However, the liquid recycling velocity here used was considerable lower than the velocities employed in other studies (Table 1).

3.2. Individual film coefficients

The determination of the individual film coefficients is useful to elucidate the location of the main resistance for mass transfer. In this context, Liss and Slater [15] correlated the type of mass transfer resistance to the H value and suggested that for VOCs exhibiting $H > 250$ atm (mole fraction)$^{-1}$ mass transfer is controlled by the liquid film. On the other hand, if $1 \leq H \leq 250$ the resistance in both films is relevant, while for VOCs holding $H < 1$ the main resistance is in the gas film. According to Sander [16], toluene exhibits an H value of 58 ± 9 atm (mole fraction)$^{-1}$ and therefore, the location of the main resistance to toluene mass transfer must be determined case by case. In our study, the individual film coefficients were estimated using the correlations proposed by Van Krevelen and Hoftijzer [17]:

$$k_G = 0.2 \left(\frac{D_G}{d_C}\right) \left(\frac{\rho_G u_G}{\mu_G a_p}\right)^{0.8} \left(\frac{\mu_G}{\rho_G D_G}\right)^{1/3} \quad (5)$$

$$k_L = 0.015 \left(\frac{D_L}{\left[\mu_L^2/(\rho_L^2 g)\right]^{1/3}}\right) \left(\frac{\rho_L u_L}{\mu_L a_e}\right)^{2/3} \left(\frac{\mu_L}{\rho_L D_L}\right)^{1/3} \quad (6)$$

Once the individual film coefficients were determined, the theoretical overall coefficients ($K_L^* a$) were calculated as [18]:

$$\frac{1}{K_L^* a} = \frac{1}{k_L a} + \frac{1}{H k_G a} \quad (7)$$

The resulting values for both k_L and K_La were constant since only one Q_L value was used in this study (Table 2). It is worth noting that the k_Ga values were 300 and 90 times higher than the corresponding K_La values at EBRTs of 7 and 50 s, respectively. This indicates that the main resistance for toluene transfer under the operational conditions evaluated was in the liquid film regardless

Table 1
Comparison of the K_La values reported for toluene in the literature.

Packing material	K_La values (h^{-1})	Q_G/Q_L ratio	EBRT (s)	$C_{G,in}$ ($g\,m^{-3}$)	u_L ($m\,h^{-1}$)	Reference
Polypropylene rings	0.9–1.2	32.8–65.7	2.4	5	15.3–50.8	[8]
PUF	12–258					
Clay pellets	10–150	0.45–90	100–6	5	2	[6]
Lava rock	10–199					
Dixon rings	11–415					
Steel pall rings	10–60	4.9–16.6	144–32	0.7–0.8	3.3–4.7	[7]
PUF	35–113	60–275	50–7	5	0.6	This study

Table 2
Individual film coefficients estimated from the Van Krevelen and Hoftijzer correlations.

EBRT (s)	k_G ($m\,h^{-1}$)	k_L ($m\,h^{-1}$)	k_Ga (h^{-1})	K_La (h^{-1})
7	5.7×10^{-3}	1.9×10^{-5}	9.5×10^{-2}	3.1×10^{-4}
11	4.4×10^{-3}	1.9×10^{-5}	7.3×10^{-2}	3.1×10^{-4}
30	2.5×10^{-3}	1.9×10^{-5}	4.2×10^{-2}	3.1×10^{-4}
50	1.7×10^{-3}	1.9×10^{-5}	2.8×10^{-2}	3.1×10^{-4}

of the EBRT used, which is in agreement with the main | of Kim and Deshusses [9]. Moreover, the K_L^*a values derived from Eq. (7) were in good agreement with the experimental K_La values ($r^2 = 0.95$) as shown in Fig. 5. As a matter of fact, the theoretical K_L^*a values based on the Van Krevelen and Hoftijzer correlations fitted very well the experimental data obtained at EBRT of 30 and 50 s, while the predicted K_L^*a values at EBRTs of 7 and 11 s were slightly lower than the experimental ones.

Dorado et al. [6], based on the characterization of toluene mass transfer in BTFs with several packing materials (including PUF) concluded that the resulting K_L^*a values obtained from the Van Krevelen and Hoftijzer correlations (and from other correlations available in the literature) were significantly lower than the experimental K_La values. This apparent mismatch was likely due to the fact that these authors fitted their experimental data to a model originally developed by Heymes et al. [8] for recycling viscous liquids and the K_La values were obtained from data linearization. Moreover, these authors used a 3 times higher u_L value than the one used in this study (2 vs 0.6 $m\,h^{-1}$). According to Cox and Deshusses [19], the superficial velocities of the recycling liquid typically range from 0.05 up to 20 $m\,h^{-1}$. On the contrary, the equations here used were based on fundamental mass balances and therefore, no particular assumptions for the recycling liquid or data linearization was required.

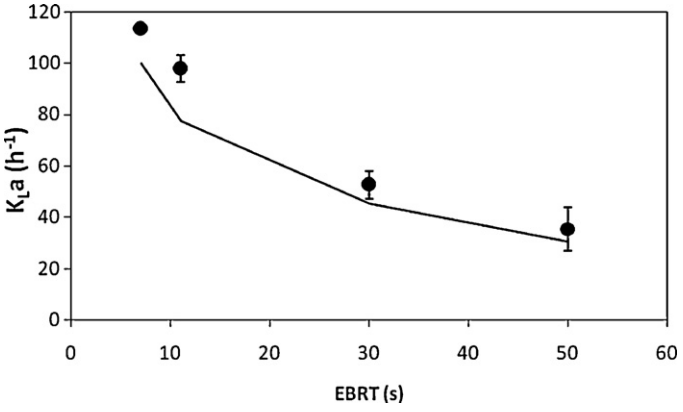

Fig. 5. Experimental (●) and theoretical (—) values for the overall mass transfer coefficient.

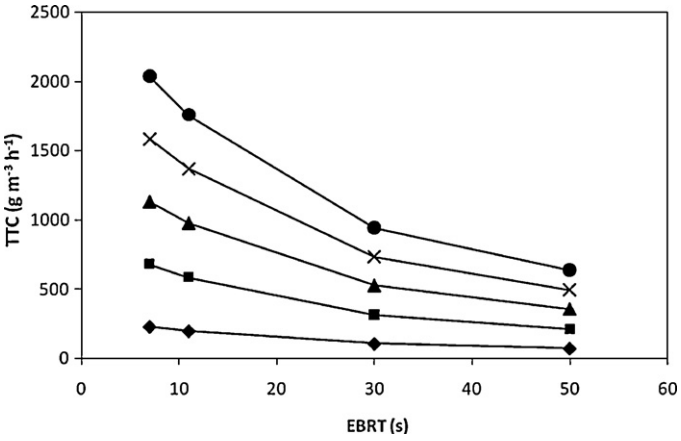

Fig. 6. Estimated TTC values for ECs of: 10% (♦), 30% (■), 50% (▲), 70% (×), 90% (●).

3.3. Assessment of the VOC mass transfer capacity

The mass transfer capacity can be defined as the mass transfer rate reached at the maximum concentration gradient available [20]. An estimation of the toluene transfer capacity (TTC) in a BTF may be done using Eq. (8):

$$\mathrm{TTC} = K_La\left(\frac{C_{G,in} - C_{G,out}}{H}\right) \quad (8)$$

The TTC is clearly a function of the removal efficiency (RE) in the BTF under biotic conditions. Thus, when $C_{G,out} = 0$ the TTC reaches a maximum value, while when $C_{G,out} = C_{G,in}$ the liquid phase is saturated and the TTC equals zero. For instance, a TTC of 354 $g\,m^{-3}\,h^{-1}$ can be attained at an EBRT of 50 s ($K_La = 35.4\,h^{-1}$) and a removal efficiency of 50% ($C_{G,in} - C_{G,out} = 2.5\ g\ m^{-3}$). The lower mass transfer performance was estimated at an EBRT of 50 s (lowest K_La value) and RE of 10%, which corresponded to a TTC value of 71 $g\,m^{-3}\,h^{-1}$ (Fig. 6). In this regard, the maximum toluene elimination capacity so far reported in BTFs for loading rates of 12–220 $g\,m^{-3}\,h^{-1}$ and EBRTs ranging from 1.2 s to 4 min is ~80 $g\,m^{-3}\,h^{-1}$ [21]. Therefore, Eq. (7) can be useful to determine whether mass transfer or biological activity is the limiting step of the process.

4. Conclusions

In brief, this study shows that mass transfer characterization is a powerful tool to optimize both BTF design and operation. The model here proposed was based on fundamental mass balances, did not require any particular assumptions or data linearization and can be used to characterize the mass transfer of VOCs in any BTF. The model accurately described the experimental data, supporting an overall correlation coefficient of 0.970. The film coefficients estimated from the Van Krevelen and Hoftijzer correlations showed that under the typical operational conditions here studied the main resistance to toluene mass transfer remained in the liquid film. Moreover, the theoretical K_L^*a values estimated from these

individual film coefficients were in agreement with the experimental $K_L a$ values, which support the validity of the location of the main resistance to mass transfer. Finally, the estimated TTC values confirmed the high mass transfer potential of BTFs even at EBRT as high as 50 s, TTC determination being useful to assess the limiting step of the process (mass transfer or biological activity).

Acknowledgements

This research was supported by the Spanish Ministry of Science and Innovation (RYC-2007-01667 and BES-2007-15840 contracts, CTQ2009-07601 and CONSOLIDER-CSD 2007-00055) projects.

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Lebrero R, Rodriguez E, Perez R, Garcia-Encina PA, Muñoz R
(2012). Applied Microbiology and Biotechnology, In Press

Chapter 8

Abatement of odorant compounds in one and two-phase biotrickling filters under steady and transient conditions

Appl Microbiol Biotechnol
DOI 10.1007/s00253-012-4247-1

ENVIRONMENTAL BIOTECHNOLOGY

Abatement of odorant compounds in one- and two-phase biotrickling filters under steady and transient conditions

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Received: 22 April 2012 / Revised: 12 June 2012 / Accepted: 14 June 2012

Abstract The removal of hydrophobic volatile organic compounds (VOCs) still remains the main restriction in the biological treatment of odorous emissions due to mass transfer limitations. The addition of a non-aqueous phase to conventional biotrickling filters (BTF) may overcome this limitation by enhancing VOCs transport from the gas to the microorganisms. This study compared the long-term and transient performance of a one- (1P) and two-liquid phase (2P; with silicone oil as non-aqueous phase) BTFs for the removal of four VOCs (butanone, toluene, alpha-pinene, and hexane) at empty bed residence times (EBRT) ranging from 47 to 6 s. Removal efficiencies (RE) >96 % were obtained for butanone, toluene, and alpha-pinene in both bioreactors regardless of the EBRT, while higher hexane REs were recorded in the 2P-BTF (81–92 %) compared to the 1P-BTF (60–97 %). The two-phase system always showed a more consistent performance, being able to better withstand step VOC concentration increases and starvation periods, although it was more affected by liquid recycling shutdowns due to a reduced VOC mass transfer. The analysis of the microbial communities showed a high biodiversity and richness despite the low C source spectrum and high community evenness and richness. In this context, the presence of silicone oil mediated the development of a highly different phylogenetic composition of the communities.

Keywords Biotrickling filter · Odor treatment · Process robustness · Two-phase partitioning bioreactor · VOC removal

Introduction

Wastewater treatment plant (WWTP) off-gases contain a wide variety of volatile organic compounds (VOCs). The emission of these VOCs, some of them air toxics and/or odorants, causes both local and global environmental problems. Today, biological techniques are the preferred option for odor control based on their cost-effectiveness and low environmental impact (Van Groenestijn and Lake 1999; Estrada et al. 2011). Among the available biotechniques, biotrickling filters (BTFs) have recently emerged as the preferred technology in terms of cost-efficiency (Estrada et al. 2011). A high removal performance in BTFs has been consistently achieved for H_2S and the highly soluble VOC fraction of the odorous emissions at empty bed residence times (EBRT) as low as 2 s (Gabriel et al. 2004). However, the removal performance of BTFs is often limited for the hydrophobic odorants, due to their low mass transfer rates from the gas to the microbial-containing aqueous phase as a result of their low aqueous solubility. In recent years, applied researchers have become increasingly interested in the use of two-phase partitioning bioreactors (TPPBs) in waste gas treatment applications. In TPPBs, a non-aqueous phase is added to improve the mass transport of hydrophobic pollutants from the gas phase to the microorganisms based on its high affinity for the target VOCs (Daugulis 2001; Darracq et al. 2010; Dumont et al. 2012). The successful applications of TPPBs were only recorded at high inlet VOCs concentrations (1–20 $g\,m^{-3}$), much higher than those typically found in WWTP odorous emissions (in the order of micrograms per cubic meter, Zarra et al. 2008), with most of the studies conducted with single VOC streams (Fazaelipoor and

Electronic supplementary material The online version of this article (doi:10.1007/s00253-012-4247-1) contains supplementary material, which is available to authorized users.

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Published online: 17 July 2012

Shojaosadati 2002; Boudreau and Daugulis 2006; Bailón et al. 2009; Montes et al. 2010). To the best of our knowledge, no single study has addressed the removal of mixtures of odorants at trace level concentrations in TPPBs, which would be of paramount relevance to mitigate the impact of the hydrophobic VOC fraction of odorous emissions. There is also a lack of information on the long-term and transient performance of TPPBs for this particular application.

Based on the need to maintain an efficient abatement performance and functional stability in off-gas treatment bioreactors, research studies aiming to correlate the specific functionality to ecological data have become increasingly popular over the last years (Marzorati et al. 2008). However, the study of the structure of microbial communities is still not straightforward (Wittebolle et al. 2009b). In the specific case of off-gas treatment bioreactors, the understanding of the microbial communities from an internal structure and composition viewpoint is still scarce. Hence, Cabrol and Malhautier (2011) recently highlighted the lack of such studies in off-gas treatment biofilters compared to other engineered ecosystems such as bioreactors for wastewater treatment.

The aim of the present paper was to compare the performance of a one- and a two-liquid phase BTFs for the removal of four VOCs (with different hydrophobicity) at trace level concentrations (in the order of micrograms per cubic meter): butanone, toluene, alpha-pinene, and hexane. The long-term performance of both systems was analyzed at different EBRTs, and the response and stability of the bioreactors to cope with common process fluctuations and operational upsets was also studied. Furthermore, the structure (evenness and richness) and composition of the microbial communities present in both bioreactors was investigated by denaturing gradient gel electrophoresis (DGGE) of 16S rRNA gene fragments.

Materials and methods

Experimental setup

The pilot plant consisted of two identical BTFs operating in parallel. The jacketed cylindrical PVC reactors had an internal diameter of 8 cm and a height of 100 cm, with a working packed bed volume of 4 L (Fig. S1, online resource). The columns were packed with Kaldness K1 rings (Evolution Aqua Ltd., UK). The packing material was characterized according to standard methods (TMECC 2002): ring diameter 1 cm, density (as received) 0.17 gmL^{-1}, void fraction 83 %, and water-holding capacity (volume basis) 11 %. The bioreactors were operated in a countercurrent flow configuration with two perforated plates acting as distributors of the odorous emission (bottom) and the recycling liquid mineral medium (top of the bioreactor). The synthetic odorous stream fed to the bioreactors was obtained by mixing a stream from a certified-custom made bottle containing butanone, toluene, alpha-pinene, and hexane (Abello Linde S.A., Spain) with a VOC-free air stream previously filtered through an activated carbon bed (2 L volume) and humidified in a 8.5-L water column. The inlet air temperature remained constant at 23.4±0.3 °C throughout the entire experimentation period. The odorant concentrations were accurately controlled by a mass flow controller (Aalborg, Denmark) at 2.72±0.33 mg m^{-3} for butanone, 1.32±0.10 mg m^{-3} for toluene, 1.31±0.14 mg m^{-3} for alpha-pinene, and 1.27±0.11 mg m^{-3} for hexane. The reactors were operated at T=25 °C (Huber water bath, Offenburg, Germany). A volume of 1.2 L of mineral salt medium (MSM) (Lebrero et al. 2011) was recycled at 1.5 mh^{-1} with a metering pump (Dosapro series G™ A, Milton Roy Ltd., USA). In the two-phase BTF (2P-BTF), 360 mL of MSM were replaced by silicon oil 20 cSt (20×10^{-6} m^2s^{-1}) (Sigma-Aldrich, 99.9 % purity) (30 % vol/vol). Previous studies demonstrated the non-biodegradability of this silicone oil by activated sludge (Lebrero et al. 2012). The trickling solutions of both BTFs were continuously agitated (300 rpm) in external 1-L tanks and their pH manually maintained at 6.9±0.1 by addition of a 10-g NaOH L^{-1} solution. All chemicals and reagents were purchased from PANREAC with a purity of +99 % (Barcelona, Spain)

Bioreactor operation: influence of the EBRT

Prior to inoculation, an abiotic test was performed to assess the adsorption and photolysis of the target VOCs in the pipelines, reactor walls, and packing material. The BTFs were operated for 72 h at an EBRT of 30 s in the absence of liquid recycling and biocatalytic activity at the above-cited concentrations. Following the abiotic test, each bioreactor was inoculated with 800 mL of activated sludge from Valladolid WWTP, previously centrifuged for 10 min at 10,000 rpm and resuspended in fresh MSM in the 1P-BTF and in fresh MSM with 30 % silicone oil in the 2P-BTF. The steady VOC removal performance of both bioreactors was evaluated at four EBRTs: 47, 25, 12, and 6 s. The corresponding inlet loads (IL) of butanone, toluene, alpha-pinene, and hexane were, respectively: IL (EBRT 47 s)= 0.21, 0.10, 0.10, and 0.10 gm^{-3} h^{-1}; IL (EBRT 25 s)=0.39, 0.19, 0.19, and 0.18 gm^{-3} h^{-1}; IL (EBRT 12 s)=0.82. 0.40, 0.39, and 0.38 gm^{-3} h^{-1}; and IL (EBRT 6 s)=1.63, 0.79, 0.79, and 0.76 gm^{-3} h^{-1}. Each steady state (average removal efficiency (RE) with a standard deviation <5 %) was maintained for at least 10 days prior to further decreasing the EBRT. MSM was daily supplied to compensate for sampling losses and water evaporation. The concentration of the four VOCs was periodically measured at the influent and effluent gas sampling ports. Liquid samples were also periodically drawn to measure the pH. The temperature and moisture of the inlet stream were also recorded.

Robustness evaluation

The response of the BTFs to surges in the inlet VOC concentrations was evaluated by increasing the inlet concentration by a factor of 3 and 6. Each step increase was maintained for 2 h, restoring the inlet concentrations to the steady values afterward. The inlet and outlet VOC concentrations were monitored every 30 min during each step increase. The systems were allowed to equilibrate for 1 day before the 6-fold step increase was conducted. The ability of both systems to recover from 1 day without liquid recycling was then investigated. Inlet and outlet gas samples were periodically taken during liquid recycling stoppage and after its restoration. Finally, the response of the systems to a 1-day starvation period was studied by monitoring the transient process performance following VOC supply restoration. Robustness analysis was performed according to Kraakman (2003) by determining the risk of negative effects (R) on the biological system associated to process fluctuations or operational failures:

$$R = \sum (p \times E)$$

where p is the probability of occurrence of a fluctuation or operation failure (number of episodes per year) and E is the negative effect that this fluctuation induces on process performance (expressed as the percentage of removal efficiency loss: the loss in total removal due to the upset divided by the total removal per year). The probability of occurrence of the inlet variation episodes and operation failures (p) were obtained from Kraakman (2003) and are shown in Table 1.

Analytical procedures

The VOC analyses were carried out by solid-phase microextraction (SPME)–gas chromatography (GC)–flame ionization detection. The gas samples were collected in 250 glass bulbs (Sigma-Aldrich) and pre-concentrated with an 85-μm PDMS/Carboxen SPME fiber (Supelco, Bellefonte, PA, USA). The adsorption and desorption times were 10 and 1 min, respectively. The GC was a Varian 3900 equipped with a SupelcoWax (15 m×0.25 mm×0.25 μm) capillary column. Oven, injector, and detector temperatures were maintained at 40, 300, and 300 °C, respectively. H_2 and air flows were fixed at 30 and 300 mL min^{-1}, respectively. N_2 was used as the make-up gas at 25 mL min^{-1}. A split ratio of 1:10 was employed. Liquid samples were filtered and the pH of the supernatant was measured using a pH/mV/°C meter (pH 510 Eutech Instruments, Nijkerk, the Netherlands). The moisture content and temperature in the influent odorous stream was recorded using a Testo 605-H1 thermohygrometer (Testo AG, Germany).

Microbial analytical procedures

To evaluate the structure (richness and evenness) and composition of the bacterial communities in both BTFs, the biomass embedded in the packing material was sampled at the end of the experiment (after system shutdown) from the upper part of each BTF. Biomass samples were vortexed and centrifuged to separate the biofilm from the packing material and concentrate the biomass. The structure and composition of the inoculum were also analyzed.

Genomic DNA was extracted according to Lebrero et al. (2012). The PCR mixture (50 μL) was composed of 25 μL of BIOMIX ready-to-use 2× reaction mix (Bioline, Ecogen), containing reaction buffer, magnesium, deoxynucleotide triphosphates, Taq polymerase and additives, 1 or 2 μL of the extracted DNA, PCR primers 968-F-GC and 1401-R (10 μM) (Sigma-Aldrich, St. Louis, MO, USA) for bacterial 16S rRNA gene amplification, and Milli-Q water up to a final volume of 50 μL. The PCR thermocycling program used was previously described in Lebrero et al. (2012).

The DGGE analysis of the amplicons was performed with a D-Code Universal Mutation Detection System (Bio Rad Laboratories) using 8 % (*w*/*v*) polyacrylamide gels with a urea/formamide denaturing gradient of 45 to 65 %. DGGE running conditions were applied according to Roest et al. (2005). The gels were stained with SYBR Green I nucleic

Table 1 Estimated overall risks (R) of the 1P- and 2P-BTFs

Failure	Probability of occurrence	1P-BTF risk (R)				2P-BTF risk (R)			
		But (%)	Tol (%)	α-Pin (%)	Hex (%)	But (%)	Tol (%)	α-Pin (%)	Hex (%)
3-fold inlet concentration increase	1/day	0	0.41	0.99	2.68	0	0.01	0	0.14
6-fold inlet concentration increase		0	1.38	2.37	0.82	0	0.15	0.15	1.95
Liquid recycling stoppage	1/year	0.01	0.18	0.22	−0.09	0.05	0.34	0.44	0.65
Starvation period	1/year	0	0	0.08	0.27	0	0	0.00	0.09
Total risk		0.01	1.97	3.66	3.68	0.05	0.50	0.59	2.83

But butanone, *Tol* toluene, *α-Pin* alpha-pinene, *Hex* hexane

acid gel stain (Sigma Aldrich, St. Louis, MO, USA) for 1 h. The obtained DGGE patterns were processed using the GelCompar IITM software (Applied Maths BVBA, Sint-Martens-Latem, Belgium). After image normalization, bands were defined for each sample using the bands search algorithm within the program. Similarity values of the compared profiles were calculated from the densitometric curves of the scanned DGGE profiles by using the Pearson product–moment correlation coefficient (Häne et al. 1993). The peak heights in the densitometric curves were also used to determine the Shannon–Wiener diversity index (H), which takes into account both the relative number of the DGGE bands (richness) and their relative intensities (evenness):

$$H = -\sum [P_i \ln(P_i)]$$

where P_i is the importance probability of the bands in a lane ($P_i = n_i/n$, n_i is the height of an individual peak and n is the sum of all peak heights in the densitometric curves).

The ranged weighted richness (R_r) (Marzorati et al. 2008), which indicates the richness and genetic diversity within a bacterial community, was calculated based on the total number of bands (N) and the denaturing gradient between the first and the last band of each pattern (D_g), according to the equation:

$$R_r = N^2 \times D_g$$

Moreover, based on the DGGE profiles, the evenness of the bacterial communities was graphically represented in Pareto–Lorenz evenness distribution curves (Lorenz 1905; Wittebolle et al. 2009b). For each DGGE lane, the bands were ranked from high to low based on their intensities. Consecutively, the cumulative normalized number of bands was used as x-axis, and their respective cumulative normalized band intensities were used as y-axis. Finally, the curves were evaluated by a horizontal y-axis projection of their intercepts with the vertical 20 % x-axis line in order to visualize the functional organization (F_o) of the bacterial communities (Marzorati et al. 2008).

Sequencing and DNA sequence analysis

The desired DGGE bands were excised from the DGGE gel in order to elucidate the bacterial composition of each bioreactor at the end of the experiment. The procedure was previously described in Lebrero et al. (2011). Some reamplified PCR products were run again on a DGGE to check their purity and mobility on the DGGE gel. The taxonomic position of the sequenced DGGE bands was obtained using the RDP classifier tool (50 % confidence level) (Wang et al. 2007). The closest matches to each band were obtained using the BLAST search tool at the National Centre for Biotechnology Information (NCBI) (McGinnis and Madden 2004). Sequences were deposited in GenBank database under accession numbers JQ914117–JQ914137.

Results

Influence of the EBRT on the VOC removal performance

No significant VOCs removal by adsorption or photolysis was recorded in the abiotic removal test (the difference between inlet and outlet concentrations was always lower than 10 %), which confirmed that microbial degradation was the only mechanism responsible for VOC removal during the entire experiment. Steady-state removals were rapidly achieved (3 days) after inoculation for butanone and toluene in both BTFs at an EBRT of 47 s and without any preliminary acclimation (Fig. 1a, b). A steady alpha-pinene RE was reached in 4 days in the 1P-BTF, while 6 days were necessary in the 2P-BTF (Fig. 1c). The stabilization of hexane removal required 7 and 13 days in the 1P and 2P-BTF, respectively (Fig. 1d).

No butanone was detected at the outlet of both bioreactors regardless of the EBRT, even at 6 s (Fig. 1a). A slight decrease in butanone RE was recorded after 13 days of operation at an EBRT of 25 s, with REs of 87 and 94 % in the 1P and 2P-BTF, respectively. However, steady REs were recovered in the following 3 days, and no further fluctuations were observed.

The 2P-BTF showed a more stable toluene removal performance compared to the 1P-BTF, with RE of 99±1 % at each EBRT tested. No significant loss in toluene RE was recorded in the 2P-BTF following each EBRT reduction (Fig. 1b). In the 1P-BTF, toluene RE remained at 98±1 % during the entire experimentation period, except for random losses in the RE at day 12 (RE=81 %) and day 55 (RE=91 %). Steady outlet toluene concentrations were also rapidly achieved (≈1 and 2 days) when the EBRT was decreased to 25 and 12 s, respectively.

No alpha-pinene was detected in the 1P-BTF outlet at EBRTs of 47, 25, and 12 s (Fig. 1c). High REs of 99±1 % were also achieved at an EBRT of 6 s, but frequent decreases in the RE were recorded from day 82 onward. Despite the RE of this terpene in the 2P-BTF was slightly lower (≈96±1 %), the performance of this bioreactor was more stable, with no deterioration in RE throughout the experimentation period.

The REs achieved for hexane in both BTFs were lower than those recorded for the other VOCs. The 1P-BTF reached hexane REs of 97±1 % at an EBRT of 47 s (Fig. 1d). However, when the EBRT was decreased to 25 s, the bioreactor performance progressively deteriorated, with a minimum value of 24 % at day 28 and a gradual performance recovery to steady REs of 71±2 % from day 37. Surprisingly, only a slight decrease in RE occurred when the EBRT was decreased to 12 s, with a minimum value of 60 % at day 55 but a stable removal performance from day 60 (RE=84±2 %). A further decrease in the EBRT to 6 s caused a transient reduction in the removal performance to finally stabilize at steady REs of 60±5 %. On the other hand, the 2P-BTF showed shorter stabilization periods and a more efficient removal

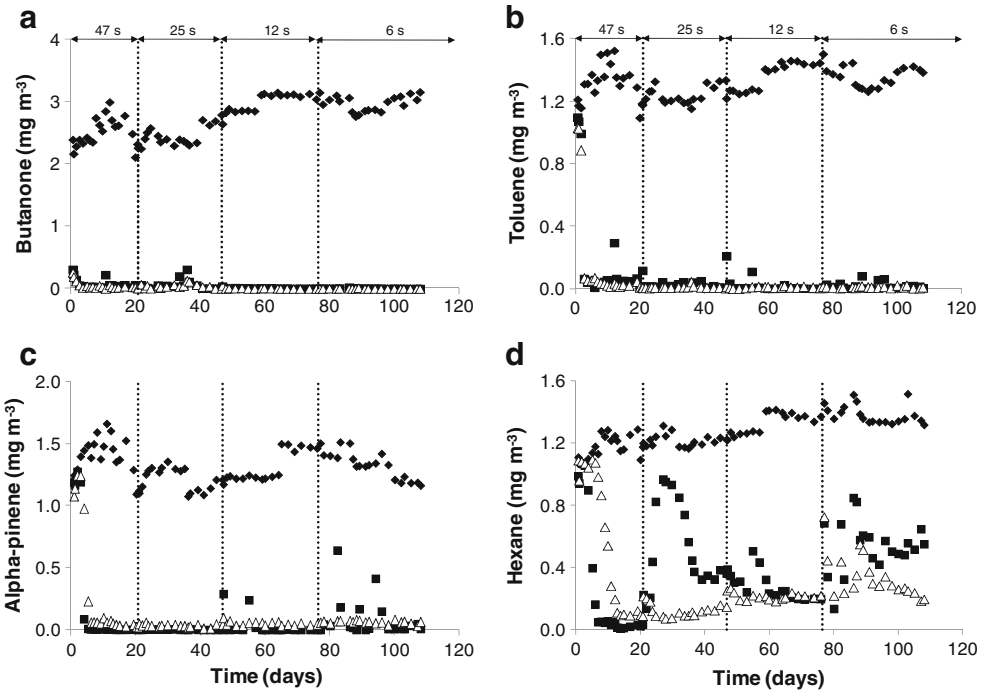

Fig. 1 Time course of the inlet (*diamond*), 1P-BTF outlet (*square*), and 2P-BTF outlet (*triangle*) concentrations of butanone (**a**), toluene (**b**), alpha-pinene (**c**), and hexane (**d**). The *vertical dashed lines* represent the changes in EBRT

performance compared to the 1P-BTF, with steady hexane REs of 92±1, 91±2, 85±1, and 81±4 % at EBRT of 47, 25, 12, and 6 s, respectively. While 3 and 4 days were necessary for process stabilization when the EBRT was decreased from 47 to 25 s and from 25 to 12 s, respectively, 2 weeks was required to achieve a steady state at 6 s.

The 1P-BTF required approximately 6 % more basic solution than the 2P-BTF to maintain the pH at 6.9±0.1 throughout the entire experimental period.

Robustness evaluation

Shock loading

Butanone was not detected in the outlet of any of the BTFs regardless of the inlet concentration increase (Fig. 2a), while the removal performance of toluene (Fig. 2b) slightly decreased to 79 and 97 % after the 3-fold increase in the 1P and 2P-BTF, respectively. The decrease in the toluene RE was, however, significant when the 6-fold surge was applied, with minimum REs of 63 and 88 % in the 1P and 2P-BTF, respectively. The increase in alpha-pinene inlet concentration did not affect significantly the performance of the 2P-BTF, decreasing the REs to 97 and 91 % during the 3- and 6-fold step increase, respectively (Fig. 2c). However, while steady-state REs were immediately recovered in the 2P-BTF when the inlet concentration was restored after the 3-fold increase, 2 h was needed to recover from the 6-fold increase. On the other hand, the alpha-pinene RE in the 1P-BTF sharply decreased from 98±2 to 54 and 38 % during the 3- and 6-fold loading raise, respectively. Finally, hexane removal was severely affected by the fluctuations in the inlet loading regardless of the BTF and concentration increase (Fig. 2d). While the RE in the 1P-BTF decreased from 64±2 to 32±1 and 27±1 %, a decrease from 82±4 to 67±2 and 43±2 % was recorded in the 2P-BTF during the 3- and 6-fold surges, respectively. However, the steady REs were always recovered in less than 30 min after the restoration of the initial inlet concentrations.

Liquid recycling stop

Both bioreactors showed a progressive deterioration in their performance as the bed dried up, this deterioration being VOC specific (Fig. 3). The REs of the four target VOCs gradually increased when liquid recycling was restored. Butanone RE decreased to 53 % in the 2P-BTF during this operational upset, while the lowest RE recorded in the 1P-BTF was 93 % (Fig. 3a). Both systems recovered complete butanone elimination 2 h after the restoration of the liquid recycling. In the case of toluene and alpha-pinene (Fig. 3b, c, respectively), the RE steadily decreased to 47 and 46 % in the 1P-BTF and to 32 and 19 % in the 2P-BTF, respectively. Two days were approximately required in both bioreactors to recover their initial toluene and alpha-pinene REs. Finally, the hexane REs decreased from 58 to 27 % in the 1P-BTF and from 87 to 16 % in the 2P-BTF (Fig. 3d). For this particular VOC, the recovery time accounted for 4 and 5 days in the 1P and 2P-BTF, respectively. It is worth noting that the steady hexane removal performance of the 1P-BTF increased noticeably from 58 to 93±1 % after the liquid stoppage.

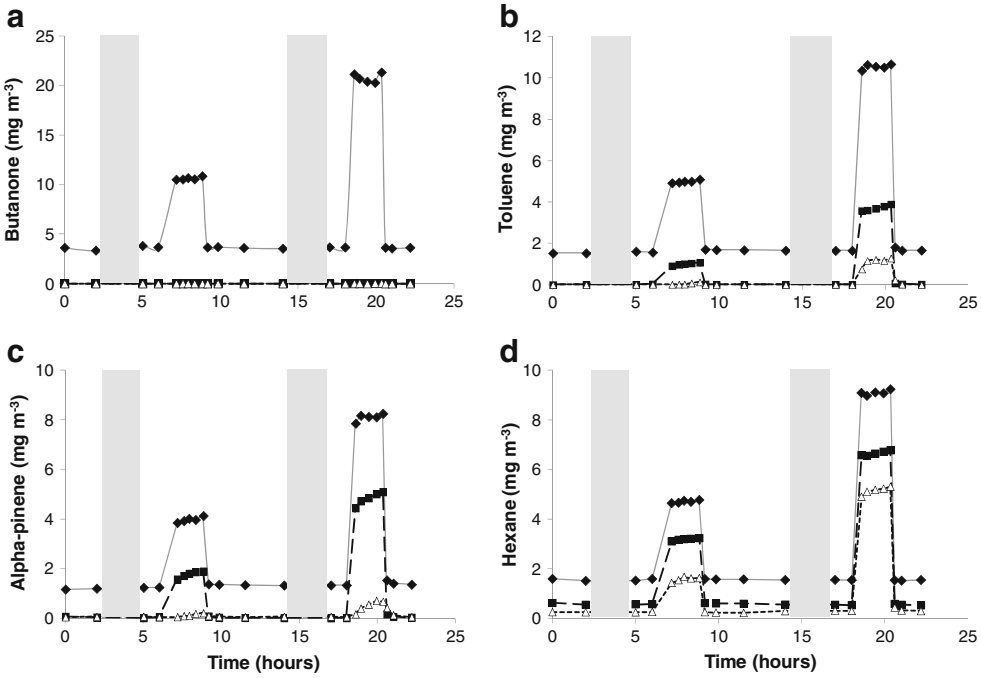

Fig. 2 Time course of the inlet (*diamond*), 1P-BTF outlet (*square*), and 2P-BTF outlet (*triangle*) concentrations of butanone (**a**), toluene (**b**), alpha-pinene (**c**), and hexane (**d**) during the 2 h 3- and 6-fold step increase in the inlet concentrations at an EBRT=6 s. The *gray bars* represent a 12-h period

Starvation period

The removal performance of both BTFs for butanone and toluene was not affected by the starvation period, with REs returning to steady-state values immediately upon the onset of gas feeding (Fig. 4a, b, respectively). Alpha-pinene REs increased from 92 % to steady-state values in less than 10 h in the 2P-BTF, while the single-phase system required 2 days to increase its RE from 78 to >99 % (Fig. 4c). Hexane removals of 47 and 53 % were recorded in the single- and the two-phase BTFs, respectively, following the restoration of the gas feeding, which progressively increased to steady-states values of 71±1 and 88±0 % within 2.8 and 1.9 days, respectively (Fig. 4c).

Internal structure and molecular composition of the bacterial communities

The Shannon–Wiener diversity index (Table 2), with typical values ranging from 1.5 to 3.5 (low and high species evenness and richness, respectively) (McDonald 2003), was high for the inoculum (3.6), 1P-BTF (3.8), and 2P-BTF (3.7).

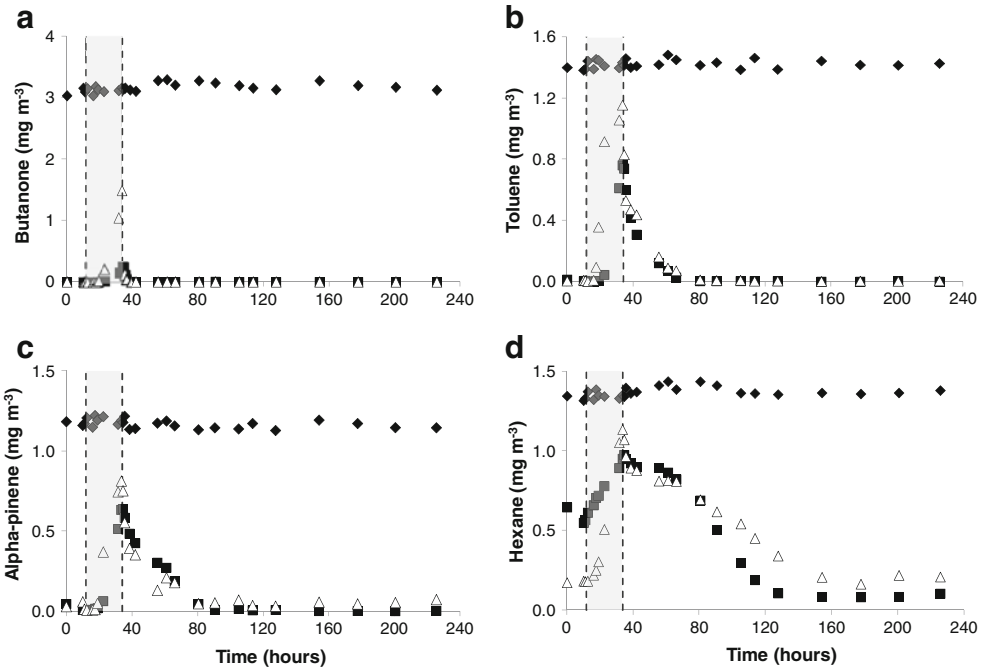

Fig. 3 Dynamic response of the 1P-BTF (*square*) and 2P-BTF (*triangle*) outlet concentrations of butanone (**a**), toluene (**b**), alpha-pinene (**c**), and hexane (**d**) to a 1-day liquid recycling stop at an EBRT=6 s. *Solid diamonds* represent the inlet VOCs concentration, and the *dashed lines* the beginning and end of the period without liquid recycling

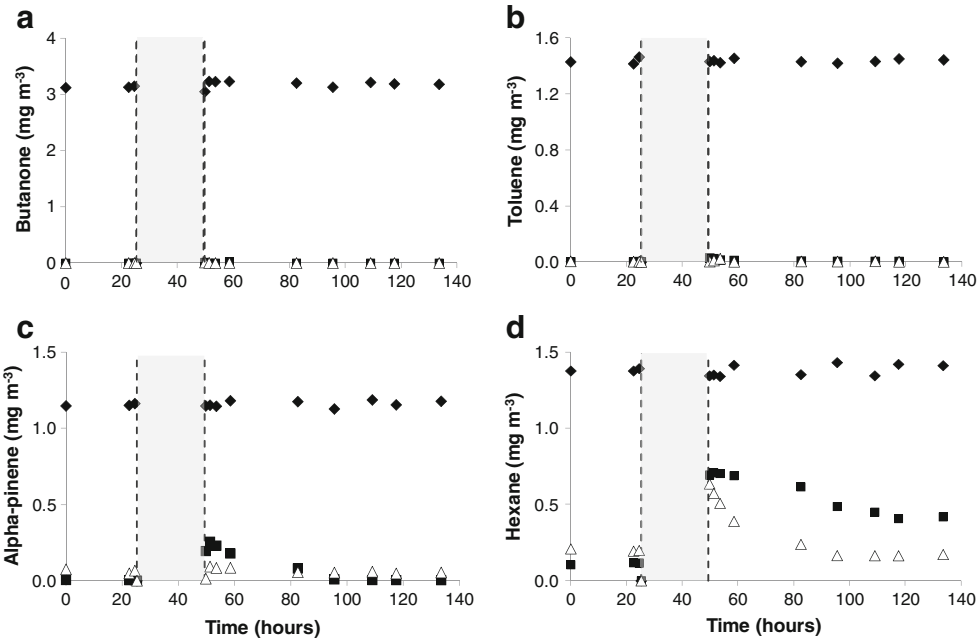

Fig. 4 Time course of the inlet (*diamond*), 1P-BTF outlet (*square*), and 2P-BTF outlet (*triangle*) concentrations of butanone (**a**), toluene (**b**), alpha-pinene (**c**), and hexane (**d**) during the 1-day feed shut-down experiment (*gray bars*) at an EBRT=6 s

Similarly, the R_r values (Table 2) were also high for the inoculum (233), 1P-BTF (268), and 2P-BTF (277) according to the classification of Marzorati et al. (2008). The functional F_o of the bacterial communities was visualized in the constructed Pareto–Lorenz evenness curves (Fig. 5b). The three samples analyzed showed an intermediate F_o (Table 2) based on the criteria of Marzorati et al. (2008), since 20 % of the bands (number based) corresponded to 33–34 % of the cumulative band intensities. Pairwise comparison of the DGGE profiles retrieved Pearson similarity values of 46 and 0 % between the inoculum and the 1P-BTF and the inoculum and the 2P-BTF, respectively. A low Pearson similarity value of 36 % was obtained between the 1P and 2P-BTF.

From the DGGE gel, 21 bands were sequenced (Fig. 5a). Six different phyla were retrieved according to the RDP classifier tool (bootstrap value of 50 %) in the RDP database: *Proteobacteria* (seven bands), *Actinobacteria* (five bands), *Acidobacteria* (four bands), *Nitrospira* (one band), *Chlamydiae* (one band), and *Verrucomicrobia* (two bands), and one band remained unclassified. *Proteobacteria* followed by *Actinobacteria* and *Acidobacteria* phyla were dominant in the 1P and 2P- BTFs. Seventeen DGGE bands were classified till the genus taxonomic level according to the RDP classification. The closest matches for each band (BLASTN) using the NCBI database are shown in Table 3, along with its similarity percentages and sources of origin.

Table 2 Shannon diversity index, ranged weighted richness (Rr) and Pareto Lorenz values (indicating the Fo of the communities) calculated from the DGGE patterns

	Inoculum	1P-BTF	2P-BTF
Shannon diversity (H)	3.6	3.8	3.7
Ranged weighted richness (R_r)	233	268	277
Functional organization (F_o) (%)	33	34	33

Rr low (<10), medium (10–30), high (>30); F_o low F_o/high evenness (25 %), medium F_o and evenness (30–70 %), high F_o/low evenness (70 %)

Discussion

This study constitutes, to the best of our knowledge, the first systematic evaluation of the performance of TPPBs for the removal of VOCs at trace level concentrations (milligrams per cubic meter), targeting the treatment of odorous emissions from WWTPs (Zarra et al. 2008). In our particular study, both 1P- and 2P-BTF showed butanone, toluene, and alpha-pinene REs higher than 96 % at EBRTs as low as 6 s, due to their relatively low mass transfer limitations from the gas to the aqueous phase (Henry's law constants at 25 °C (H)=C_{aq} C_g^{-1}=4.1×10^2 for butanone, 4.0 for toluene, and 1.2 for alpha-pinene, where C_{aq} and C_g are the concentration in the aqueous and gas phase, respectively) (Sander 1999). These high REs can be also explained by the high microbial diversity present in the inocula (as discussed later) and the presence of specific microorganisms able to degrade aromatic compounds (*Rhodopseudomonas*-related organisms, members of the *Betaproteobacteria* and *Actinobacteria* phyla, *Nitrospira*, etc.) (Lebrero et al. 2011, 2012). However, the 2P-BTF outperformed the conventional BTF for the removal of hexane, one of the most hydrophobic VOCs (H=0.014) (Sander 1999), at low EBRTs and supported a more stable removal performance for the other VOCs. This superior removal performance was likely driven by the lower partition coefficient

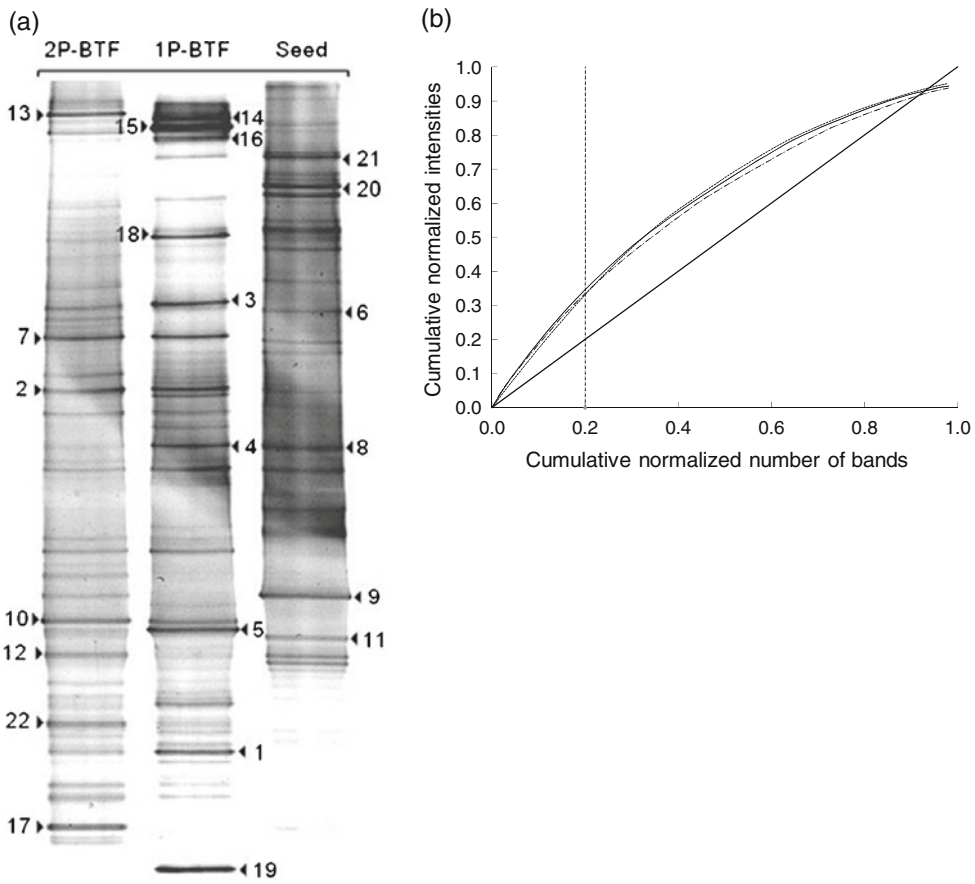

Fig. 5 **a** DGGE patterns of the inoculum and final biomass from 1P and 2P-BTFs. The *numbers* correspond to the 21 bands sequenced. **b** Pareto–Lorenz distribution curves derived from the DGGE patterns. The *dashed vertical line* at the 0.2 *x*-axis level is plotted to numerically interpret the PL values

of hexane in silicone oil ($K=C_{SO}\ C_g^{-1}=1.7\times10^2$, where C_{SO} is the concentration in silicone oil, Hernández et al. 2010a) compared to that of water, which enhanced the mass transfer of this VOC from the gas phase to the microorganisms (which might be present both in the aqueous and silicone oil phase). Besides, the progressive deterioration of the hexane removal performance observed after each decrease in the EBRT in the 1P-BTF was not recorded in the 2P-BTF as a result of its improved mass transfer and the capacity of the organic phase to absorb any accumulation of VOCs.

Under real operation, process fluctuations and operational upsets are more frequently encountered than constant operational conditions. Therefore, the dynamic stability of the system and the ability to rapidly recover after operational upsets have to be validated (Nielsen et al. 2005). The results here reported demonstrated that the 2P-BTF was able to better withstand step VOC concentration increases and starvation periods when compared to the 1P-BTF. For example, the risk associated to a 6-fold step surge was 2.37 and 0.15 % of loss of total alpha-pinene removal per year in the 1P- and 2P-BTF, respectively (Table 1). In the particular case of hexane, although lower REs were achieved in the 1P-BTF during the 6-fold step loading increase, the recovery time for the 2P-BTF was slightly higher, probably due to the faster hexane adsorption compared to its biodegradation rate, increasing the outlet hexane concentration when hexane was desorbed following the restoration of the initial inlet concentrations, therefore increasing the loss of hexane removal per year (1.95 % in the 2P-BTF vs. 0.82 % in the 1P-BTF). The fast mass transfer and the high solubility of most VOCs in the non-aqueous phase ($K_{toluene}=3.5\times10^2$ (Poddar and Sirkar 1996), $K_{alpha\text{-}pinene}=5.5\times10^3$ (Muñoz et al. 2008), $K_{hexane}=1.7\times10^2$ (Hernández et al. 2010b)) prevent microorganisms present in the aqueous phase from being exposed to high and toxic concentrations, improving the stability of TPPBs toward surges in inlet VOC concentration or emission flow rates. However, the transient behavior of 2P-BTFs has been scarcely investigated so far (Nielsen et al. 2005; Rene et al. 2011).

On the contrary, the 2P-BTF was more affected by the shutdown in the liquid recirculation than the single-phase BTF, with lower REs during the dry period, while both systems presented similar recovery times after liquid resumption. Since approximately 50 % of the silicone oil was recycled through the bed together with the aqueous phase as a stable emulsion (the 50 % remaining embedded in the packing material), the liquid shutdown likely reduced the mass transfer of the VOCs in this system. A high hexane RE was recorded in the 1P-BTF after liquid recycling resumption (Fig. 3) compared to the previous steady REs (lower outlet concentrations which resulted in a negative risk of −0.09 % loss of hexane removal; Table 1). However, after the feed shutdown, the

Table 3 RDP classification of the DGGE bands sequenced and corresponding matches (BLASTN) using the NCBI database with indication of the similarity percentages and sources of origin

Taxonomic placement (50 % confidence level)	Band no.	Seed	R1	R2	Closest relatives in Blast Name (accession number)	Similarity (%)	Source of origin
Proteobacteria							
	1		x	x	Uncultured bacteria (HQ440055)	99	WWTP receiving multi-classes of antibiotics
					Uncultured *Alphaproteobacteria* (HQ132470)	98	Heavy metal-contaminated estuarine sediment
Alphaproteobacteria							
Rhizobiales							
Bradyrhizobiaceae							
Rhodopseudomonas							
	2		x	x	Uncultured *Alphaproteobacteria* (CU926988)	99	Anaerobic digester which treats municipal wastewater sludge
					Uncultured bacteria (AY491632)	99	Microbial fuel cell enriched with artificial wastewater
Rhodobacterales							
Rhodobacteraceae							
	3		x		*Rhodobacteraceae* bacterium D3-7bb (AM403177)	99	Marine aquaculture biofilter
					Pararhodobacter aggregans^{T} (AM403160)	99	Marine aquaculture biofilter
Gammaproteobacteria							
Xanthomonadales							
Xanthomonadaceae							
Stenotrophomonas							
	4		x	x	*Stenotrophomonas acidaminiphila* (JF894171)	99	Petroleum-polluted soil
					Stenotrophomonas acidaminiphila (NR025104)	99	Upflow anaerobic sludge blanket (UASB) reactor
Dokdonella							
	5		x	x	Uncultured *Dokdonella* sp. (JF808836)	99	Activated sludge in a membrane bioreactor
					Dokdonella sp. (NR044554)	99	Soil
					Uncultured bacteria (JQ038783)	98	BTF treating VOCs at low concentrations (MeSH, toluene, α-pinene, hexane)
Betaproteobacteria							
Burkholderiales							
Comamonadaceae							
Simplicispira							
	6	x	x	x	*Simplicispira limi*^{T} (NR043773)	98	Activated sludge
					Uncultured *Betaproteobacteria* (CU466883)	98	Anoxic basin of a municipal WWTP
Deltaproteobacteria							
Myxococales							
Polyangiaceae							
	7		x	x	Uncultured *Myxococcales* (JF515259)	98	Soil
Actinobacteria							
Actinobacteria							
Acidimicrobiales							
Iamiaceae							
Iamia							
	8	x			Uncultured bacteria (JN606102)	99	Reactors inoculated with activated sludge treating toluene at different concentrations
					Candidatus "*Microthrix parvicella*" (FJ638889)	99	WWTP (activated sludge)
	9	x		x	Uncultured bacteria (JN606102)	100	Reactors inoculated with activated sludge treating toluene at different concentrations
	10		x	x	Uncultured bacteria (JN606102)	100	Reactors inoculated with activated sludge treating toluene at different concentrations
	11	x			Uncultured bacteria (JN606102)	99	Reactors inoculated with activated sludge treating toluene at different concentrations
					Candidatus "*Microthrix parvicella*" (FJ638889)	99	WWTP (activated sludge)

Table 3 (continued)

Taxonomic placement (50% confidence level)	Band no.	Seed	R1	R2	Closest relatives in Blast Name (accession number)	Similarity (%)	Source of origin
	12	x	x	x	Uncultured bacteria (JN606102)	99	Reactors inoculated with activated sludge treating toluene at different concentrations
					"*Candidatus Microthrix parvicella*" (FJ638889)	99	WWTP (activated sludge)
Acidobacteria							
*Acidobacteria*_Gp4							
Genus Gp4							
	13		x	x	Uncultured bacterium (GQ264413)	99	Simulated low level radioactive waste site
	14		x	x	Uncultured bacterium (HQ119859)	99	Loamy sand collected from a field planted with tomatoes
	15		x		Uncultured bacterium (HQ119859)	99	Loamy sand collected from a field planted with tomatoes
	16		x	x	Uncultured bacterium (HQ119859)	99	Loamy sand collected from a field planted with tomatoes
Nitrospira							
Nitrospira							
Nitrospirales							
Nitrospiraceae							
Nitrospira							
	17	x	x	x	Uncultured bacteria (JN606106)	99	Reactors inoculated with activated sludge treating toluene at different concentrations
					Uncultured bacteria (HQ147611)	99	Activated sludge system treating VOCs
					"*Candidatus Nitrospira defluvii*" (EU559167)	98	WWTP
Chlamydiae							
Chlamydiae							
Chlamydiales							
Waddliaceae							
Waddlia							
	18	x	x	x	*Estrella* sp. (EU363463)	93	Drinking water treatment plant
Verrucomicrobia							
Verrucomicrobiae							
Verrucomicrobiales							
Verrucomicrobiaceae							
Luteolibacter							
	19		x	x	Uncultured *Verrucomicrobiales*	97	Upper sediment (wetland)
					Luteolibacter pohnpeiensis^{T} (NR041625)	95	Wood piece
Prosthecobacter							
	20	x			Uncultured bacterium (JN606104)	99	Reactors inoculated with activated sludge treating toluene at different concentrations
					Prosthecobacter fluviatilis^{T} (NR041608)	99	Soil and river water
Unclassified bacteria							
	21		x	x	Uncultured bacteria (HQ682010)	98	Biofilm in zeolite biological aerated filters

The presence/absence of each band in each sample tested is also shown

steady RE dropped again to values similar to the former steady states (Fig. 4). The reason underlying this phenomenon is unknown and deserves further investigation. The low REs achieved in the 2P-BTF resulted in a higher total risk (1.48 % loss of total VOCs removal per year vs. 0.32 % in the one-phase system).

The effect of the 24-h starvation period was negligible for butanone and toluene, the easiest biodegradable compounds, whereas the removal performance for alpha-pinene and hexane slightly decreased after feed restoration. The 2P-BTF showed not only higher REs but also shorter recovery times compared to the 1P-BTF, probably due to the availability of VOCs in the silicone oil during the starvation period, which likely mediated a higher metabolic activity of the communities in the 2P-BTF. However, both systems recovered the steady removals in less than 3 days. This ability to rapidly recover steady removal performance was also reported by Nielsen et al. (2005) during a 24-h benzene-feed stoppage in a two-phase stirred tank bioreactor. The risk related to this shutdown was low in spite of the decrease in the REs due to its low probability of occurrence (0.35 and 0.09 % total loss of VOC removal in the 1P- and the 2P-BTF, respectively) (Table 1).

The overall risk during the operation of the 1P- and the 2P-BTF was negligible for butanone, while for toluene the risk associated was a yearly loss of 1.97 and 0.50 %, respectively. On the other hand, the risk associated to alpha-pinene accounted for a yearly loss of 3.66 and 0.59 % in the 1P- and 2P-BTF, respectively, while a total loss of hexane removal of 3.68 and 2.83 % was calculated for the 1P- and 2P-BTF (Table 1). The daily step increase in VOC concentration was the major contributor to the risk in both bioreactors, mainly due to its high probability of occurrence (1/day).

Solvent loss has been often pointed out as one of the main disadvantages of TPPBs. In the present study, almost 50 % of the silicone oil remained embedded in the packing material even after bed washing. Besides, a previous study in a BTF containing silicon oil confirmed that the loss of oil was <3 %/year (Van Groenestijn and Lake 1999).

Similar levels of R_r, F_o, and Shannon index were obtained for the bacterial communities in the 1P-BTF, 2P-BTF, and inoculum (Table 2). Both BTFs exhibited a high biodiversity and richness (R_r>30) despite being fed with a limited C source spectrum. Highly diverse populations have also been found in bioreactors treating VOCs at low concentrations (Bayle et al. 2009; Lebrero et al. 2011, 2012). As depicted by the y-intercept at 0.2 calculated from the Pareto–Lorenz curves (Fig. 5b), the bacterial communities showed a medium–low F_o, which indicates a medium–high evenness. Communities with high species diversity often possess a high degree of functional redundancy, which confers them a higher robustness toward process fluctuations (Girvan et al. 2005). Likewise, community evenness is a key factor conferring it a high functional resilience (Wittebolle et al. 2009a). Hence, the community structure parameters here obtained showed populations with a strong carrying capacity and adapted to changing operational conditions in both BTFs. In this context, the high initial community evenness and richness of the inoculum may have been a determinant factor for the selection of communities with such characteristics, since previous studies demonstrated the key influence of inoculum evenness (Witebolle et al. 2009a) and diversity (Cabrol et al. 2012) on the functional stability and robustness of the bioreactors. In spite of the high similarity of 1P- and 2P-BTF in terms of community structure, the differences in the phylogenetic composition of the communities caused by the absence/presence of silicon-oil (low Pearson similarity values between 1P- and 2P-BTF) might explain the different macroscopic performance of both reactors.

In this work, the analysis of DGGE bands rendered six different phyla (*Proteobacteria*, *Actinobacteria*, *Acidobacteria*, *Nitrospira*, *Chlamydiae*, *Verrucomicrobia*). These phyla have been previously found in biological odor abatement systems (Lebrero et al. 2011, 2012). Moreover, the VOC biodegradation ability of some of the microorganisms identified in this work has been already reported in literature. For instance, in the *Proteobacteria* phylum, *Rhodopseudomonas*-related organisms (DGGE band 2) are able to degrade aromatic compounds (Larimer et al. 2004), *Stenotrophomonas*-related bacteria (DGGE band 4) can use BTEX as a carbon and energy source (Lee et al. 2002), and members in the *Burkholderiales* order (DGGE band 6) of the *Betaproteobacteria* show an extraordinary catabolic potential for aromatic compounds (Pérez-Pantoja et al. 2011). Microorganisms in the *Actinobacteria* phylum (DGGE fragments 8–12), which includes aromatic and aliphatic hydrocarbon-degrading bacteria, were detected with high intensity in the 2P-BTF and in the inoculum. Similarly, based on the intensity of the DGGE bands, *Nitrospira*-like bacteria (DGGE band 17) likely played a key role in the removal of VOCs in the 2P-BTF. Highly similar sequences (accession numbers: JN6060106, HQ147611) to DGGE band 17 were retrieved from bioreactors treating different VOCs. In fact, nitrifying organisms have been shown to degrade aromatic and non-aromatic hydrocarbons (Shinoda et al. 2004; Silva et al. 2009). Other phyla such as *Acidobacteria* (DGGE bands 13–16), *Verrucomicrobia* (DGGE band 19), and *Chlamydiae* (DGGE band 18) were more abundant in 1P-BTF than in 2P-BTF (based on band intensities), but their role in the degradation of the different VOCs is still unknown.

In brief, the high REs supported by the 2P-BTF and its superior robustness demonstrate the potential of TPPB as a high-performance alternative for the treatment of odorous waste gases, which constitutes a step further in the implementation of this technology. There was no significant influence of the presence of silicone oil on community richness, evenness, and diversity, although it severely impacted the phylogenetic composition of the communities as shown by their low Person similarity value.

Acknowledgments This research was supported by the Spanish Ministry of Science and innovation (RYC-2007-01667 contracts and projects CTQ2009-07601 and CONSOLIDER-CSD 2007-00055). The Regional Government of Castilla y León is also gratefully acknowledged for its funding (Project VA004A11-2 and GR76).

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Chapter 9

A membrane bioreactor for the simultaneous treatment of acetone, toluene, limonene and hexane at trace level concentrations

A membrane bioreactor for the simultaneous treatment of acetone, toluene, limonene and hexane at trace level concentrations

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Abstract

The performance of a flat-membrane biofilm reactor (MBR) for the removal of acetone, toluene, limonene and hexane at concentrations ranging from 1.3 to 3.2 mg m^{-3} was investigated at different gas residence times (GRT): 60, 30, 15 and 7 s. A preliminary abiotic test was conducted to assess the mass transport of the selected volatile organic compounds (VOCs) through the membrane. A reduced transport of limonene and hexane was observed with water present over the dense side of the membrane. The presence of a biofilm attached on the dense side of the membrane following bioreactor inoculation significantly increased VOC transport. High acetone and toluene removals (>93%) were recorded in the MBR regardless of the GRT. To remediate the low hexane removal performance (RE< 24 %) recorded at the initial stages of the process, a re-inoculation of the membrane with a hexane-degrading consortium embedded in silicon oil was performed. Although hexane removal did not exceed 27%, this re-inoculation increased limonene removals up to 90% at a GRT of 7s. The absence of inhibition of hexane biodegradation by substrate competition confirmed that hexane removal in the MBR was indeed limited by the mass transfer though the membrane. Despite the low carbon source spectrum and load, the microbiological analysis of the communities present in the MBR showed high species richness (Shannon-Wiener indices of 3.2-3.5) and a high pair-wise similarity (84-97 %) between the suspended and the attached biomass.

Keywords: Membrane bioreactor, Odorous VOCs, Trace level concentrations, Waste gas treatment

1. Introduction

Biological technologies such as biofilters, biotrickling filters and bioscrubbers are nowadays the best available techniques for odour abatement from both an economical and environmental perspective (Estrada et al., 2011). In addition to their lower operating costs, biotechnologies exhibit lower energy/chemical consumptions and CO_2 emissions than their physical-chemical counterparts (e.g. activated carbon adsorption, chemical scrubbing, incineration, etc.). However, these biotechnologies face severe mass transfer limitations when treating hydrophobic odorants. In bioscrubbers, the gaseous pollutants are absorbed in a water recycling phase prior to its biodegradation and thus only odorants with a low Henry constant ($H = C_g\ C_{aq}^{-1} < 0.01$, where C_g and C_{aq} are the pollutant concentrations in the gas and aqueous phases, respectively) are efficiently treated. Similarly, the presence of a trickling and a stagnant water layer over the packing bed of biotrickling filters and biofilters, respectively, also limits the odorant mass transfer, although in a lesser extent (odorant with $H < 0.1$ for biotrickling filters and $H < 10$ for biofilters; Mudliar et al. 2010). Hence, biotechnologies for odour treatment usually

present low removal efficiencies (RE) for the less water soluble odorants (i.e. terpenes, volatile organic sulfides, alkanes, hydrocarbons, etc.) (Iranpour et al. 2005). This mass transfer limitation directly impacts on the footprint of biotechnologies: lower mass transfer rates entail higher gas residence times and therefore higher bioreactor volumes.

Membrane bioreactors for waste gas treatment (MBR) can overcome these mass transfer limitations due to the high permeability and affinity of some particular membranes for hydrophobic pollutants (Kumar et al. 2008). In MBRs, the membrane also serves as a support for the growth of the microbial population responsible for pollutant biodegradation (although biomass might be also suspended in the aqueous phase), which significantly increases the pollutant concentration gradients available for mass transport (Kumar et al. 2008). In a typical membrane bioreactor configuration, the volatile organic compound (VOC) and O_2 laden gas stream circulates through one side of the membrane, while on the other side, an attached biofilm is submerged into a mineral salt solution that provides the water and nutrients required for microbial growth. This mineral salt solution is usually recycled, buffered to maintain a suitable pH and replaced periodically with fresh solution to replenish nutrients and avoid toxic by-products accumulation. The performance of MBRs is determined by the membrane material (polydimethylsiloxane (PDMS), polypropylene (PP), polyethylene (PE), polyvinylidene difluoride (PVDF), etc., Kumar et al. 2008) and the type of membrane configuration (plate and frame, spiral wounded, tubular, capillary or hollow fiber modules) (Mulder, 1997). To date, most of the studies on MBRs focused on the removal of individual compounds such as toluene, propene, benzene, etc. at high concentrations (g m^{-3}) (Kumar et al., 2008), while research on the performance of MBRs for the removal of mixtures of VOCs is scarce. In this context, since odorous emissions are complex mixtures of sulphur/nitrogen derived compounds and VOCs at concentrations in the order of mg m^{-3}- μg m^{-3}, the results reported in literature studies for MBRs cannot be directly applied to odour abatement. Besides, it has been hypothesized that the low substrate concentrations is one of the main limitations of MBRs since they might not sustain an active microbial population (Kumar et al. 2008).

The present study aims at investigating the performance of a flat MBR for the treatment of a mixture of VOCs (acetone, toluene, limonene and hexane) at trace level concentrations in order to evaluate: i) the influence of VOC nature on the transport through the membrane and on the biodegradation, ii) the performance of MBRs at the low VOC loads typically found in WWTP odorous emissions (mg m^{-3}), iii) the dynamics of microbial biodiversity linked to the MBR performance.

2. Materials and Methods

Chemicals and reagents

Acetone was purchased from Chem-Lab (+99%), toluene and limonene from Sigma Aldrich with a purity >99% and >97%, respectively, and hexane (purity +99%) from Acros Organics (USA). All chemicals for mineral salt medium (MSM) preparation were purchased from Acros Organics (USA) with a purity of at least 98%, and vitamins were obtained from Laboratories Vitarmonyl (France).

Bioreactor set-up

A laboratory-scale flat membrane bioreactor made of Perpex was used (Fig. 1). Although hollow fibre MBRs offer higher specific gas-liquid surface areas, a flat-sheet configuration is preferred due to its easier operation (cleaning and membrane replacement) (Ergas and McGrath, 1997). A commercially available composite flat membrane was provided by GKSS Forschungszentrum Geesthacht (Germany). The hydrophobic dense top layer material was polydimethylsiloxane (PDMS) with an average thickness of 0.3 μm while the porous hydrophobic support layer was polyacrylonitrile (PAN) with a thickness of 50 μm. The membrane was clamped between the two identical compartments of the reactor and placed in an isothermal chamber at 23ºC. The total volume of the reactor was 16 mL (8 mL of gas volume and 8 mL of liquid volume) and the contact area of the membrane was 40 $cm^{2.}$

The MSM solution was continuously recycled along the dense side (liquid side) of the membrane at a velocity of 30 mL min^{-1}

by a peristaltic pump (Masterflex, Cole Parmer, USA). The necessary macro and micronutrients were supplied via a buffered nutrients solution containing KNO_3 53.6 g L^{-1}, KH_2PO_4 3.0 g L^{-1}, K_2HPO_4 3.0 g L^{-1}, $MgSO_4$ 2.5 g L^{-1}, micronutrients (P, Ca, Fe, Zn, Co, Mn, Mo, Ni, B) and vitamins at trace concentrations (Álvarez-Hornos et al. 2011). The fresh nutrients solution was periodically supplied to the MBR to maintain nitrogen concentration above 20 mg L^{-1} in the recycling solution. The total liquid volume in the reservoir, maintained under continuous agitation in a thermostatic bath at 23ºC, was 800 mL.

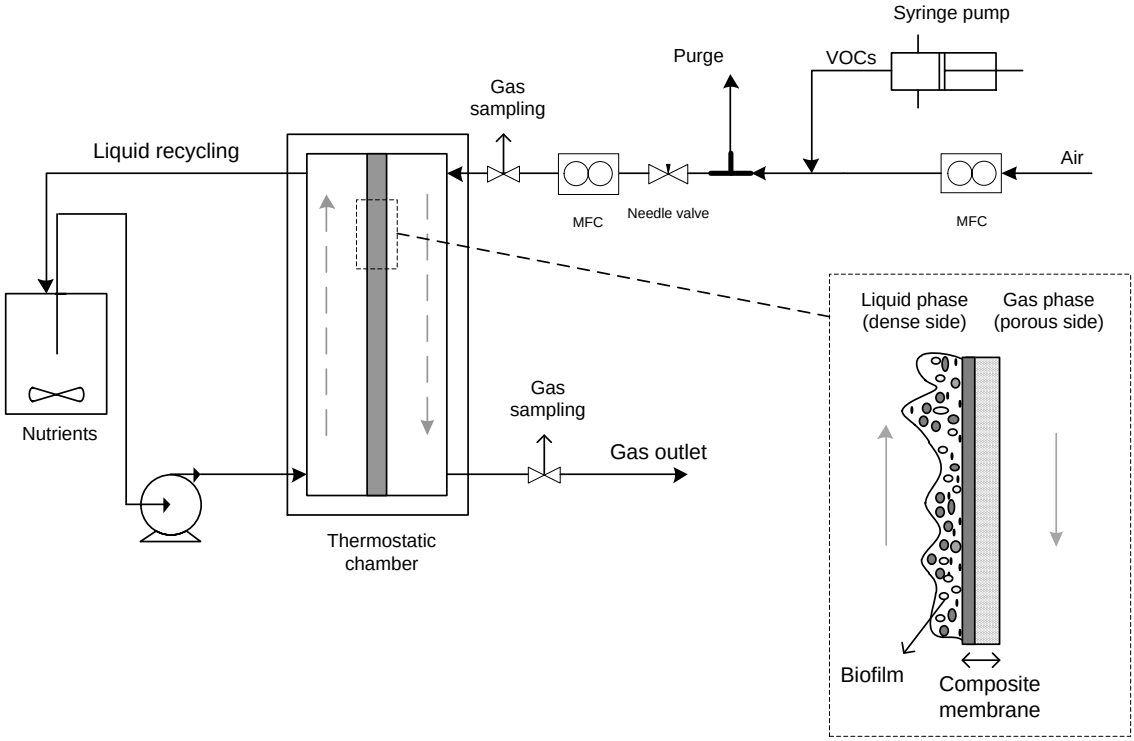


Fig 1. Schematic representation of the experimental setup

The contaminated air stream was obtained by evaporating a mixture of the target VOCs. The liquid VOC mixture was loaded in a syringe (Hamilton Gastight, Switzerland) and dosed into the air stream by means of a syringe pump (model NE 1000, Qis, USA). The pumping velocity was controlled to maintain the inlet concentrations of acetone, toluene, limonene and hexane at 2.5±0.1, 2.4±0.1, 3.2±0.1 and 1.3±0.0 mg m^{-3}, respectively. The volumetric loading rates (based on gas side reactor volume) fed to the MBR ranged between 0.1-10.2 g m^{-3} h^{-1} for acetone, 0.2-13.4 g m^{-3} h^{-1} for toluene, 0.1-5.6 g m^{-3} h^{-1} for limonene and 0.1-9.6 g m^{-3} h^{-1} for hexane. The gas flow rates (in a counter current configuration) through the porous side (gas side) of the MBR were accurately controlled by a mass flow controller (Brooks, Holland).

Abiotic mass transfer characterization

The mass transport of the four VOCs through the membrane was determined according to Kumar et al. (2009) under two different scenarios in the dense/porous sides of the membrane: air/air and air/water. In the air/air scenario, the polluted air containing the target VOCs was introduced through the porous side at three different gas residence times (GRTs, defined as the volume of the gas chamber divided by the gas flowrate): 30, 16 and 7 s, while clean air passed through the dense side at a constant velocity of 30 mL min^{-1}. The inlet and outlet concentrations of the VOCs in the polluted stream and the outlet concentration of the clean air were periodically measured. Each experimental condition was maintained until the standard deviation of three consecutive measurements was lower than 10% and the mass balance over the reactor was evaluated to ensure the accuracy of the results obtained. In the air/water scenario, MSM instead of clean air was continuously fed to the dense side of the membrane at 30 mL min^{-1}. In this case, the transport of the VOCs was determined at 4 different GRTs: 60, 30, 16 and 7 s.

Inoculation and bioreactor operation

The bioreactor was inoculated with aerobic activated sludge from the Ossemeersen WWTP (Ghent, Belgium) previously stored at 4ºC for one month. The initial biomass concentration in the recycling nutrients solution was 0.6 g of total suspended solids (TSS) L^{-1}. The reactor was operated at 60 s of GRT for the first 23 days. At day 24, the MBR was re-inoculated with fresh activated sludge from Valladolid WWTP (Spain) (TSS in the recycling liquid = 3.4 g L^{-1}) and operated under similar conditions until day 42. At day 42, the MBR was stopped and re-inoculated with a hydrophobic microbial consortium due to the low limonene and hexane removal efficiencies. This hydrophobic bacterial consortium consisted of hexane-degrading bacteria immersed in silicon oil (Hernández et al., 2012). The silicone oil containing the hydrophobic bacteria was spread on the membrane surface of the liquid side (dense layer). The reactor was operated for 2 days with no liquid recycling to allow bacteria to grow on the membrane surface and avoid their removal by liquid shearing. At day 44 the liquid recycling was restarted and the performance of the MBR was then evaluated at GRTs of 60, 30, 15 and 7 s. Each steady state was maintained for at least 8 days. Finally, in order to assess any potential inhibition of hexane biodegradation in the MBR by the presence of the other VOCs, hexane (1.6±0.3 mg m^{-3}) was directly fed to the MBR, which contained a new membrane impregnated with the hydrophobic microbial consortia. This MBR was operated under these conditions at a GRT of 7 s for 21 days. The inlet and outlet gas concentrations were daily measured by SPME-GC-FID.

Biodegradation tests

At days 18 and 33, two sets of VOC biodegradation tests were performed to assess the catabolic potential of the biomass present in the MBR. In both tests, 5 mL of bacterial suspension from the recycling liquid were added to 12 serological bottles of 120 mL. The bottles were maintained under continuous magnetic agitation (100 min^{-1}) at 22ºC. In 2 serological bottles, 5 mL of distilled water instead of bacterial culture were added to serve as control. The bottles were sealed with mininert valves (Sigma-Aldrich, USA) and the VOCs were added to the headspace at initial concentrations of 0.05 and 0.4 mg m^{-3} of acetone, 2.3 and 2.3 mg m^{-3} of toluene, 2.5 and 3.1 mg m^{-3} of limonene and 1.7 and 1.8 mg m^{-3} of hexane in the first and second tests, respectively. The concentration of the VOCs was periodically measured for 9 hours by SPME-GC-FID by removing a test bottle each time due to the destructive nature of the analysis.

Analytical Methods

Gas samples from the inlet and outlet sampling ports of the experimental setup were periodically collected in 125 mL glass bulbs (Alltech, USA) and pre-concentrated for 15 min by SPME using a 75 µm PDMS-Carboxen fiber (Supelco, USA). The VOC concentrations were then determined in a GC-FID (Agilent 4890, USA) equipped with a HP-1 column (30 m × 0.53 mm × 5 µm). The injector and detector temperatures were 300ºC and 250 ºC, respectively. The oven temperature was maintained at 35ºC for 2 min, then increased at 10ºC min^{-1} up to a temperature of 75ºC, at 20 ºC min^{-1} up to 220 ºC and finally hold at this temperature for 1 min. The He flow was 5.2 mL min^{-1}.

Liquid samples of 20 mL were periodically collected from the nutrients storage bottle to analyze the concentration of phosphate, nitrate, total nitrogen and COD by Nanocolor Test Tubes (Macherey-Nagel, Germany). The pH was analyzed by a pHmeter (Jenway, UK; electrode from Hamilton, Switzerland).

Microbiological procedures

To evaluate the richness and composition of the bacterial communities, biomass samples of the three inocula (activated sludge from Ossemeersen WWTP -sample A-, activated sludge from Valladolid WWTP -sample B-, and the hydrophobic microbial consortium -sample F-), of the liquid recycling media at day 28 (sample C), 42 (sample D) and 80 (sample G) and of the membrane biofilm at day 42 (sample E) and 80 (sample H) were collected and stored immediately at – 20ºC. The biofilm samples were retrieved by removing the membrane from the reactor and scrapping part of the biofilm from the membrane surface.

The genomic DNA was extracted according to Lebrero et al. (2012b). The PCR mixture (50 µL) was composed of 25 µL of BIOMIX ready-to-use 2× reaction mix

(Bioline, Ecogen), containing reaction buffer, magnesium, deoxynucleotide triphosphates (dNTPs), Taq polymerase and additives, l or 2 µL of the extracted DNA, PCR primers 968-F-GC and 1401-R (10µM) (Sigma-Aldrich, St. Louis,MO, USA) for bacterial 16S rRNA gene amplification, and Milli-Q water up to a final volume of 50 µL. The PCR thermo-cycling program used was previously described in Lebrero et al. (2012b).

DGGE analysis of the amplicons was performed with a D-Code Universal Mutation Detection System (Bio Rad Laboratories) using 8% (w/v) polyacrylamide gels with a urea/formamide denaturing gradient of 45 to 65%. The DGGE running conditions were applied according to Roest et al. (2005). The gels were stained with SYBR Green I nucleic acid gel stain (Sigma Aldrich, St.Louis, MO, USA) for 1 h. The obtained DGGE patterns were processed using the GelCompar IITM software (Applied Maths BVBA, Sint-Martens-Latem, Belgium). After image normalization, bands were defined for each sample using the bands search algorithm within the program. Similarity indices of the compared profiles were calculated from the densitometric curves of the scanned DGGE profiles by using the Pearson product–moment correlation coefficient (Häne et al. 1993). The peak heights in the densitometric curves were also used to determine the Shannon–Wiener diversity index (H), which considered both the relative number of the DGGE bands (richness) and their relative intensities (evenness):

$$H = -\sum \left[P_i \ln(P_i) \right]$$

where P_i is the importance probability of the bands in a lane ($P_i = n_i/n$, n_i is the height of an individual peak and n is the sum of all peak heights in the densitometric curves).

Sequencing and DNA sequence analysis

Some bands were excised from the DGGE gel in order to identify the microorganisms present both in the inocula and in the MBR. The procedure was previously described in Lebrero et al. (2011). The taxonomic position of the sequenced DGGE bands was obtained using the RDP classifier tool (50% confidence level) (Wang et al. 2007). The closest matches to each band were obtained using the BLAST search tool at the NCBI (National Centre for Biotechnology Information) (McGinnis and Madden, 2004). Sequences were deposited in GenBank Data Libray under accession numbers JX627815-JX627846.

3. Results and Discussion

Abiotic mass transfer characterization

Acetone was completely transferred at a GRT higher than 16 s when air was present at both sides of the membrane. The transfer efficiency decreased to about 60 % at a GRT of 7 s. When water was flowing at the dense membrane side RE decreased to 89% at GRT of 16 and 30 s. However, at 7 s of GRT, the acetone transport through the membrane with water was superior than with air flowing through the dense side (Fig. 2a). The transport of toluene in the air/air experiments was similar to that of acetone, but decreased noticeably in the air/water scenario: 64%, 52% and 13% at GRTs of 30, 16 and 7 s, respectively (Fig. 2b). These results were in agreement to those obtained by Kumar et al. (2009) with a similar composite membrane (PDMS 0.3µm/PAN 185µm) and toluene as the only pollutant in the gas phase. Hexane and limonene presented the lowest percentages of mass transfer through the membrane (Fig. 2c and 2d). The mass transfer percentages of hexane (53%, 68% and 94% in an air/air scenario at GRTs of 7, 16 and 30 s, respectively) decreased noticeably with water in the dense side (4% at a GRT of 7 s and <35% at GRTs <60 s). Similarly, 50%, 90% and 98% of the limonene was transferred at GRTs of 7, 16 and 30 s in an air/air scenario, respectively, while its transport severely decreased when water was recycled through the dense side of the membrane (<10% at GRTs <30 s and 40% at a GRT = 40 s). The presence of a biofilm significantly increased the transport of acetone, toluene and limonene at 7 s of GRT compared to the gas/gas scenario, whereas no improvement was recorded for hexane.

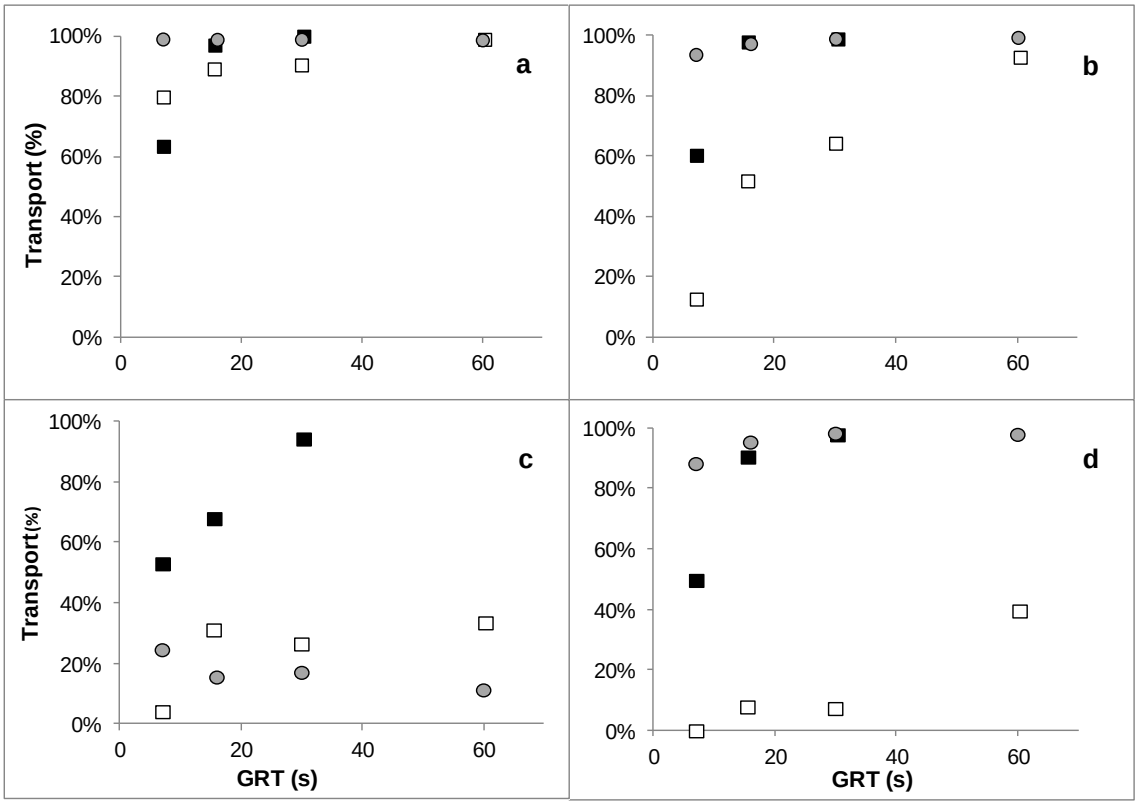


Fig 2. Influence of the gas residence time on the transport efficiency of acetone (a), toluene (b), hexane (c) and limonene (d) through the membrane reactor in the air/air (■), air/liquid (□) and air/biofilm (◍) scenarios.

A summary of the gas-water ($K_{g/w}$), octanol-water ($K_{o/w}$) and octanol-gas ($K_{o/g}$, calculated from $K_{g/w}$ and $K_{o/w}$) partition coefficients of the target compounds is shown in Table 1 (data collected from Sander 1999, Schwarzenbach et al. 2002, Copolovici and Niinemets, 2005). Mass transfer in the system can be conceptually described by a number of transfer resistances in series. Moving from the air towards the biofilm there are: a stagnant laminar boundary layer at the bulk air/porous membrane interphase, diffusion trough the stagnant air in the pores; air-membrane transfer, diffusion across the membrane, membrane-air transfer and a laminar boundary layer at the dense membrane-bulk air output. Since the interphase at both sides of the membrane is the same in the air/air situation, a compound with greater affinity for PDMS will benefit at the input side but not at the output side. Besides, whereas in the output side the resistance is constant because the velocity, and hence the thickness of the stagnant layer are constant, at the input side the lower GRTs (higher air velocity) reduce the thickness of the layer and subsequently the resistance to mass transfer. Therefore, it is difficult to predict whether the behaviour of the components is dominated either by equilibrium constant or by the flow dynamics, since the mass transfer is a combination of the different resistances at the different GRTs.

Table 1. Partition coefficients for the target VOCs (C_g: concentration in the gas phase, C_{aq}: concentration in the aqueous phase, C_{oct}: concentration in an octanol phase)

Compound	$\log(K_{g/w} = C_g\, C_{aq}^{-1})$	$\log(K_{o/w} = C_{oct}\, C_{aq}^{-1})$	$\log(K_{o/g} = C_{oct}\, C_g^{-1})$
Acetone	-2.82	-0.24	2.58
Toluene	-0.59	2.69	3.29
Limonene	0.06	4.23	4.17
Hexane	1.77	4.11	2.33

In the air/water scenario, the driving force will be determined by $K_{o/g}$ at the input side, which is highest for limonene and lowest for acetone. However, at the output side, the VOC transfer will be determined by the partition coefficient between the membrane and water (estimated by $K_{o/w}$), improving acetone transport and hindering that of hexane. Therefore, there are two

driving forces with opposed effects on the transfer of the VOCs, and hence the limiting step cannot be directly elucidated from the experimental data. Nevertheless, the substitution of the air phase by an aqueous phase in the dense side mediated an enhancement in the transport of acetone, followed by toluene, limonene and hexane, which corresponds to the relative order of $K_{g/w}$.

When biofilm was present on the dense side of the membrane, the experimental data suggested that the transport depended on the existing concentration gradients and was not likely correlated to $K_{o/w}$ or $K_{g/w}$, due to the addition of a biodegradation step (VOC sink) to the physical transport. In this case, physical and biological processes cannot be separately considered.

Membrane bioreactor performance

The formation of a thin biofilm over the dense side of the membrane was visually observed four days after the inoculation of the MBR. REs higher than 99% were recorded for acetone already one day after the inoculation of the membrane. It can be hypothesized that pollutant biodegradation in MBRs is not only due to the microorganisms present in the biofilm but also to the suspended biomass, especially for highly water soluble VOCs. A high acetone removal performance was observed during the entire experimentation period, regardless of the inoculation strategies and the GRTs tested, probably due to its high biodegradability (Fig. 3a).

Four days were necessary to achieve toluene REs higher than 99% following the MBR start-up (Fig. 3b). Toluene removals higher than 99% were maintained during operation at GRTs of 60 s and 30 s. When the GRT was decreased to 15 and 7 s, the REs slightly declined to 97 ± 1% and 93 ± 0%, respectively. Steady REs (considering a steady state as the operation period with a STD<5% in the average removal efficiency) were always achieved immediately after each change in the operating conditions, except for the last decrease in GRT when the MBR required 1 day to achieve steady state performance. Several studies on the performance of membrane bioreactors for the treatment of VOCs-contaminated gas streams used toluene as the model compound. An efficient removal of toluene as single pollutant in MBRs has been consistently shown in these laboratory studies at high inlet concentrations (30-4650 mg m^{-3}) and GRTs as low as 1.4 s (Ergas and McGrath 1997, Ergas et al. 1999, Jacobs et al. 2003, Kumar et al. 2008b). Toluene is relatively easy to degrade and elimination capacities (ECs) up to 2520 g m^{-3} h^{-1} have been obtained in a hollow fiber membrane reactor configuration at a GRT of 1.8 s (Ergas et al. 1999). However, at such high ECs, the corresponding REs were much lower than those observed in the present study (RE = 35%, Ergas et al. 1999). To the author's knowledge, the only study testing low toluene inlet concentrations (4 mg m^{-3}) was performed by Jacobs et al. (2003) in a flat MBR with a composite membrane inoculated with *Pseudomonas putida* TVA8. These authors recorded REs of ~75%, ~55% and 53% at GRTs of 8, 4 and 2 s, respectively, which were lower than those here achieved at a GRT of 7 s (RE = 93%).

Hexane was the VOC with the lowest REs at all GRTs evaluated. Steady REs of 15±4% were achieved six days after the inoculation of the membrane. However, the biodegradation tests showed that none of the microbial communities present in the MBR (the bacterial community in the recycling liquid presented the same structure than in the biofilm) were able to degrade hexane (Fig. 4.1c and 4.2c). At day 42, the MBR was inoculated with a hydrophobic hexane-degrading consortium growing immersed in silicon oil by spreading it on the dense side of the membrane surface (Hernández et al. 2012). The addition of the silicon oil consortium slightly improved the removal performance to 24±3%, the highest values observed throughout the experimentation period. When the GRT was further decreased to 30, 15 and 7 s, the RE remained at 14 ± 3% regardless of the GRT tested. Based on the proven hexane degrading capacity of this microbial consortium (Hernández et al. 2012), it could be hypothesizes that the low hexane removal performance recorded in the MBR could be caused by either a substrate competition between the different microorganisms present in the biofilm and/or in the liquid suspension or by a mass transport competition of the 4 VOCs in the membrane. In this regard, Zhao et al. (2011) found interactions during the simultaneous biotreatment of two volatile pollutants, toluene and hexane, in a hollow fiber MBR, although at higher inlet concentrations (30-1100 mg m^{-3}). To clarify the reasons underlying this consistently low

performance, the membrane was operated with the silicon-oil inoculum and fed only with hexane for 30 days. However, the recorded REs were always lower than 27%, which suggests that hexane mass transport through the membrane was the limiting factor during hexane biodegradation.

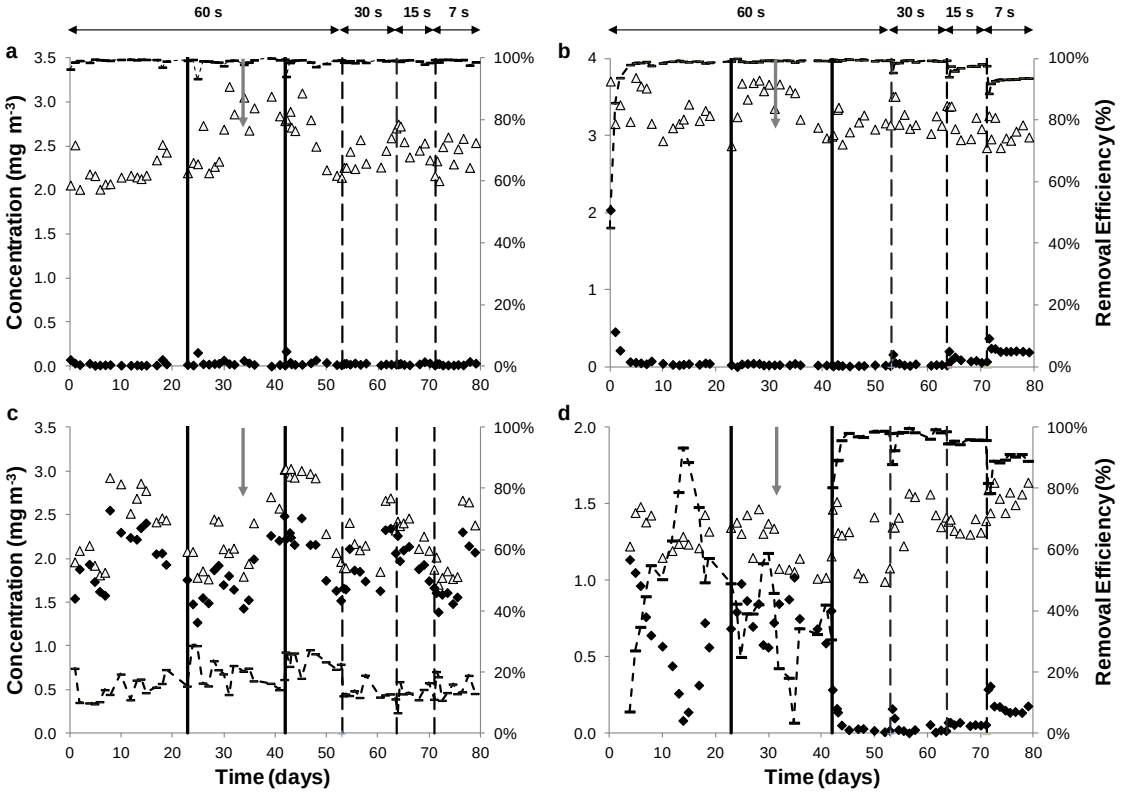
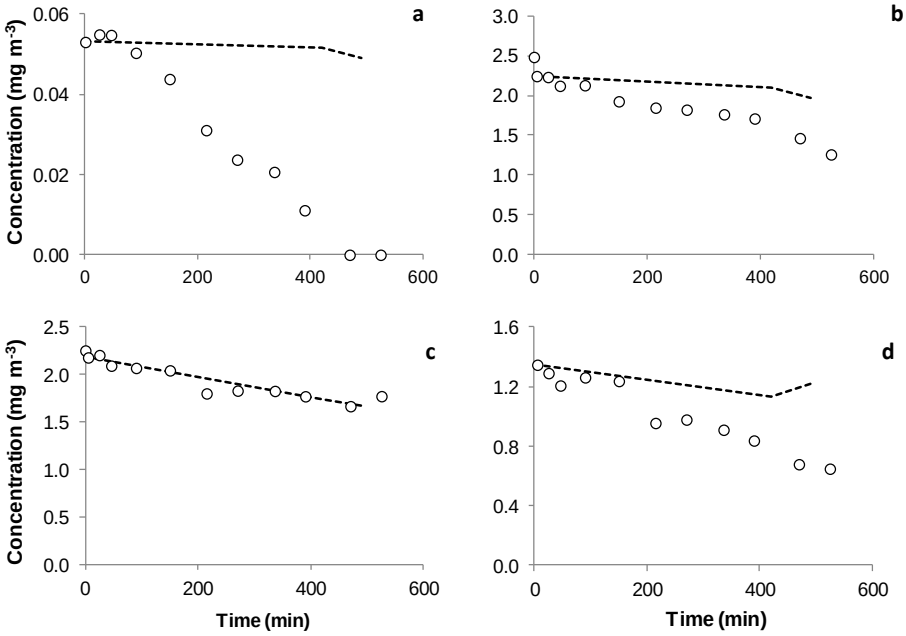


Fig 3. Time course of the inlet (△) and outlet (♦) concentrations, and removal efficiency (▬) of acetone (a), toluene (b), hexane (c) and limonene (d) in the MBR. The re-inoculation of the MBR is represented by continuous vertical lines, while vertical dashed lines correspond to the changes in GRT. Membrane cleaning is represented by a vertical grey arrow.

Limonene removal efficiency increased gradually from the start-up of the bioreactor to finally achieve a maximum RE of 93% at day 14 but, surprisingly, the limonene removal performance decreased progressively to values of 52 ± 5% by day 23 (Fig. 3d). This deterioration in the removal performance of MBRs during the start-up period has been already observed by other authors in fixed-film bioreactors degrading VOCs (Arcangeli and Arvin, 1992; Reij et al. 1998) and also in hollow fiber MBRs (Ergas et al. 1999). This drop in the RE was attributed to the starvation of the suspended microbial community in the aqueous phase following biofilm formation in the membrane. A change in the hydrophobicity of the membrane was also pointed out as a probable reason underlying this behavior (Ergas et al. 1999). This decrease in the membrane hydrophobicity is often due to the coating of the membrane pores by polysaccharide materials excreted by the biomass, although this mechanism was unlikely in our particular case since the biofilm was formed on the dense side of the composite membrane. The re-inoculation of the MBR with fresh activated sludge did not improve the limonene removal performance, and indeed, a minimum RE of 4% was recorded by day 35. This deterioration was attributed to the formation of a thick biofilm on the membrane surface (which was visually noticeable and likely induced mass transfer limitations in the process). At day 35 the membrane was partially cleaned by increasing the velocity of the recycling pump for a few seconds to promote biofilm sloughing due to the increased shear forces, and the removal performance immediately increased to steady REs of 35±5%. The biodegradation tests performed at days 18 and 33 with the suspended culture demonstrated the capacity of the existing

microorganisms to degrade limonene (Fig. 4.1d and 4.2d). The degradation curves are notably different in both tests, which can be attributed to a change in the structure or concentration of the microbial community. However, the limonene degradation line is clearly below the control, which suggested that the MBR was indeed mass transfer limited for limonene in the absence of a biofilm. The high similarity coefficient (83.8%, further discussed in the *Internal structure and molecular composition of the bacterial communities* section) between the microbial population in the suspended culture and in the biofilm validated this assumption.

4.1



4.2

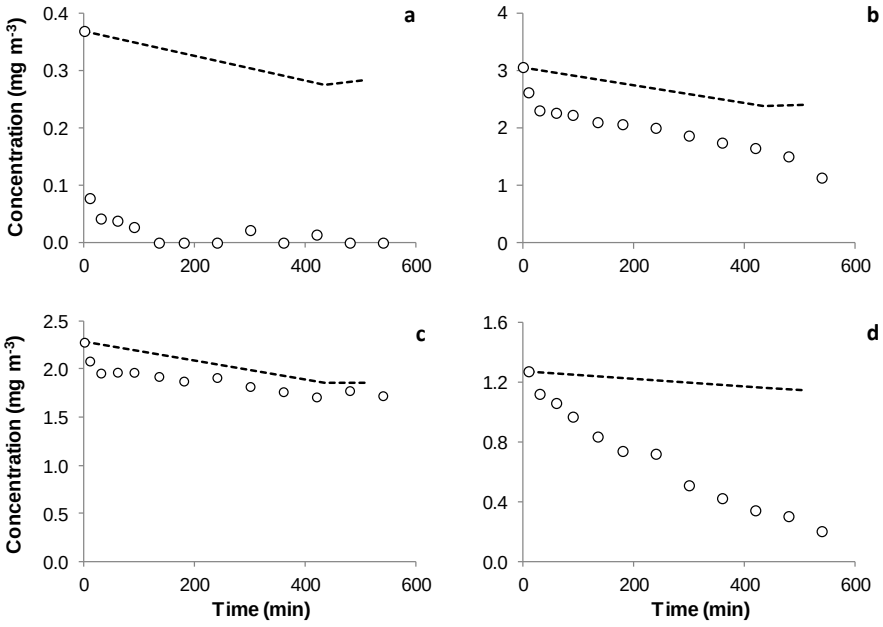


Fig 4. Time course of the acetone (a), toluene (b), hexane (c) and limonene (d) headspace concentration (○) in the batch biodegradation tests conducted with MBR biomass at days 18 (Fig. 4.1) and 33 (Fig. 4.2). The dashed lines represent the VOC concentrations in the control bottles.

The re-inoculation of the membrane by day 42 with the hydrophobic microbial consortium increased the limonene RE up to 98 ± 1% in the following 3 days. Similar REs were recorded only 1 day after decreasing the GRT to 30 s, while the removal performance slightly decreased to 95 ± 1% and 90 ± 1% when the gas flow rate was further increased to GRTs of 15 and 7 s, respectively. These high elimination efficiencies (much higher than the mass transfer rates recorded under abiotic conditions) were likely related to the presence of a hydrophobic silicone-oil layer on the membrane surface, which eventually improved the mass transfer of limonene (a low water soluble compound with high affinity for silicone oil, Table 1) to the degrading microorganisms present in the biofilm (the inoculum was spread on the membrane surface, without replacing the existent microbial suspension). It can be also hypothesized that a highly active limonene-degrading bacterial population was present in the inoculated silicon-oil layer. This, together with the improved mass transport of limonene, could explain the high limonene removal observed in our MBR. To the authors knowledge, this is the first study where a non-aqueous phase (here silicone oil) was combined with a biological membrane bioreactor. Considering the promising results obtained for limonene, this two-phase system deserves further investigation.

The REs here obtained for the less hydrophobic compounds (acetone, toluene) and for the moderately hydrophobic limonene were comparable to those observed in previous studies in activated sludge systems, biofilters and biotrickling filters under similar inlet concentrations and gas residence times (Lebrero et al. 2011, 2012a, Prenafeta et al. 2012). For instance, Prenafeta et al. (2012) recorded high REs for limonene (RE > 99%) during the biofiltration of a real odorous emission from a composting plant. However, hexane REs up to 70% were recorded under comparable conditions in a biotrickling filter at GRTs as low as 11 s, in contrast with the low efficiencies obtained in this study.

Finally, it was observed that microbial activity (either in the form of biofilm or suspended culture) mediated a higher concentration gradient for acetone, toluene and limonene over the membrane (thus increasing the driving force), as shown by the increased mass transport efficiencies of these VOCs through the membrane compared to those measured under abiotic conditions (Fig. 2a, b and d). However, in the particular case of hexane, the transport was only slightly increased at the lowest GRT (7 s) and it was hypothesized that the biofilm established over the membrane could create an additional resistance to hexane transport at the highest GRTs. The selection of a membrane material with a higher affinity for hexane is therefore mandatory to achieve higher eliminations for this particular VOC.

Internal structure and molecular composition of the bacterial communities

The activated sludge inoculum from Valladolid WWTP showed the highest species evenness and richness among the inocula evaluated as demonstrated by its high Shannon-Wiener diversity index (3.3, with values usually ranging from 1.5 to 3.5, McDonald 2003). The biodiversity of the inoculum from Ossemeersen WWTP (A) was slightly lower (2.9), while the microbial inoculum contained in the silicone oil (F) presented the lowest diversity (H = 2.2). The samples retrieved from the liquid phase recirculation (C, D and G) and the biofilm (E, H) on the membrane surface also presented a high species evenness and richness during the whole experimentation period (H varying from 3.2 to 3.5) despite the low carbon source spectrum. This high biodiversity and richness in bioreactors fed with low VOCs concentrations has been previously reported (Estrada et al. 2012, Lebrero et al. 2012a, 2012b). In this context, Estrada et al. (2012) observed that low toluene concentrations mediated a higher biodiversity in a suspended bioreactor, while the biodiversity was significantly reduced at high toluene loadings.

The pair-wise similarity indices showed a high correspondence between the community profiles of the samples from the recirculation liquid and the biofilm (83.8% between samples D and E and 97.5% between samples G and H), which confirmed that most microorganisms developed in both the suspended culture and the biofilm. After the second re-inoculation of the membrane, similarity coefficients of 66.3% and 51.7% were observed between the inoculum (sample B) and the suspended cultures on days 28 and 42 days, respectively (samples C and D).

These empirical findings demonstrated the progressive acclimation of the microorganisms to the operating conditions.

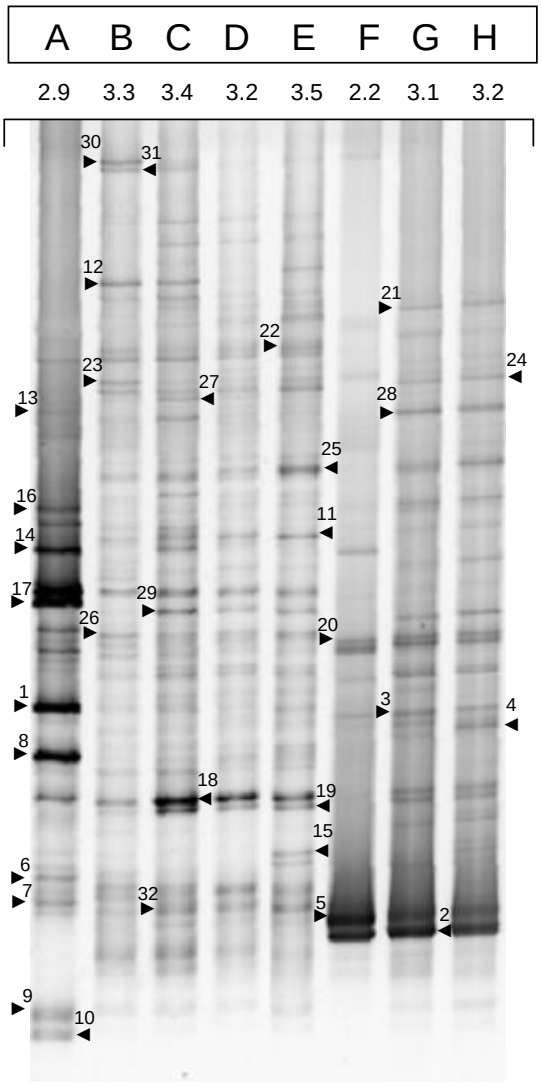


Fig 5. Bacterial DGGE profiles in the MBR. The samples names and the Shannon-Weiner diversity indices are shown in the upper part of the gel.

Finally, the highest similarity in the phylogenetic composition of the communities (~95%) was obtained between the silicon oil inoculum (sample F) and the biofilm sample retrieved from the membrane at the end of the experiment (sample H).

From the DGGE gel, 32 bands were sequenced (Fig. 5). Seven different phyla were retrieved according to the RDP classifier tool (bootstrap value of 50 %) in the RDP database: *Actinobacteria* (10 bands), *Proteobacteria* (10 bands), *Chlamydiae* (5 bands), *Acidobacteria* (2 bands), *Chlorobi* (2 bands), *Firmicutes* (2 bands), and *Chloroflexi* (1 band). The closest matches for each band (BLASTN) using the NCBI database are shown in Table 2, together with the similarity percentages and the sources of origin.

Most of these phyla have been previously found in biological odour abatement systems (Lebrero et al., 2011, 2012a, 2012b), exhibiting a demonstrated VOC biodegradation ability. Microorganisms in the *Actinobacteria* phylum (DGGE fragments 1-10), which includes aromatic and aliphatic hydrocarbon-degrading bacteria, were detected with high intensity in samples A, F, G y H. The DGGE fragments 3 and 4 showed a 99% similarity with *Rhodococcus phenolicus* (NR042950), a species capable of degrading aromatic compounds (Rehfuss and Urban, 2005). Similarly, microorganisms belonging to the *Mycobacterium* genus (fragment 5), known as slow-growing bacteria, are able to degrade toluene at low concentrations (Juteau et al., 1999). Previous literature studies have detected members of the *Proteobacteria* phylum in biological gas treatment systems (Bayle et al., 2008). In our particular study, the *Gammaproteobacteria* class is significantly present in all samples analyzed. Fragments 18 and 19 were affiliated to the *Dokdonella* genus, which has been previously detected in bioreactors treating sulfurous compounds, ammonia and VOCs (Maestre et al. 2010, Lebrero et al. 2012a, 2012b). Member of the *Chlamydiae* phylum (DGGE bands 21-25) were also found in a biorreactor treating gaseous toluene (Estrada et al. 2012). Despite present in our MBR, the ability of members of the phyla *Acidobacteria* (DGGE bands 26 and 27), *Chlorobi* (DGGE bands 28 and 29) and *Firmicutes* (DGGE bands 30 and 31) to degrade VOCs has not been reported yet. This fact clearly confirms the scarce knowledge available on the microbiology of off-gas treatment biotechnologies. Microorganisms classified into the *Chloroflexi* phylum (DGGE band 32) have been commonly retrieved from a wide variety of biological systems, but information about their functional role is scarce. Finally, it must be stressed that the biodiversity of the microbial community present in the bioreactor remained constant over time for the phyla *Actinobacteria, Proteobacteria* and *Chlamydiae,* while *Acidobacteria, Chlorobi, Firmicutes* and *Chloroflexi* were no longer found after day 42 of operation (Fig. 5 and Table 2).

Table 2. RDP classification of the DGGE bands sequenced and corresponding matches (BLASTN) using the NCBI database with indication of the similarity percentages of sources of origin. The presence (x) / absence of each band in each sample tested is also shown.

Taxonomic placement (50% confidence level)	Band n°	A	B	C	D	E	F	G	H	Closest relatives in Blast Name (accession number)	Similarity (%)	Source of origin
Phylum *Actinobacteria*												
Class *Actinobacteria*												
Subclass *actinobacteridae*												
Order *actinomycetales*												
Suborder *Actinomycineae*												
Family *Actinomycetaceae*												
Genus *Actinomyces*												
	1	**X**								Uncultured bacterium (AY953348)	100	Anaerobic sludge
Suborder *Corynebacterineae*												
Family *Nocardiaceae*												
	2						**X**	**X**	**X**	*Rhodococcus phenolicus* (NR_042950)	97	Culture collection
Genus *Rhodococcus*												
	3					X	X	**X**	X	*Rhodococcus phenolicus* (NR_042950)	99	Culture collection
										Rhodococcus sp.(EU0174049)	99	Soil
	4								**X**	*Rhodococcus phenolicus* (NR_042950)	99	Culture collection
										Rhodococcus phenolicus (JN180180)	99	Soil
Family *Mycobacteriaceae*												
Genus *Mycobacterium*												
	5	X		X	X		**X**	**X**	**X**	*Mycobacterium fortuitum* (JF734327)	99	Soil
Suborder *Propionibacterineae*												
Family *Propionibacteriaceae*												
Genus *Propionibacterium*												
	6	X								*Propionibacterium sp.*(AB540663)	95	Ditch sludge
	7	**X**								Uncultured bacterium (EU186882)	97	Cachaca yeast
	8	**X**				X				*Propionibacterium jensenii* (AY883044)	100	Culture collection
										Propionibacterium sp. (AB540663)	98	Ditch sludge
Order *Bifidobacteriales*												
Family *Bifidobacteriaceae*												
Genus *Bifidobacterium*												
	9	**X**	X	X	X	X		X	X	Uncultured bacterium (JN620462)	97	Activated sludge from a bioreactor treating synthetic wastewater
	10	**X**								Uncultured bacterium (JN620462)	97	Activated sludge from a bioreactor treating synthetic wastewater
Phylum *Proteobacteria*												
	11		X	X	X	X		X	X	Uncultured bacterium (JQ426388)	96	Soil
Class *Alphaproteobacteria*												
	12		X	X	X	X				Uncultured bacterium (CT574092)	99	Evry municipal wastewater treatment plant
Order *Rhodobacterales*												
Family *Rhodobacteraceae*												
Genus *Rhodobacter*												
	13	X						X	X	Uncultured bacterium (AB286495)	98	Activated sludge
	14	**X**		X			X			Uncultured bacterium (AB286495)	97	Activated sludge
Order *Rhodospirillales*												
	15	X				X				*Acetobacteraceae* bacterium (HQ687487)	97	Culture collection
										Uncultured bacterium (FN667149)	97	Full scale municipal waste compost
Class *Gammaproteobacteria*												

Order *Xanthomonadales*												
Family *Xanthomondaceae*												
	16	**X**		X	X	X		X	X	Uncultured *Xanthomonadales* bacterium (AM936405)	95	Bioremediation process of a hydrocarbon-contaminated soil
Genus *Pseudoxanthomonas*												
	17	**X**								Uncultured *gamma proteobacterium* (AB669240)	99	Anaerobic digester sludge
Genus *Dokdonella*												
	18	**X**	X	**X**	**X**	**X**		**X**	**X**	Uncultured bacterium (JQ038783)	100	Biotrickling filter (BTF) treating low concentrations of VOCs
										Uncultured *Dokdonella sp.*(JN679149)	99	Membrane bioreactor
										Uncultured bacterium (FM213064)	99	Biotrickling filter removing H2S from water treatment sludge
	19		X	**X**	X	**X**		**X**	**X**	Uncultured bacterium (JQ038783)	100	Biotrickling filter (BTF) treating low concentrations of VOCs
										Uncultured bacterium (FJ660574)	99	Activated sludge
										Uncultured *Dokdonella sp.*(JN679149)	99	Membrane bioreactor
										Uncultured bacterium (FM213064)	99	Biotrickling filter removing H2S from water treatment sludge
Family *Sinobacteraceae*												
Genus *Steroidobacter*												
	20		X				**X**	**X**	**X**	Uncultured *Pseudomonadales* bacterium (EU193058)	92	Agricultural soil
Phylum *Chlamydiae*												
Class *Chlamydiae*												
Order *Chlamydiales*												
Family *Parachlamydiaceae*												
	21				X	X		X	X	Uncultured bacterium (JQ056534)	95	Soil
Genus *Parachlamydia*												
	22		X		X	X				Uncultured bacterium (JQ050078)	92	Soil
	23		X	X	X	X	X	X	X	*Criblamydiaceae* bacterium (JF706725)	94	Culture collection
	24		X	X	X	X		X	X	Uncultured bacterium (JN606107)	99	Reactors treating toluene at different concentrations
	25		X	X	X	X		X	X	Uncultured bacterium (JQ053179)	100	Soil
Phylum *Acidobacteria*												
	26		X	X	X	X				Uncultured bacterium (FQ659784)	100	PAH-contaminated soil; retention systems which treat road runoffs
Class *Acidobacteria_Gp4*												
Genus Gp4												
	27			X	X	X				Uncultured bacterium (FN827223)	99	Activated sludge from a membrane bioreactor
Phylum *Chlorobi*												
Class *Ignavibacteria*												
Order *Ignavibacteriales*												
Family *Ignavibacteriaceae*												
Genus *Ignavibacterium*												
	28		X	X						Uncultured bacterium (GQ397077)	98	Soil
	29			**X**	X	X				Uncultured bacterium (FN824912)	98	Biofilm sampled in a treatment system for groundwater contaminated with BTEX, MTBE and ammonium
Phylum *Firmicutes*												
Class *Clostridia*												
Order *Clostridiales*												
Family *Lachnospiraceae*												
Genus *Clostridium XlVa*												
	30		**X**	X						*Clostridiaceae* bacterium (AB298726)	100	Rice straw residue in a methanogenic reactor of cattle farm waste
										Uncultured bacterium (CR933122)	99	Evry municipal wastewater treatment plant
	31		X	X	X					Uncultured bacterium (CR933122)	95	Evry municipal wastewater treatment plant
Phylum *Chloroflexi*												
	32	X	X	X	**X**	**X**				Uncultured bacterium (AB630830)	98	Aquatic moss pillars
										Uncultured bacterium (JQ800911)	96	Soil

4. Conclusions

This work confirmed the efficiency of MBRs for the treatment of water soluble and moderately soluble VOCs. Whereas the abiotic test showed that the presence of an aqueous phase over the dense side of the membrane induced a higher overall mass transfer resistance, biofilm activity mediated higher concentration gradients over the membrane and therefore a more efficient VOC transport. Thus, REs higher than 93% were always obtained in the MBR for acetone and toluene at GRTs as low as 7 s. In the particular case of limonene, the inoculation of the membrane with an inoculum embedded in silicon-oil increased its removal performance up to 90% at 7 s of GRT. Nevertheless, hexane biodegradation was limited by its mass transfer over the membrane regardless of the GRT (RE < 24 %), which pointed out towards the selection of the optimum membrane material as a key design criterion determining the performance of MBRs for the treatment of highly hydrophobic VOCs. Finally, the microbiological analysis of the communities present in the MBR showed a high species richness despite the limited C source spectrum, and a high structural similarity between the microbial populations present in the suspended culture and in the biofilm.

Acknowledgements

The authors acknowledge the support of Ghent University under the GOA-project: Ugent BOF10-GOA010, the Spanish Ministry of Science and Innovation (RYC-2007-01667 contract and projects CTQ2009-07601 and CONSOLIDER-CSD 2007-00055) and the Regional Government of Castilla y Leon (VA004A11-2). Francisco J. Álvarez-Hornos is gratefully acknowledged for his technical support at the early stages of the project.

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Chapter 10

Comparative assessment of a biofilter, a biotrickling filter and a hollow fiber membrane bioreactor for odour abatement

Comparative assessment of a biofilter, a biotrickling filter and a hollow fiber membrane bioreactor for odour abatement

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Introduction

Malodorous emissions are complex mixtures of odorants including sulfur derived and volatile organic compounds (VOCs). Biotechnologies have consolidated as the best available technologies for odour treatment in the last decades due to their lower environmental impact and operating costs compared to physical-chemical techniques (Estrada et al. 2011). However, conventional biotechniques such as biofiltration or biotrickling filtration are reported to support low removal efficiencies for the hydrophobic fraction of the odorous emissions, whose elimination is mandatory for effective odour abatement (Iranpour et al. 2005). In membrane bioreactors a hydrophobic layer is placed between the polluted air emission and the liquid phase where the biofilm is formed. The presence of the membrane has been reported to increase the mass transfer of the less water soluble odorants and to provide larger gas-liquid interfacial areas, thus improving their removal performance compared to that achieved by its biological counterparts. The application of these membrane bioreactors for gas treatment is very recent and no single application for odour abatement has been performed so far (Kumar et al. 2008). Therefore, a comparative study between conventional biotechnologies and membrane bioreactors for odour treatment is necessary to assess their performance under comparable operating conditions.

Materials and methods

A biofilter (BF, 2 L), a biotrickling filter (BTF, 2 L) and a hollow-fiber membrane bioreactor (HF-MBR, 400 mL) were operated in parallel at a constant temperature of 25ºC. The BF was packed with compost and the BTF with 1 cm^3 cubes of polyurethane foam. The HF-MBR was a commercial module from Medarray®. The odorous stream was prepared by mixing a concentrated methyl-mercaptan (MeSH), toluene, alpha-pinene and hexane mixture from a calibration bottle with a previously humidified VOC-free air stream. The odorous emission was then equally split and fed to the BF, the BTF and the HF-MBR from the bottom of the reactors (counter current configuration) at concentrations of 4.9±0.5, 0.82±0.07, 0.91±0.10 and 0.75±0.08 mg m^{-3} for MeSH, toluene, alpha-pinene and hexane, respectively. A mineral salt medium (MSM) was periodically irrigated to the biofilter, and continuously recycled through the BTF and the HF-MBR at 1.5 m/h and 200 mL/min, respectively.

The bioreactors were inoculated with activated sludge. The influence of the empty bed residence time (EBRT) on the VOC RE of the BF and the BTF was evaluated at EBRTs of 48, 18 and 8s. At day 78, the packing material of the BF was removed and half of the compost was mixed with plastic rings due to the high pressure drop (ΔP) recorded in this system. The BF was stopped at day 95, while the EBRT of the BTF was further decreased to 4s. The HF-MBR was also operated at EBRTs of 43, 34 and 16s

for 95 days. However, due to the instability of its performance and the low REs recorded in this system, the EBRT was increased to 84s in the last operating period.

The VOCs concentration in the inlet and outlet gas streams was periodically measured. The samples were pre-concentrated by means of a solid-phase microextraction fiber and subsequently analyzed in a GC-FID. The ΔP of the three systems was measured by means of a water U-tube.

Preliminary Results and Discussion

MeSH removal efficiencies (REs) > 97% were recorded in both BF and BTF regardless of the EBRT (Fig. 1a). Toluene and alpha-pinene steady REs > 99% were also observed during the complete experimentation period in the BF and the BTF (Fig. 1b, c). When the EBRT was decreased from 18 to 8s in the BF low REs were initially recorded due to a reduced humidity in the bed. An increase in the irrigation frequency mediated a rapid system recovery. The lowest removal performance was obtained for hexane in both the BF (REs ranging from 94±1% to 98±1%) and the BTF (REs varying from 88±1% to 95±2 %) likely due to the mass transfer limitations caused by the low solubility of this pollutant. Surprisingly, the change in the packing material of the BF (and the subsequent removal of half of inoculated compost) did not affect MeSH and alpha-pinene removal, whereas toluene and hexane REs noticeably decreased. Nevertheless, previous REs were restored within 6 days.

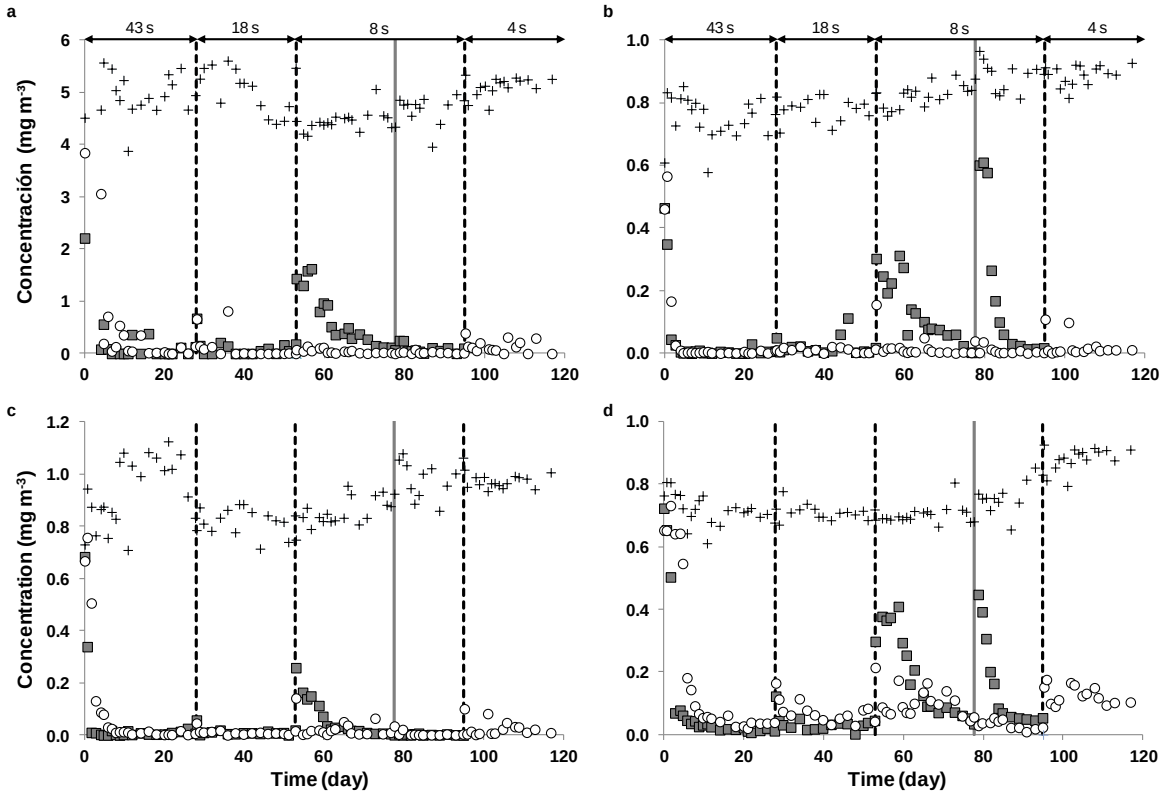

Fig. 1. Time course of the inlet (+) and outlet concentrations in the BF (■) and the BTF (○) for MeSH (a), toluene (b), alpha-pinene (c) and hexane (d). Dashed vertical lines represent the changes in EBRT and the vertical grey line the change of BF packing material

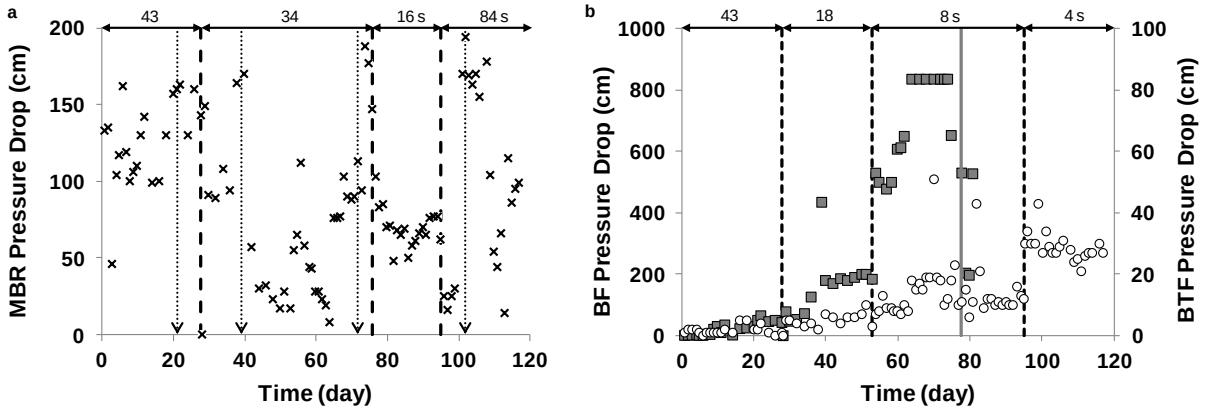

Fig. 2. Time course of the pressure drop (expressed in cm of water column) in the MBR (a), in the BF (b, left axis) and in the BTF (b, right axis). Dashed vertical lines represent the changes in EBRT and the dotted vertical arrows in figure 2a the membrane cleanings.

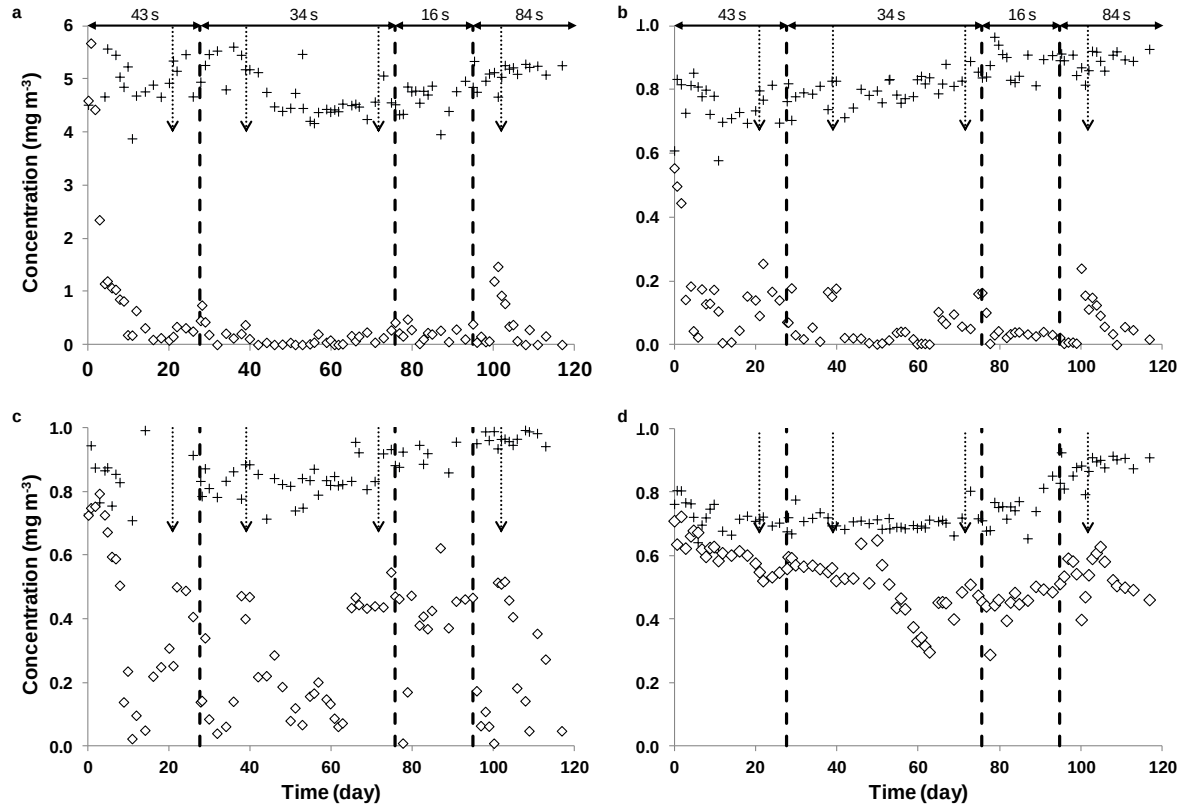

Fig. 3. Time course of the inlet (+) and outlet concentrations in the HF-MBR (◊) for MeSH (a), toluene (b), alpha-pinene (c) and hexane (d). Dashed vertical lines represent the changes in EBRT and the dotted vertical arrows the membrane cleanings

Both systems demonstrated their capability for treating odorous air emissions with high REs even at EBRTs as low as 8 and 4s in the BF and the BTF, respectively. However, while maximum ΔPs of 30 cm H_2O were recorded in the BTF at 4s of EBRT (Fig. 2b), ΔPs of up to 1 m H_2O were observed in the BF at 8s of EBRT, even after addition of the plastic rings, which clearly pointed out towards a rapid compost deterioration.

The MBR performance was characterized by its instability during the complete experimentation period. Although REs > 95% were recorded for MeSH and toluene regardless of the EBRT (Fig. 3a, b), alpha-pinene REs varied between 39% and 99% in spite of the operating conditions (Fig. 3c). These fluctuations correlated with the changes in ΔP and were attributed to mass transfer limitations associated to membrane biofouling. Surprisingly, after membrane cleaning the ΔP did not decrease immediately but gradually (Fig. 2a). Finally, maximum hexane REs = 45±3% were recorded at an EBRT of 84s (Fig. 2d).

In brief, the BTF exhibited the best cost-effective performance despite exhibiting slightly lower REs than the BF, while the HF-MBR was not able to support a consistent odorant abatement performance. A microbial analysis is being performed to evaluate the dynamics of microbial biodiversity linked to the bioreactors performance.

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Chapter 11
Conclusions

Several conventional and innovative biotechnologies for odour abatement were herein reviewed, focusing on the removal of both sulfur derived compounds and VOCs within a wide range of hydrophobicity.

The results here obtained confirmed BFs as a reliable and robust technology for sustained H_2S and VOC abatement able to recover from the negative impact of common process fluctuations or operational failures (**Chapters 3, 4 and 10**). The operation of a compost-based BF fed with an odorous emission from WWTP sludge resulted in high and stable REs for most of the emitted odorants (mainly formed by hydrolysis and acidogenesis processes) in spite of their fluctuating concentrations (**Chapter 5**). However, BFs still exhibit high land requirements due to the high EBRTs required for an efficient odour abatement (>40 s), while their long-term operation leads to an increased compaction of the packing material and therefore to high pressure drops through the bed (**Chapter 10**). Besides, the likely development of anaerobic zones within the BF organic packing media resulted in negative removals of specific compounds such as dimethyl sulfide or acetic acid (**Chapter 5**).

Activated sludge systems also exhibited REs comparable to those of BFs, likely mediated by their high mass transfer potential and a high robustness towards typical operational fluctuations (**Chapters 3 and 4**). This good performance was further confirmed by the high stability of the bacterial population structure as shown by the DGGE profiles. In addition, the supply of wastewater to the AS unit contributed to an enhanced process stability by preventing biomass compaction. Based on the results here obtained, a complete VOC and H_2S removal can be expected in aerated tanks with fine bubble diffusers in WWTPs under the typical operating conditions.

The subsequent studies were devoted to analyze the performance of BTFs for odour abatement. The results confirmed the potential of BTFs to treat hydrophilic and moderately hydrophobic VOCs and sulfur compounds (RE > 99%) while supporting an efficient abatement for highly hydrophobic VOCs (>80%) at EBRTs as low as 7 s (**Chapters 6, 8 and 10**). This good performance was attributed to the high volumetric mass transfer coefficients recorded for the system (113 ± 2 h^{-1} at 7 s of EBRT). However, the long term operation of the BTF resulted in the accumulation of toxic metabolites, which likely caused an irreversible damage to the microbial community (**Chapter 6**). In this context, the addition of an organic non-aqueous phase (i.e. silicone oil) to the recycling solution allowed for a rapid process stabilization. The comparative evaluation of a conventional and a two-phase BTF showed similar REs in both systems

for the water soluble and moderately hydrophobic VOCs, while the two-phase BTF outperformed the conventional BTF for the removal of the highly hydrophobic VOC at EBRTs as low as 6 s (**Chapter 8**). Moreover, this system supported a superior robustness performance. The addition of silicone oil enhanced the mass transfer potential of BTFs and severely impacted the phylogenetic composition of the bacterial communities grown in the biofilm.

The mathematical model developed was able to accurately characterize the VOC mass transfer in BTFs by means of the overall mass transfer coefficient (**Chapter 7**). This model did not require any particular assumption or data linearization, and accurately described the experimental data obtained (r^2=0.97). Moreover, the estimation of the film coefficients confirmed the location of the main resistance for VOC mass transfer in the liquid film. Hence, the model could be applied for BTF design optimization and operation under mass transfer limiting conditions.

The performance of MBRs for odorant abatement was also assessed as a potential compact, high-performance alternative for hydrophobic VOC treatment (**Chapter 9**). The work confirmed the efficiency of flat MBRs for the removal of water soluble VOCs at low gas residence times (7 s), while the inoculation of a microbial community embedded in silicone-oil on the membrane surface increased the removal performance of moderately soluble odorants such as limonene. However, low REs were observed for the hydrophobic fraction of the gas emission due to mass transfer limitations.

Finally, a comparative study of a BF, a BTF and a hollow fiber MBR confirmed BTFs as the best cost-effective biotechnology despite exhibiting slightly lower REs than the BF, which underwent a rapid compost structural deterioration resulting in unsustainable pressure drops (**Chapter 10**). On the other hand, the hollow fiber MBR was not able to support a consistent odorant abatement performance due to membrane fouling.

In brief, the present studies confirmed the high performance and robustness of conventional biotechnologies for efficient odour treatment, with BTF standing out as the most cost-efficient and robust. The higher odour abatement potential and robustness of two-phase BTFs was validated under steady and transient conditions. On the other hand, MBRs supported an instable VOC removal performance and further research is necessary before their application for odour abatement.

Chapter 12
Curriculum Vitae

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16-18 June Barcelona, Spain, 2010

3rd IWA Conference on Odour and VOCs

Attendee. Barcelona, Spain, 8-10 October 2008

Short coursers and seminars

Technical symposium: Caracterización y Gestión de Olores en Estaciones Depuradoras de Aguas Residuales.

Department of Chemical Engineering and Environmental Technology. University of Valladolid.
Speaker and member of the organizing committee
Valladolid, 7-8 June 2010

Course: Control y Solución a la Contaminación Ambiental por Olores

Assistant. Cámara Oficial de Comercio e Industria de Madrid, Madrid, 19 Noviembre 2009.

Course: Tecnologías Avanzadas para el Tratamiento de Aguas Residuales.

Organizer: University of Santiago de Compostela.
Speaker
Santiago de Compostela, 16-17 September 2009

Summer School "Model Based Design Operation and Control of Wastewater Treatment Plants"

Attendee. Organizer: Centro de Estudios e Investigaciones Técnicas de Guipuzkoa (CEIT).
San Sebastián, Spain, 13-17 Junio 2009

Tecnologías y Estrategias para el Rediseño de EDARs

Attendee. Organizer: University of Santiago de Compostela (Proyecto Novedar-Consolider)
Madrid, Spain, 24 November 2008

Other merits

Stays abroad

August – December 2010
UNSW Water Research Center. New South Wales University, Sydney, Australia.
Professor Richard Stuetz. Head of the UNSW Water Research Center
Name of the project: Odour measurement and assessment, evaluation of odour treatment technologies

August-December 2011
EnVOC Research Group. Faculty of Bioscience Engineering, University of Gent, Belgium.
Professor dr. ir. Herman Van Langenhove. Head of the EnVOC group.
Name of the project: Membrane bioreactors applied to odorous VOCs treatment

Reviewer

Reviewer for: Water Research, Journal of Chemical Technology and Biotechnology, African Journal of Biotechnology, Ekologija, Chemical and Biochemical Engineering Quaterly

Project evaluator for the Innovation and Technology Commission (Government of Hong Kong), May 2011

Member of the Organizing committee of:

- The Water and Industry Conference 2011, held in the University of Valladolid,
- Technical symposium: Caracterización y Gestión de Olores en Estaciones Depuradoras de Aguas Residuales**.**

Final Year Projects

Mª Gabriela Lira Rangel (August 2011 – December 2011) "Design and Evaluation of a system for the treatment of gaseous emissions from a wastewater treatment plants" Universidad de Valladolid

Master Thesis and Research Projects

Ana Celina Gondim Santos (Feb 2012-July 2012) "Comparison of three bioreactors for the treatment of odours: biofilter, biotrickling filter and membrane bioreactor". Valladolid University (Spain).

Mª Gabriela Lira Rangel (March 2011 – July 2011) "Characterization of odours from Wastewater Treatment Plants" Valladolid University (Spain).

Teaching

Environmental and Process Technology (2010-2013). Lecturer. Industrial Engineering Degree. 1st Course. Valladolid University (Spain).

Coordinator of the Electronically Engineering Degree