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Performance of innovative PU-foam and natural fiber-based composites for the biofiltration of a mixture of volatile organic compounds by a fungal biofilm

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ABSTRACT

The performance of perlite and two innovative carriers that consist of polyurethane (PU) chemically modified with starch; and polypropylene reinforced with agave fibers was evaluated in the biofiltration of a mixture of VOCs composed of hexane, toluene and methyl-ethyl-ketone. At a total organic loading rate of 145 gC m^{-3} h^{-1} the elimination capacities (ECs) obtained were 145, 24 and 96 gC m^{-3} h^{-1} for the biofilters packed with the PU, the reinforced polypropylene, and perlite, respectively. Specific maximum biodegradation rates of the mixture, in the biofilters, were 416 mgC $g^{-1}_{protein}$ h^{-1} for the PU and 63 mgC $g^{-1}_{protein}$ h^{-1} for perlite, which confirms the highest performance of the PU-composite. 18S rDNA analysis from the PU-biofilter revealed the presence of *Fusarium solani* in its sexual and asexual states, respectively. The modified PU carrier significantly reduced the start-up period of the biofilter and enhanced the EC of the VOCs. Thus, this study gives new alternatives in the field of packing materials synthesis, promoting the addition of easily biodegradable sources to enhance the performance of biofilters.

1. Introduction

Packing material, in biofiltration systems, is defined as a solid phase on which the adhesion of microorganisms takes place, resulting in the development of a biofilm [1]. The main function of the packing material is to promote contact between contaminants and microorganisms allowing the generation of a stable aggregate. Thus, the packing material is considered the core of a biofilter, since its nature influences both removal performance and operational costs [2]. Packing materials must have specific physicochemical properties such as a high porosity, a high surface area, a good mechanical resistance and water retention capacity to improve microbial growth and biofilm adhesion [3–6]. Several types of biofilter packing materials have been reported in the literature, including inorganic and organic materials, as well as synthetic materials in which additives are often incorporated to improve their physicochemical properties [1–6].

When organic packing materials are used, if the water content in the system is too low, the packing bed becomes dry and brittle, developing cracks and preferential channels through which the gas can pass without being treated. In contrast, high moisture content in the packing bed could limit the transfer of oxygen and hydrophobic pollutants toward the biofilm, promoting the development of anaerobic zones within the bed. In general, the majority of biofiltration studies report bed moisture contents ranging from 20% to 60% by weight [7,8].

In addition, organic packing materials have a limited lifetime as they tend to compact, increasing the pressure drop in the system, which increases the operational costs of biofilters due to higher energy consumption [8,9]. Inert packing materials have advantages over organic materials since they maintain their structural resistance. However, also inert materials can also lead to problems if excessive biomass growth is present in biofilters [10,11]. In recent years, diverse packing materials have been synthesized to improve the performance of biofilters [12–14]. Synthetic materials are of great importance because they simplify the modeling of biofilters. Also, when they are chemically modified, the physicochemical properties of the synthetic materials such as porosity, surface area, buffer capacity and water retention capacity can be improved [15]. It is possible to improve the characteristics of synthetic materials in terms of water retention capacity and biofilter start-up performance by the addition of easily biodegradable carbon sources to the polymer [16,17]. In a previous study, polyurethane foam cubes were modified with polyethyleneimine to improve cell attachment to treat H_2S polluted airstreams [16]. It was found that the reactor packed with the modified material eliminated higher

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loads than the one packed with non-modified polyurethane [16]. Polyurethane foam has been found to be an effective biofilter packing medium by several researchers during the treatment of both volatile organic and inorganic compounds [18–23]. Polyurethane has shown improved performance over other types of packing media, especially at high gas flow rates and low concentrations due to its open structure, high porosity, low density, large specific area, non-biodegradability, physical strength, easiness of inoculation, ability to absorb water and to its capacity to develop low pressure drops across the bed [18–21]. In addition to PU-foam, other materials have also been modified with additive substances to improve their physicochemical properties, in order to be applied as packing material in biodegradation or biofiltration systems [17,23]. Selection of the biofilter carrier is thus crucial in order to maintain the long-term operational stability of the bioreactor.

Considering the previous statements, this study was focused on the comparison of a novel modified PU-foam, a novel modified polypropylene and a perlite carrier (aluminum silicate) as packing materials for the biofiltration of a synthetic mixture of volatile organic compounds (VOCs) containing toluene, hexane and MEK.

2. Materials and methods

2.1. Chemicals, inoculum and mineral medium

Toluene, methyl-ethyl-ketone (MEK) and n-hexane were obtained from Tecsiquim Company (Mexico). All chemicals used were of analytical grade. The microbial consortium used in this research was obtained from a previous work, where it was supplemented with hexane, toluene and trichloroethylene over 6 months [24]. The mineral medium composition used in this study was reported previously [25]. For this work, the medium was adjusted to pH 5.

2.2. Packing materials

The packing materials used were perlite (Perlita La Laguna, Mexico); polypropylene reinforced with agave fibers, and a modified PU-foam (Patent application MEX/a/2009/013966 and PCT/MX2010/000155) containing starch as an additional carbon source and whose synthesis was reported in a previous study [26]. The polypropylene composite was prepared in a twin screw extruder at 160 °C, with a material composition of 70% of agave fibers and 30% of polypropylene (w/w). The obtained composite was cut in cubic particles of approximately 7 mm. The physicochemical properties of the packing materials used are shown in Table 1.

2.3. Biofiltration experiment

The first experiment (PB) was carried out in a stainless steel column (9 cm diameter and 60 cm length) packed with perlite with a working volume of 2.6 L. 200 mL of mineral medium solution containing an acclimatized consortium of microorganisms for the consumption of VOCs was used as the inoculum, with an initial biomass concentration of 63.4 $mg_{protein}\,L^{-1}$. Air saturated with water, containing the mixture of VOCs at a concentration of 1 $g\,m^{-3}$ of each pollutant, with a total organic loading rate (OLR) of 180 $g\,m^{-3}\,h^{-1}$ (60 $g\,m^{-3}\,h^{-1}$ of each hydrocarbon) was introduced into the biofilter. The PB was operated in down-flow mode, with a flow rate of 2.6 $L\,min^{-1}$, giving an empty bed residence time (EBRT) of 1 min. 100 mL of mineral medium was added to the biofilter every 3 days in order to supply nutrients and to control the pH. During the first 55 days the biofilter was operated at a neutral pH. After day 55 of the biofilter operation the biofilm was mainly composed by fungi. Hence, in order to improve the activity of the fungal biofilm the pH was reduce to 5 by the continuous addition of mineral medium.

Subsequent experiments were carried out in glass columns (2.6 cm diameter and 25 cm length) with an effective volume of 90 mL. The biofilters were packed with the PU-composite (PUB) and reinforced polypropylene (PPB), respectively. The packing materials were inoculated with 40 mL of a mixture of mineral medium and the microorganisms described above, and the bioreactors were operated at the same OLR and EBRT conditions as the biofilter described above. All biofilters were operated at a pH of 5. The pH, density and bed void fraction were determined as mentioned in another study [25,26]. Approximately 10 mL of mineral medium were added to the PU and PP biofilter every 3 days in order to supply nutrients and to control the pH.

The effect of the inlet loading rate on elimination capacity (EC) was tested after 65 days of the PU and PB biofilters operation. For both the PU-composite and the perlite biofilter, equi-mass loadings rates of all VOCs were tested, giving total loading rates between 120 and 650 $g\,m^{-3}\,h^{-1}$. In the case of the PUB biofilter, the effect of the individual VOC loading rate on EC was also tested by interrupting the addition of the other VOCs.

The concentrations of toluene, hexane and MEK in the gas phase were monitored daily in all experiments (PB, PUB and PPB). Once the experiments were finished, samples of each packing material were taken to determine the morphology of the biofilm by scanning electron microscopy (SEM). The protein content of the biomass was also measured at the end of the experiments [26].

2.4. Identification of hydrocarbon degrading fungi

Samples of biomass were extracted from the polyurethane cubes from the first 5 cm in the upper part of biofilter PUB on day 65. These samples were allowed to serial dilutions in water, plated on PDA medium, placed at 28 °C and transferred several times until the isolation of different microorganisms, obtaining only fungal species. Total DNA was extracted as described by Reader and Broda [27].

Total DNA was used as template to amplify the intergenic transcribed spacer (ITS) from 18S rDNA using the primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) [27,28]. Amplicons were cloned in pGEM-T Easy (Promega) and sequenced by the Sanger reaction in an ABI sequencer (Applied Biosystems). Nucleotide sequences were deposited in GenBank under the following accession numbers: BK1-1 (JN882255), BK2-1 (JN882256), BK3-2 (JN882257), OB1-1 (JN882258), OB2-2 (JN882259), OB3-1 (JN882260), and OB4-2 (JN882261).

2.5. Analytical methods

Toluene, MEK and n-hexane concentrations were determined by injecting 200 μL gas samples into a 6890 series gas chromatograph (Agilent Technologies) with a flame ionization detector (FID) and a capillary column (DB-624). The carrier gas was nitrogen at a flow rate of 25 $mL\,min^{-1}$. The temperature of the injector, oven and detector were maintained at 230, 60 and 230 °C, respectively.

CO_2 production was measured during steady state operation of the biofilters using a TCD gas chromatograph (Agilent Technologies GC-6850) equipped with a capillary column HP-PLOT Q. Helium at a flow rate of 10.1 $mL\,min^{-1}$ was used as the carrier gas. The temperature of the injector, column and detector were 180, 40 and 180 °C, respectively.

Protein concentrations were determined by the previously reported Lowry method [26]. Samples were hydrolyzed with sodium hydroxide (0.2 M). Serum bovine albumin was used as the standard (Sigma–Aldrich).

Table 1
Physicochemical properties of the packing materials used in this study.

Material/parameter	Perlite	Polyurethane	Polypropylene
Water retention capacity (%)	42.8 ± 2.25	33.3 ± 1.44	21.7 ± 0.63
pH	7.5 ± 0.08	6.7 ± 0.10	4.1 ± 0.20
Initial bed void fraction (ε) (%)	56 ± 0.50	68 ± 0.20	62 ± 0.20
Bed density (g mL^{-1})	0.115 ± 0.015	0.073 ± 0.00	0.262 ± 0.019
Particle size (mm)	3.35 ± 0.16	5 ± 0.15 (cubes)	7 ± 0.17 (cubes)

Water retention capacities were evaluated using a thermogravimetric analyzer (Thermo-Cahn TGA) at 10 °C min^{-1} up to 100 °C. The analysis was held for 20 min under isothermal conditions at 100 °C. Afterwards, the same temperature ramp continued up to 550 °C. The TGA analyzer was operated with nitrogen and helium flows of 20 and 40 mL min^{-1}, respectively.

Material morphology was analyzed by SEM, using a microscope ESEM-QUANTA 200 (FEI Company). Samples were set on an aluminum plate without any preparation and were analyzed using a LFD (large fill detector) electron detector. The microscope was operated at 15 kV and 90 Pa under low vacuum mode.

3. Results and discussion

3.1. Biofiltration experiments

Three different packing materials were tested independently in the biofilters in order to evaluate their physicochemical properties during the attachment of microorganisms and their effect on the biofiltration of a synthetic mixture of VOCs.

All the tested biofilters were operated for 75 days. Fig. 1 shows the total EC of the VOC mixture as a function of time for the three different packing materials. Pollutant biodegradation in the PB biofilter (packed with perlite) started slowly on day 5 and increased with time. The PB biofilter showed steady state behavior between days 33 and 55, which probably means that microorganisms were already acclimated to the reactor conditions. After day 55, the pH was adjusted to 5 which leads to the improvements of the EC to 145 gC m^{-3} h^{-1} (RE = 100%). This improvement might be related with fungal predominance at low pH [4,25]. The CO_2 production at day 60 of the experiment was 260 g_{CO_2} m^{-3} h^{-1} which represents the 50% mineralization.

In the case of the PUB biofilter (polyurethane composite), there was no lag phase probably due to a faster biofilm formation. This might be a consequence of the starch present in the packing material, which probably was used as energy source by the microorganisms. In addition, the water retention capacity exhibited by this packing material might have promoted the interaction between microorganisms and VOCs (Table 1). A rapid increase of the EC occurred during the first 14 days which indicated that the activity of microorganisms was substantially high. On day 21, the EC increased until steady state conditions were reached, and almost 100% of removal efficiency (RE) was achieved. CO_2 production on day 64 was 434 g_{CO_2} m^{-3} h^{-1}, corresponding to a mineralization of 82%.The EC of the PPB biofilter (packed with the reinforced polypropylene) was not steady with time. There was no biodegradation of pollutants until day 23, perhaps due to the physicochemical characteristics of the corresponding packing material as it is mentioned in detail in Section 3.3. At the mid term of the reactor operation a maximum EC of 35 gC m^{-3} h^{-1} was obtained. At the end of the experiment, the EC decreased to 10 gC m^{-3} h^{-1}, suggesting a reduction of the active biofilm. Furthermore, SEM photomicrographs of the biofilm samples indicated that the attachment of microorganisms to the modified polypropilene carrier failed (discussed later). The RE attained with the PPB bioreactor was lower than those obtained with the others packing materials, since 100% of RE of VOCs was attained at the end of the experiments for PB and PUB.

It is important to mention that the initial EC obtained with the PU-foam composite, was higher than the EC achieved with the PB and PPB packing materials, giving a notable difference in the start-up of the reactors performance.

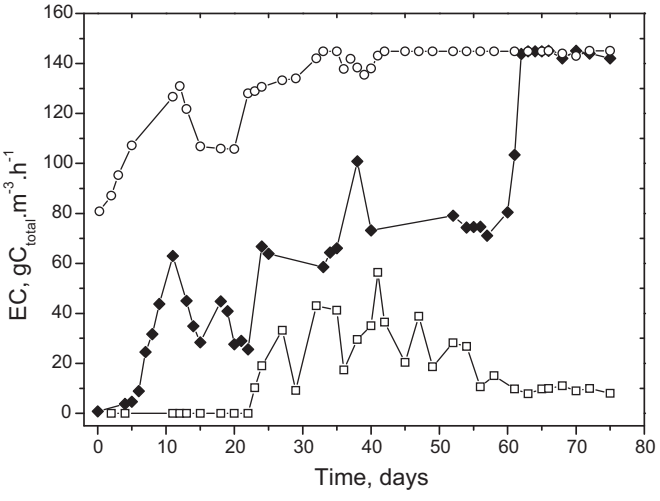

Fig. 1. Total elimination capacity of VOCs mixture reached with different packing materials in the biofilters PPB (□), PB (♦) and PUB (○).

3.2. Effect of inlet load rate on biofilters performance

The effect of the inlet load on EC was studied only for the PB and PUB packing materials due to their better performance in biofiltration experiments. The EC for PUB and PB as a function of total inlet load are shown in Fig. 2A and B. In addition, the effect of inlet load of each hydrocarbon on EC for the PUB biofilter was studied (Fig. 2C). The maximum EC obtained for the PB and PUB biofilters were 160 and 350 gC m^{-3} h^{-1}, respectively. The experiments regarding the effect of the load clearly indicate the better performance obtained with the PUB biofilter, as the total maximal EC was enhanced by a factor of 2 compared to the PB biofilter (Fig. 2A and B). Also, it could be noted that a reduction of EC can be observed at OLRs higher than 175 gC m^{-3} h^{-1} for the PB. This could be related to a microbial inhibition at high concentrations of VOCs. Therefore, the biodegradation capacity of the microorganisms was exceeded [29]. As it can be noted, the highest ECs obtained with the PUB biofilter were 300, 360 and 460 g m^{-3} h^{-1} for hexane, toluene and MEK vapors, respectively. The level of biodegradability of the VOCs tested in the PUB biofilter is consistent with previous studies and follows the trend MEK > toluene > hexane [18,26,30].

Table 2 shows reported studies for biofilters packed with various materials used to remove the VOCs tested in the present study. The results show that in the case of MEK, the maximum EC obtained in this study was 2.5 times higher than the EC reported by Raghuvanshi and Babu [31] and Lee et al. [32], using coal/compost and ceramic cubes carriers, respectively. For toluene vapors biofiltration, the

Table 2
Summary of previous studies of MEK, toluene and hexane vapors biofiltration with various packing materials.

VOC	Packing material	EC_{max} ($g\,m^{-3}\,h^{-1}$)	Characteristics	Reference
MEK	PU-foam	2.1	VOC mixture MEK, toluene, ethylbenzene, xylene	[18]
	Coal and compost	191	Experiments for shock loadings conditions and kinetic modeling	[32]
	Composite	42	Experiments with acetone Composite PVA/Peat/GAC/KNO_3	[17]
	Fern chips	160	Field biofilter of 128 L Treating a mixture of vinyl acetate, toluene, acetone and MEK from synthetic resin and solvent manufacturing processes	[30]
	Ceramic cubes	200	Study with mixture of acetone and MEK	[32]
	Composite PU	480	Mixture of toluene and hexane	This study
Toluene	Activated carbon	205	Toluene in mixture with H_2S	[2]
	Coconut fiber	90	Fungal biofiltration at low pH 1.5–5	[4]
	Compost	95		
	Peat	72		
	Pine leaves	85		
	Activated carbon (GAC)	100	Mixture of GAC and GTR with compost (1:1, 2:1, 4:1)	[6]
	Ground tire rubber (GTR)	45		
	Compost	60		
	PU-foam	6.8	VOC mixture MEK, toluene, ethylbenzene, xylene	[18]
	Perlite	50	Fungal biofiltration *Cladophialophora* sp.	[19]
	PU-foam	37		
	Celite	100	Pressure drop problems	[20]
	Lava	130		
	GAC/celite	110		
	PU	350		
	PU-foam	90	EBRT effect	[21]
	PU foam	230	Mixture with benzene, additions of yeast extract (YE) medium, operated at 60 °C	[33]
		520	Value obtained with YE addition	
	Ceramic raschig rings	290	Fungal biofilter *Paecilomyces variotii* CBS 115145	[34]
	Porous ceramic	75	EBRT 27 s	[35]
	Perlite	40		
	PU-foam	22		
	Crab shells	56	Leaf mold solution used as nutrient solution	[36]
	Composite PU	360	Mixture of toluene and hexane	This study
Hexane	Perlite	150	Bacterial consortium	[25]
	Perlite	60	*Fusarium solani*	[38]
	Peat	70	Consortium of fungal species	
	Peat moss	90	The carriers were chemically modified with poly(ethylene ether carbonate)	[37]
	Pine sawdust	100		
	Mixture of perlite and wheat bran	30	*Rhinocladiella similis*, the packing material consist in 17% of wheat bran	[39]
	Diatomaceous earth pellets	40	Biotrickling filter	[40]
	Composite PU	300	Mixture of toluene and hexane	This study

PU: polyurethane foam; VOC: volatile organic compound; MEK: methyl-ethyl-ketone; CBS: Centraalbureau voor Schimmelcultures; and EBRT: empty bed retention time.

maximum EC obtained in the present study ($360\,g\,m^{-3}\,h^{-1}$) was similar to those reported by Ryu et al. [20] and Cho et al. [33] using a PU-foam carrier. Also, the maximal toluene EC is greater than various studies reported of biofilters packed with ceramic Raschig rings, porous ceramic, perlite and crab shells [34–36]. In the case of hexane biofiltration, the maximum EC obtained with the PUB biofilter was twice that of previously reported values using perlite and organic composites even using fungal biofilters [25,37–40]. Thus, our results suggest that the PU-foam carriers are promising materials for the biofiltration of VOCs due to their superior physicochemical properties with respect to other materials. In addition, our work shows that the chemical modification of PU-foam represents an interesting area of study since the incorporation of the starch into the chemical structure of the polymer improved the physicochemical properties (discussed later), enhanced the reactor performance by increasing the EC and significantly reduced the biofilter start-up time. This hypothesis is supported by a previous work in which it was shown the synthesis of the modified polyurethane used for packing the PUB biofilter [26].

3.3. Influence of packing material on biofilter performance

Packing materials properties showed a significant effect on the efficiency of the biofilters. Table 1 shows the physicochemical properties of materials evaluated before the biofiltration experiments started.

The bed density was lower with polyurethane than with perlite and polypropylene, respectively. The measured densities were appropriate for all studied materials since low densities ensure better hydrodynamic properties and help to prevent bed compaction [14]. These densities are comparable with those of other materials used in biofiltration systems such as peat ($0.21\,g\,mL^{-1}$), vermiculite ($0.15\,g\,mL^{-1}$), and hydroballs ($0.27\,g\,mL^{-1}$) [41]. The water retention capacity of each material is also shown in Table 1. Perlite demonstrated the highest value (42.8%) comparable with some materials used by Gaudin [14]. The polyurethane composite showed a water retention capacity of only 33.3%, almost the same obtained by Hirai et al. [15]. Even tough polyurethane is hydrophobic, its open cell structure helps to retain water. The material

Table 3
Analysis of packing materials at the end of the experimental period.

Biofilter	ε final (%)	Biomass ($mg_{protein}/g_{dry\ material}$)	Water content (%)	Bed compaction (cm)	Specific biodegradation rates ($mgC_{total}\ g^{-1}_{protein}\ h^{-1}$)
Perlite (PB)	26	22.5 ± 1.62	85 ± 1.30	3	63.2 ± 4.57
Polyurethane (PUB)	60	11.3 ± 1.12	78 ± 0.82	0	416 ± 41.60
Polypropylene (PPB)	59	ND	51 ± 2.40	0	NM

ND: no detectable; and NM: not measured.

with the lowest water capacity was the polypropylene composite (21.7%) due to its hydrophobic character.

Water content and pH are crucial parameters for the performance of the biofilter [4,41]. These two material properties were likely the cause of the poor performance of the PPB biofilter. The biofilter packed with this material did not start to remove VOCs until day 23, and the removal efficiency declined with time, possibly due to the acidic pH of the material (see Table 1). The pHs of modified polyurethane and perlite were closer to neutral values, giving a shorter start-up time and better performance (Fig. 1).

Furthermore, the polyurethane used in this study was modified with starch in a previous work, to provide an alternative carbon source for microorganism growth [26]. Such an additive has a high hydroxyl group content, which could interact with microorganisms and water molecules by electrostatic forces and hydrogen bonds. Thus, the attachment of microorganisms and the development of the biofilm were better in this modified PU material. The modified polyurethane acted as an alternative carbon source readily accessible to the inoculated microorganisms on the material. This carbon source together with the already mentioned characteristics of the composite may have accelerated the formation of active biomass, and consequently, shortening the start-up time of the biofilter. In addition, this composite could have improved the adsorption of VOCs due to the presence of hydroxyl groups from the starch added, thus the adsorption of VOCs onto the modified PU packing material would have helped to attenuate the load variations that can occur during the operation of a reactor.

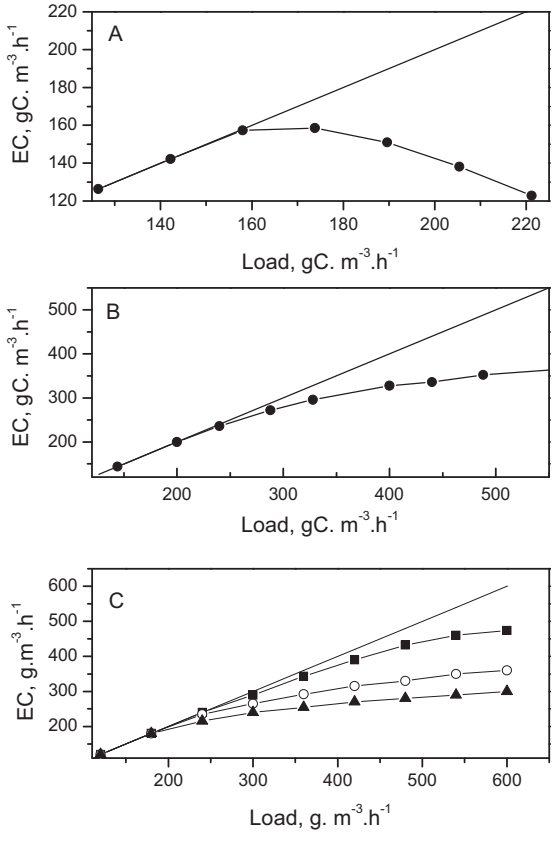

Fig. 2. Effect of the total inlet load on the elimination capacity in biofilters (A) PB and (B) PUB; (C) PUB for MEK (■), toluene (○) and hexane (▲).

Table 3 shows the bed void fraction, water and biomass content, and the specific degradation rates of the three biofilters at the final day of operation. The low performance of the perlite packed biofilter could be related to the reactor clogging when the bed void fraction was reduced from 0.6 to 0.26. When a packing material does not have sufficient porosity for microorganisms to go into the pores, the biofilm develops around the particles restricting the flow of polluted air more quickly [4,8]. After some days, the bed starts to compact reducing the contact area between the gas phase and the biofilm. Eventually, the efficiency of the reactor decreased. In contrast, the modified polyurethane has mechanical properties that facilitated the development of the biofilm in the pores which reduced clogging and prevented bed compaction (Table 3).

Biofilter performance is related to the amount of microorganisms in the biofilm. However, it has to be taken into account that not all biomass in the packing material is active. This depends on the availability of the carbon source (contaminants) and the electron acceptor (oxygen) needed for the complete oxidation of VOCs. The PB biofilter contained the highest amount of protein, followed by the PUB biofilter. However, the biofilm was more active in the PUB biofilter than in the others biofilters since the specific biodegradation rate was 6.6 times greater than the value obtained with the PB biofilter (Table 3). For the PB biofilter, the generated biomass was abundant which limited mass transfer toward the inner particles reducing the diffusion of oxygen and promoting zones of non-active biomass as other authors have mentioned [8].

3.4. Scanning electron microscopy analysis

In order to have an idea of the influence of the packing material on the biofilm structure, scanning electron micrographs were taken on day 65 of the biofilters operation. Fig. 3 shows a dramatic effect of the type of packing material on microorganism growth. In the case of the PB packing material, a dense biofilm completely covering the particle was observed. This corroborates the assumption that such dense biofilms limit the diffusion of pollutants toward inner particles. For the PPB, the fiber can be observed but a biofilm surrounding the fiber was not detected, probably due to the lower water retention capacity of this packing material. Regarding the PUB packing material, it can be assumed that there was adequate biofilm growth since the biofilm was well structured but not too dense which allows diffusion toward the interior. Thus, a larger area was available to carry out the biodegradation of pollutants. These results were consistent with the specific biodegradation rates obtained for the PUB and PB reactors (Table 3).

3.5. rDNA 18S analysis of microorganisms

Microorganisms' samples were taken from the upper part of the PUB biofilter. Microbiological analysis by serial dilutions showed the typical growth and morphology of filamentous fungi, which

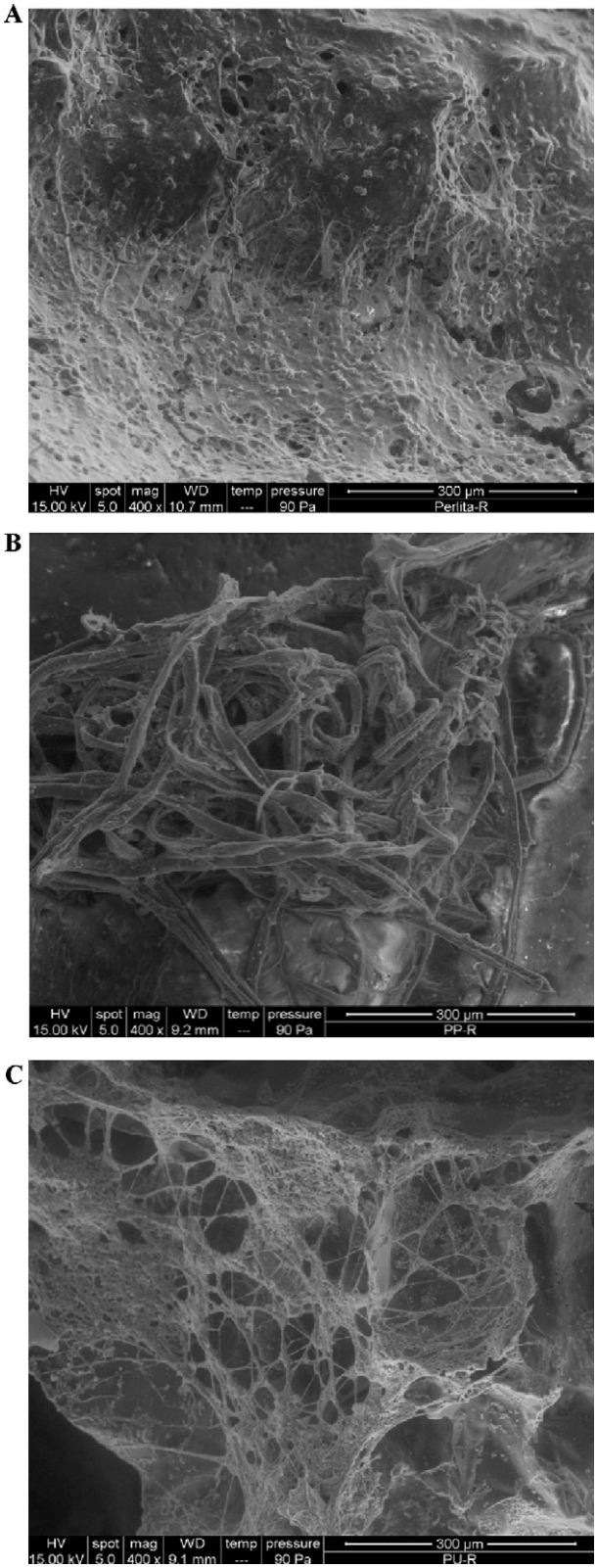

Fig. 3. Scanning electron micrographs of packing materials taken on day 65. Biofilm can be observed at 300 μm for perlite (A), reinforced polypropylene (B) and the modified polyurethane (C), corresponding to biofilters PB, PPB and PUB, respectively.

were isolated for further ITS analysis. Two colony morphologies were observed, however, analysis under microscope of these fungi showed the typical structures belonging to the *Fusarium*/*Nectria* genera, such as macro and micro conidia which have a rod shape (Fig. 4A) and conidiophores (Fig. 4B). On the other hand, scanning

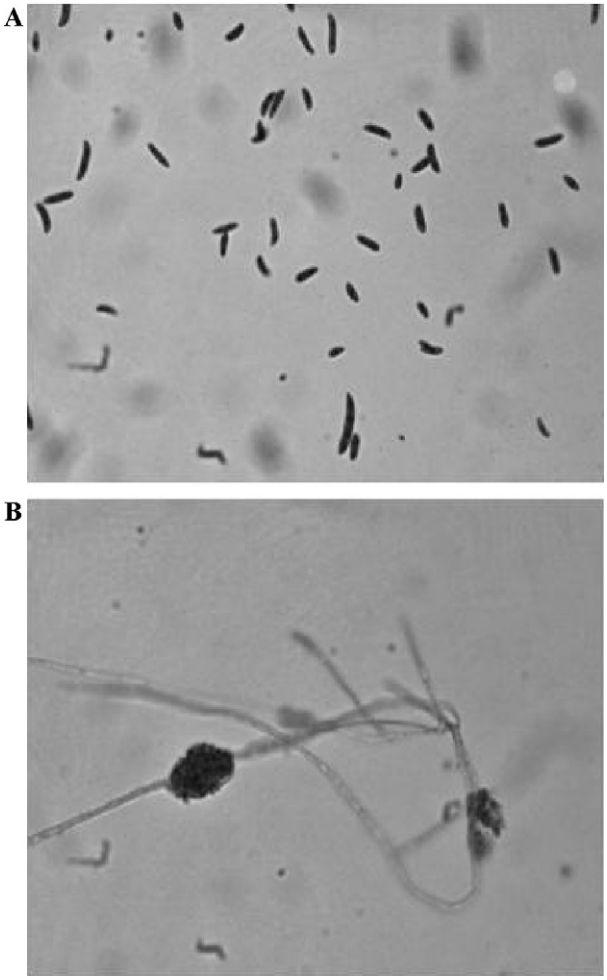

Fig. 4. Macroconida and microconida of *Nectria haematococca* (A) and conidiophores of *Fusarium solani* (B).

electron micrographs depicted only the typical filaments growth of fungi. We were unable to detect bacterial shapes such as rod, bacilli or coccus, which allow us to conclude that filamentous fungi were probably responsible of VOCs degradation. Total DNA from two fungal isolates were extracted, and their ribosomal ITS were amplified, sequenced and compared against a non-redundant NCBI database using the BLAST algorithm [28]. A 97–100% identity was found between sequences BK1-1, BK2-1, BK3-2 and OB3-1 with sequences of different strains of *Fusarium solani* in the NCBI database, whereas the sequences OB1-1, OB2-2 and OB4-2 matched 97–100 identity with sequences of different strains of *Nectria haematococca*. *F. solani* and *N. haematococca* are the same fungal strain, the separate names denoting the teleomorph (sexual) and anamorph (asexual) states, respectively.

It has been reported that *F. solani* is able to live under stressful conditions like water stress, chemical toxicity, and recalcitrant carbon sources and sometimes under anaerobic conditions. Our results suggest that *F. solani* was able to use VOCs to growth under stressful conditions, probably using these compounds as carbon sources. We are unable to confirm this, due to the fact that the solid sample taken from the biofilter to performed the ITS analysis was not representative, considering the total size of the biofilter. Also, probably *F. solani* grew at expenses of the starch content of the PU-carrier. Thus, based on the results obtained, we cannot confirm that VOCs were used as a main or a secondary carbon sources, more experiments are need to confirm this. Additionally, the degradation of VOCs is probably related to the

genetic information of microorganisms which encodes for the synthesis of the catabolic enzymes that degrade these compounds to use them as energy sources and to tolerate adverse environmental conditions. Also, the VOCs concentration in the biofilter ($180\,g\,m^{-3}\,h^{-1}$) was probably toxic for most of other species.

4. Conclusions

The physicochemical properties of packing materials, such as porosity, water retention capacity, density and pH, have a strong influence on the attachment of microorganisms and development of biofilms. These parameters are also important to avoid clogging and pressure drops in biofilters which can increase the operational costs of treatment. According to the results, the PU-composite showed appropriate physicochemical properties for the degradation of the VOC mixture. Therefore the performance of this biofilter was superior to the perlite and polypropylene reinforced with agave fibers biofilters. Furthermore, the additive in the PU-foam was key factor for enhancing the performance of the biofilter since the EC was improved and a shorter start-up period for the biofilter was achieved. Fungi from the genus *F. solani* was probably the responsible of pollutants' biodegradation. Finally, this study gives new insights into the addition of easily biodegradable carbon sources to polymeric materials for use as carriers, such as PU-foam, with the objective of enhancing the performance of biofiltration processes.

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