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Characterization of artificially dried biofilms for air biofiltration studies

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One of the main problems associated with the operation of air biofilters is the loss of performance caused by drying of the bioactive support, as the removal capacity of contaminants by the microorganisms is dependent on their water content. In this work, biofilms from a microbial consortium adapted to toluene were grown on stainless steel slides. The biofilms were dried in stoppered flasks with saturated saline solutions to obtain final water activities of 97.4 %, 83.9 %, 74.8 % and 32 %. The biofilms were characterized by a sorption isotherm Type III with toluene; the water desorption isotherm was fitted to the BET model and the biofilm hydrophobicity was also determined. Specific oxygen consumption rates decreased at lower Aw from 60 µg O2/mg protein/h to zero activity. Biofilm activity, represented by a toluene consumption rate, and others physical properties presented a critical point between Aw 0.84 and 0.97. Biological activity of dried biofilms was restored either partially or completely, depending on the extent of drying and rewetting method.

Keywords: Biofilters, biofilm, drying isotherm, sorption isotherm, toluene degradation.

Introduction

In air biofiltration, a contaminated air stream is passed through a porous support material where the pollutant-degrading microorganisms are immobilized.1 Although biofilters can function steadily for long periods of time, one of the most critical and troublesome operational requirements is to maintain a proper water content in the biofilter packing that generally is not saturated and does not have a free-flowing aqueous phase. Water content affects both the physics and biology of the system. Water in excess, retained by capillarity, restricts the air flow limiting the mass transfer in the inter-particle spaces and yielding reductions in the real residence time, increments in the pressure drop and, in extreme cases, may cause anaerobic conditions. Conversely, low water content can reduce the microbial degradation capacity which is dependent of the water activity (Aw) in the biofilm; furthermore, drying can also cause limitations in the metabolite excretion and in the bio-availability of soluble nutrients.

Drying of the support material is caused by different conditions: heat generation by microbial activity, low humidity in the inlet air flow, inadequate wetting procedures and by heat and mass exchange between the support medium and the environment in open systems under variable atmospheric conditions.1,2 Water is the principal component of the biofilm and is distributed both inside and outside the cells and its availability is critical for the biological activity by facilitating the transport of substrates and products.3 Diverse studies dealing with the effect of drying on biofilter performance have been performed. The influence of initial water content on ethanol degradation rates and the minimum water content necessary to sustain the biofilter performance were determined by Auria et al.4 A peat biofilter for toluene removal was operated without water addition and the performance diminution was associated with the formation of channels in the bed material.5

A comprehensive model was developed to predict water evaporation by the metabolic heat generated by the contaminant oxidation and variations of relative humidity, temperature and water content of both the incoming airflow and support.6 In an industrial compost biofilter for VOCs (volatile organic compounds) removal, highly hydrophobic biomass aggregates hinder the re-humectation of the support material compromising the biofilter performance.7 A study on the influence of water content and water activity, Aw, on styrene degradation in a perlite
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biofilter showed the formation of gradients along the filter bed.

This condition caused variations in microbial activity, also, the ammonia removal was strongly dependent on the water content in the yard waste compost with a critical value of 0.5 g H2O/g solids. Chang and Halverson studied a Pseudomonas putida biofilm which was subjected to reduced water availability in a soil matrix. Dehydration resulted in biofilms with smaller cells and a thicker extracellular polysaccharide layer at the air interface. Else et al. reported that low relative humidity affected the biofilm formation on metal slides to a point that biofilm cell counts were reduced to the values near their detection level when the relative humidity was 32 to 84 %.

In the studies describing the effect of water on the biofilter performance, generally the biofilm is considered being integrated into the humid organic support material. The characterization of biological and physical properties of controlled dried biofilm is then necessary to further understand the implications of the drying process in air biofilters. In this study, a biofilm capable of consuming toluene was initially grown on stainless steel slides. To study the effect of Aw, the biofilms were equilibrated at five levels of relative humidity and then characterized by their physical properties and biological activity. Finally, some re-humectation assays were performed to evaluate the recovery of biofilm's activity.

Materials and methods

Inoculum and medium

A mixed culture formed by five bacteria and two yeast strains was used as inoculum. The strains were stored at 4°C on Saboureaud agar plates for yeasts and nutrient agar for bacteria. The mixed culture was grown in flasks by daily adding liquid toluene (to a final concentration of 300 mg/L) agitated at 200 rpm and maintained at 30°C. The mineral medium contained (g/L): KH2PO4 (0.2), K2HPO4 (0.8), MgSO4·7H2O (0.5), CaSO4·2H2O (0.05), FeSO4·7H2O (0.01), (NH4)2SO4 (1.0), Ca(OH)2 (0.4).

Biofilm development

The biofilm on stainless steel slides (1.5 cm × 7 cm) was developed in an apparatus specially designed for this purpose (Figure 1). The device allowed for the formation of a biofilm on the slide by sequentially exposing the formed biofilm to nutrients in solution and to air. Two acrylic containers (30 cm × 30 cm × 30 cm) were placed one on the top of the other, the stainless steel slides were supported on a rack that was placed in the top container; both the inoculum and nutrients (5.4 L) were added to the bottom container. The suspension was pumped to the top container, allowed to rest for few minutes and then returned to the bottom container by gravity.

The procedure was repeated every 30 min for 7 to 14 days to allow for a biofilm development. The device was operated at 30°C in a temperature controlled room; the medium pH was adjusted at 7.0 with 1N NaOH, and an air stream (2800 ml min−1) was supplied to both containers. The microbial suspension with nutrients had an initial optical density of OD540 = 0.2. Glucose (1.37 g L−1 d−1) was added as a carbon source. Biomass growth in the liquid culture in the bottom container was monitored. Once the stationary growth phase was attained, the biofilm on the slides in the top container was allowed to develop for 8 days more and then used in the experiments.

Fig. 1. Biofilm formation system. 1) Biofilm reactor; 2) Rack for biofilm slides; 3) Medium and inoculum container; 4) Sample and control port; 5) Liquid pump; 6) Liquid drainage; 7) pH measurement; 8) Timer.

Experimental procedure

The assays were carried out in nominal 125 mL Erlenmeyer flasks (total volume of 146 ± 4 mL) stoppered with Mininert® valves. Five milliliters of a desiccating salt solution was placed in the flask to obtain an atmosphere with a defined relative humidity ( % RH). Low desiccation
levels were achieved with polyethylene glycol solutions (PEG-400) at 10 %, 20 %, 30 %, 40 % and 50 % to attain RH values in the 99.6 % to 91.8 % range. Extended desiccation was obtained with the following saturated saline solutions: K₂SO₄ (97.4 % RH), KCl (83.9 % RH), NaCl (74.8 % RH) and MgCl₂ (32 % RH). Experiments were performed simultaneously with distilled water (100 % RH) as a control.

The drying process was achieved by partitioning between the water in the biofilm layer and the specific atmosphere in the flask. At thermodynamic equilibrium, the % RH is equal to the Aw of the solid. The biomass colonized slides were placed on a support in the Erlenmeyer flask to avoid its contact with the drying solutions and the growth was monitored during 120 h. The flasks were kept at 30°C. Successive toluene additions (30 g/m³ flask volume) were made at 0, 24 and 120 h. All assay conditions were performed in quadruplicate; two flasks with two slides each were used for each drying level.

Biofilm re-humectation procedures were implemented to evaluate the recovery of biofilm activity after drying. Slides containing biofilm were desiccated to different Aw as previously described in flasks with saturated saline solutions during 68 h. Three humectation procedures were performed: by contact with a 100 % RH atmosphere for 24 hours, by spraying water on the biofilm slide and by immersion in distilled water for 1 hour. The restored biological activity was determined by toluene and oxygen consumption as is described in the section on respirometry. Tests were done in duplicate.

**Biofilm characterization**

**Biomass measurements**

Three methods to determine biomass were used: microbial suspension absorbance at 540 nm, Lowry protein and dry weight assays. The biofilm was scrapped from the stainless steel slides with 100 mL of a 1M phosphate buffer at pH 7, then it was homogenized for 30 s with an Ultraturrax mixer at level 50 (Ika-werk OH USA); the microbial suspension was analyzed by absorbance and by measuring its protein content with the Lowry method.

**Respirometry**

Biofilm activity was followed by toluene and oxygen depletion and CO₂ production in Erlenmeyer flasks stoppered with Mininert® valves. The initial toluene concentration in the headspace was 30 g/m³. Simultaneous assays without toluene addition were carried out to determine endogenous respiration. Toluene consumption was followed by using a gas chromatograph (HP 5890 Series II, PA USA) equipped with a FID (flame ionization detector). Carbon dioxide and oxygen were simultaneously evaluated in a TCD-equipped (thermal conductivity detector) gas chromatograph (Gow-Mac Instruments Co. Madison, NJ USA). The results were fitted to the Gompertz model, Eq. 1.

\[ Sc = \alpha \exp \left[ -\beta \exp \left( -kt \right) \right] \]  

where \( Sc \) (g toluene-m⁻³-mg protein⁻¹) is the substrate consumed, \( \alpha \) (g toluene-m⁻³-mg protein⁻¹) is the maximum substrate concentration consumed, \( \beta \) is a parameter related to initial conditions, \( k \) (h⁻¹) is the specific substrate consumption rate and \( t \) is the time (h). The maximal substrate consumption rate \( V_{max} \) was obtained by Eq. 2 [12]

\[ V_{max} = \frac{dSc}{dt} = -k \left( \frac{\alpha}{e} \right) \log \left( \frac{(\alpha/e)}{e} \right) = \frac{k\alpha}{e} = 0.368k\alpha \]  

**Toluene sorption isotherm**

Biomass from the slides was scrapped and dried for 24 h at 70°C. Increasing amounts of dried biomass were placed in 250 mL Erlenmeyer flasks; 250 µL of toluene were added to each flask, which was then stoppered with Mininert® valves and kept at 30°C for 48 hours. The toluene concentration in the headspace was measured from a 100 µL injection with a FID gas chromatograph. Isotherms were obtained in duplicate. The toluene sorption isotherm on stainless steel slides were also obtained as controls.

**Water in biofilm**

Water desorption and Aw in the biofilm were determined. The biofilm colonized slides from the biofilm reactor and from the controlled relative humidity flasks were weighed (Ohaus Galaxy 160, USA) at room temperature and humidity. The weight was recorded periodically until a constant value was obtained. Aw in the biofilm colonized slides was simultaneously measured in a Decagon Aqualab CX-2 (WA, USA) which uses a dew point method. The drying rate and the water desorption isotherm were obtained from water desorption data. Applying the condition % RH = 100Aw at equilibrium, the relative humidity used to dry the biofilm was substituted by Aw and the data were fitted to the BET model, Eq. 3.

\[ \frac{a}{M(1-a)} = \frac{1}{M_i C} + \frac{a(C-1)}{M_i C} \]  

where \( a \) corresponds to Aw, \( M \) is the biofilm water content (g H₂O/100 g dry biomass), \( M_i \) is the water content in the monomolecular film and \( C \) is a constant, Eq. 4.

\[ C = k \cdot \exp(Qs \cdot R^{-1} \cdot T^{-1}) \]  

where Qs is the sorption heat (cal mol⁻¹), R is the ideal gas constant (1.986 cal mol⁻¹ K⁻¹) and T is the absolute temperature (K).
Biofilm hydrophobicity

The method based on the microbial sorption to the water-hydrocarbon interface was used. The assays were performed with suspended biomass obtained from the biofilm formed on the slides.[13] The biomass was washed three times with a 1 M phosphate buffer at pH 7. The absorbance of the aqueous suspension was determined at 540 nm against a buffer blank (Spectronic 20D, Milton Roy Co. USA). Toluene, (3.0 ml), were added to 3.0 ml of a microbial aqueous suspension in a spectrophotometer cell (diameter 1.0 cm), then mixed in a vortex for 60 seconds. After 15 min, the final absorbance in the aqueous phase was measured. The % hydrophobicity of the biofilm was calculated by Eq. 5.

\[
\% \text{Hydrophobicity} = \frac{A_i - A_f}{A_i} \times 100 \quad (5)
\]

where \( A_i \) and \( A_f \) are the initial and final absorbance in the aqueous phase, respectively. The measurement was repeated in six samples for each biofilm drying level.

Results and discussion

The suspended biomass growth in the biofilm forming device showed a lag phase of one day, followed by the exponential growth phase for 5 days and finally a stationary phase for 6 to 14 days. The maximal biomass production was around 1500 mg protein L\(^{-1}\). The biomass formed on the slides after 14 days of cultivation had an average of 0.48 ± 0.21 mg protein cm\(^{-2}\) for 70 slides.

Toluene sorption isotherm

Figure 2 shows the toluene sorption on the dried biofilm biomass. The interaction between the gaseous toluene and the biomass can be described as a combination of absorption (i.e., solubilization of toluene in the biomass which is favored by the presence of lipid components) and of adsorption (i.e., solubilization of toluene in the biomass which is favored by the presence of lipid components) and of adsorption in the biofilm were measured simultaneously on 70 slides. The biomass formed on the slides after 14 days of cultivation had an average of 0.48 ± 0.21 mg protein cm\(^{-2}\) for 70 slides.

For 10 to 20 g toluene m\(^{-3}\) of head space, the sorbed toluene was in the range from 2 to 3 mg g\(^{-1}\) dry biomass, while for 20 to 25 g toluene m\(^{-3}\) range it increased abruptly to 12 mg g\(^{-1}\) dry biomass. This behavior suggests a type III toluene sorption isotherm, indicating that as sorption proceeds; additional sorption is facilitated because the interaction of the sorbate with the sorbed layer is greater than the interaction with the sorbent surface.[14]

In air biofiltration, toluene removal occurs by biodegradation of the contaminant that has been solubilized in the biofilm allowing then a further sorption in the aqueous biotic film. As shown in Figure 2, sorption on dry biofilm could reach 12 mg mg\(^{-1}\) dry biomass at a toluene concentration in the gas phase of around 25 g m\(^{-3}\), which is two orders of magnitude higher than the equilibrium concentration in water (around 0.1 mg g\(^{-1}\) water at this toluene concentration in the gas phase and considering a Henry coefficient of 0.4).

This observation confirms that biomass constituents, such as membrane lipids, are important contributors to VOCs removal in biofilters by favoring the partition equilibrium and hence improving mass transfer. There is sparse work dealing with sorption isotherms on biofilms; polychlorinated biphenyls, gaseous mercury and hexane adsorption isotherms have been reported recently.[15,16,17]

Isotherms for different VOCs on biofilm can be useful in order to determine the effect of the abiotic removal in the performance in air biofilters, specially under transient conditions.[17]

Water activity, drying rate and water desorption isotherm

Water content in the support material, including the biomass, may vary due to both external abiotic variables, such as those associated with the operating conditions, and variables related to the biotic phase, such as the heat generated during the substrate oxidation. The local water content, and more precisely the water activity, determines the viability and activity of the biomass. Aw and water evaporation in the biofilm were measured simultaneously on colonized slides previously equilibrated in a 100 % RH atmosphere.

The curves of water evaporation and water activity were obtained (Fig. 3). Two drying phases were observed, in the first phase it took around 30 minutes to reduce the water content from 15 to around 0.9 g H\(_2\)O g\(^{-1}\) dry biomass but the Aw changed only from 0.99 to 0.87. High Aw values correspond to water loosely bound to the biofilm that is easily evaporated and therefore the water losses are large without a significant reduction in the Aw. In the second phase, the water layer on the biofilm evaporated.

Consequently, the drying rate diminished and depended on the water diffusion through the biofilm, with the Aw which decreasing quickly from 0.84 to 0.32. The reduction
in Aw with biofilm-colonized slides at a constant weight indicates that equilibrium was not reached.

The Aw curve shows a critical change at around 0.84. Similar observations have been reported by Schmitt & Flemming. The authors studied dried biofilms using FTIR spectroscopy and found that the drying process was separated in two phases; the first was completed in 49 seconds and the second in 21 minutes, which indicated different mechanisms of water binding in the biofilm.

In Figure 4, the linear decay of the drying rate as the biofilm dries demonstrated that the water transfer was in this case diffusion limited. The rate tends to zero after around 50 minutes as the remaining water is tightly bound to the biomass.

The water desorption isotherm was obtained for the biofilm subjected to varied relative humidities. The water content in the biofilm (g H$_2$O·100 g db$^{-1}$, where db is dry biomass) and the drying level ( % RH), expressed as Aw, were adjusted to the BET equation. The adjusted result was $\frac{M}{M_1(1-A_w)} = -0.024 + 0.121 Aw$ with a correlation coefficient $r^2 = 0.905$. The isotherm data and fit to the BET model are shown in Figure 5. The water desorption experimental data showed a suitable fit to the BET isotherm model in a range from 0.32 to 0.84 Aw. Several models like BET and GAB (Guggenheim, Anderson, De Boer) assume a well defined monomolecular water layer and consequently they adjust better to low Aw values.

The BET parameters were obtained by regression data, the constant C was -4.041, and the water content in the monomolecular layer was $M_1 = 10.309$ g H$_2$O·100 g db$^{-1}$. The water content in the support medium is often determined gravimetrically and reported as concentration; however, using Aw is more appropriate because it represents the amount of water available for microbial activity. Cox et al. reported 32 % of the water content for a value of 1.0 Aw in a desorption isotherm of E. jeanselmei growing on perlite. These values are low compared to those obtained in the present work, but the difference appears to be due to the presence of the inert support.

Biofilm hydrophobicity

It has been reported that restoring the proper humidity to the support and recovering the biofiltration activity is more difficult when the support has been subject to various drying cycles. This has been attributed to an increase in the biofilm’s hydrophobicity that may be due to irreversible modification of the extracellular polymeric materials upon drying. The hydrophobicity of the dried biomass, indicated by the hydrophobicity percentage, increased as the biomass was equilibrated at lower % RH.

The values were 12 ± 6 %, 33 ± 4, 39 ± 2 and 51 ± 11 for the biomass subject to 97, 84, 75 and 32 % RH, respectively. The increased hydrophobicity exhibited by the biomass that was more intensively dried may explain why it is difficult to re-hydrate those biofilter supports that were subjected to extensive drying. On the other hand, biofiltration of hydrophobic VOCs may be improved by this increased hydrophobicity by favoring their solubility (i.e., partition coefficient) in the biofilm. Hydrophobicity of the biofilm was also shown to be a result of adaptation to the substrate, which is beyond the scope of the present study.

Toluene degradation and respirometry

The biofilm developing on the support and being in contact with the gas phase is responsible for the transport and transformation of VOCs, so its water content strongly affects these processes. Consequently, it is critical to determine the conditions that optimize the VOC degradation. Toluene consumption rates by biofilms were evaluated at different desiccation levels. A preliminary desiccation experiment was conducted for 20 h using PEG-400 solutions, corresponding to 91.8 to 99.6 % RH (or Aw in the biofilm of 0.918 and 0.996, respectively).
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Fig. 5. Biofilm water content (db, dry biomass) as function of drying level RH % (bars) and water desorption isotherm fit to BET model (circles).

The profiles of toluene consumption for each drying level showed initial adaptation periods of variable duration followed by an intermediate rapid consumption and, finally, a stage with a decreasing substrate consumption rate. The kinetic parameters from Gompertz model $k$ (specific substrate consumption rate) and $V_{\text{max}}$ (maximal substrate consumption rate) showed no statistical differences; $k$ and $V_{\text{max}}$ were $0.39 \pm 0.06 \, \text{h}^{-1}$ and $37.7 \pm 15 \, \mu\text{g toluene} \cdot \text{mg protein}^{-1} \cdot \text{h}^{-1}$, respectively. The experiment with the same biofilm colonized slides was extended to 50 h with fresh toluene addition. Under these conditions, the toluene consumption rate decreased in the samples that had been subjected to a more extended drying (Fig. 6).

Three consumption profiles were identified: complete toluene consumption for Aw 0.996 and 0.993, slower toluene consumption for Aw 0.972 and 0.962, and no detectable consumption at Aw 0.946 and 0.918. The biofilm activity, measured as $k$ and $V_{\text{max}}$, did not show significant differences between the various drying levels when the biofilm was dried for a short period (20 h); however, the specific substrate consumption rate ($k$) was similar to that obtained in a peat biofilter for toluene removal.[12] Acuña et al.[12] reported the effect of water content on the biofilter elimination capacity; the $k$ values obtained for 70 %, 60 % and 55 % RH were 0.46 h$^{-1}$, 0.48 h$^{-1}$ and 0.43 h$^{-1}$ respectively. The relative humidity percentages reported were low but the $k$ values were similar to those found for Aw values between 0.918 and 0.996 (91.8–99.6 % RH) in the present work. Perhaps, the water retained in the organic support masked the real water content in the biofilm.

The second experiment was carried out with samples that had been more extensively dried. Slides with recently formed biofilm were placed in flasks with saturated saline solutions (Aw from 0.32 to 1); toluene was added at 0, 24 and 48 h and substrate concentration in the headspace was followed for 45 or 120 h. The acclimation period needed to start toluene consumption was directly related to the biofilm desiccation degree. Initially, toluene was not consumed but oxygen uptake confirmed the microbial viability. On the second substrate addition, toluene consumption was dependent of the biofilm drying level. Observed kinetics at Aw 0.974, 0.748 and 0.320 are shown in Figure 7.

The effect of drying was dependent not only on Aw but also on the time exposure to drying conditions as occurs in air biofilters. Oxygen consumption was observed throughout the entire experiment, except for the lowest Aw value, reflecting the biomass endogenous respiration.

Biological activity was affected at low Aw because the cells require more energy to maintain the intracellular water content required by the anabolic and catabolic enzymatic reactions. Some authors pointed out that cell damage after dehydration occurs even at the proteins or DNA level.[20]
In addition, the limited water content may reduce the toluene bioavailability by hindering the molecular diffusion through the dry exopolymeric substances around the cells.

Specific oxygen consumption rates \( (q_s) \) were calculated from the slope between two substrate additions in the oxygen consumption plots. Slopes were calculated at 20, 51 and 114 h to evaluate the effect of the drying period. The calculated \( q_s \) were plotted versus relative humidity percentage at which the biofilm was dried (Fig. 8).

The oxygen consumption rate at 20 h of desiccation was not related to the \( Aw \), which the biofilm was subjected to, reflecting an incomplete atmosphere-biofilm equilibrium. Elsewhere, the data at 51 h showed a clear relationship between the \( q_s \) and the desiccation degree. The specific oxygen consumption rate increased with \( Aw \). Moreover, \( q_s \) changed sharply in a range of \( Aw \) from 0.84 to 0.97.

The values at 114 h showed the same trend as at 51 h but with lower values, reflecting the importance of extended drying on microbial activity. It has been reported that the endogenous respiration contributes significantly to the overall oxygen requirements. The \( q_s \) determined in the present work results not only on the desiccation level but on the endogenous metabolism and toluene exposure time. It has been reported that cell damage by long exposure to solvents caused cryptic growth (cellular growth based on cellular debris) and consequently higher endogenous metabolism. Increasing the extent of drying led to lower biofilm activities with a critical point at around \( Aw \) 0.97.

This work shows that biological activity remains in the biofilm beyond those \( Aw \) values generally reported for bacteria. Auerbach et al. [22] studied the morphology of *Pseudomonas* biofilm desiccated at 75.5 % RH air atmosphere and observed only a minor change in the biofilm caused by drying treatment. Holden et al. [23] investigated the toluene depletion by *Pseudomonas putida* in soils over a water potential range from 0 to −1.5 MPa and observed that the toluene consumption was reduced after partial dehydration, but cellular growth was less affected. In both studies, the water stress was not lower than 75 % RH. Contrarily, Else et al. [11] observed biofilm formation on metals at different relative humidity levels using saturated salt solutions in microcosm chambers (KCl 83.6 %, KI 67.9 % and MgCl₂ 32.4 % RH). The microorganisms did not show desiccation tolerance and relative humidity values below 100 % completely hindered biofilm formation over a period of 18 months. As it was mentioned before, the biofilm performance is influenced not only by the drying degree but also by exposure time to the specific % RH atmospheres.

**Biofilm re-humectation**

Restoring the water to the dried support is important for recovering the performance in biofilters. Three different strategies were studied to restore the water content to the artificially dried biofilm: contact with a 100 % RH atmosphere by 24 h, spraying water, or by immersion in water by 1 hour. Toluene consumption in biofilm equilibrated at 100
% RH atmosphere showed a similar profile to that of the biofilm previously desiccated at AW 1.0 and 0.974. The lag phase was 7 h and toluene was completely consumed in 21 and 24 h. For the other dried biofilms, there was no toluene consumption, but the oxygen consumption continued by endogenous metabolism in the biofilm (Table 1).

In the biofilm rewetted by spraying water, the toluene consumption rate was faster than for the biofilms at AW 1.0 and 0.97, toluene was consumed in 12 hours, but a lag phase was not observed. The biofilm dried from AW 0.84 to 0.32 did not show toluene consumption.

The biological activity in biofilm rewetted by immersion in water diminished as drying levels increased. Toluene consumption was completed at 13, 25 and 30 h for biofilm at AW 1.0, 0.97 and 0.32 respectively; moreover, the lag phase was longer at higher desiccation levels. Biofilm at AW 0.97 had an 8 h phase lag and biofilm at AW 0.32 had a 16 h phase lag.

In the present work, all rewetting methods were effective for slightly dried biofilm, i.e., AW > 0.97. For drier biofilms, the effectiveness of the rewetting strategy decreased in the following series: immersion > spraying > 100 % RH atmosphere. The strategy of allowing the dried biofilm to recover its water content under a saturated environment would be equivalent in biofilters to assuring that the incoming air is saturated after a drying event. Biofilms have relatively high water demands considering that, besides the basal cell water content, they contain 50 % to 80 % in weight of exopolymereic substances. These form a hydrogel with up to 98 % of water content and consequently the recovery of activity may be controlled by the slow water diffusion through this hydrogel. Restoring water content by equilibration with water saturated air proves to be a limited method considering that there is small driving force (activity was shown to be affected below AW of 0.97).

Small temperature increments caused by metabolic heat further impede the attaining of sufficient humidity in the biofilm. Spraying water on the support was a more efficient method but there may be problems in assuring a regular water distribution in practical biofiltration. Rewetting by immersion proved to be the best method to recover the biological activity as it guarantees that all the biofilm is in contact with water. Unfortunately this method may not be generalized as biofilters are not necessarily designed to be inundated with water. Probably the best practical method is water spraying with intermittent mixing of the support to assure a regular water distribution as has been shown previously.

### Conclusions

Biological activity, represented by gaseous toluene and oxygen consumption rates, diminished down to zero as the biofilm dried. A critical region of AW was between 0.84 and 0.97. Restoring the activity was possible, but its feasibility was dependent on the rewetting method. The extent of drying also affects the biofilm hydrophobicity, which influences the re-wetting efficiency and hence the activity recovery. In general, biofilms that did not suffer extensive drying recovered more promptly their biological activity allowing a more robust and efficient biofilter operation.

### Acknowledgments

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### References


### Table 1. Biological activity as oxygen consumption rate in rewetted biofilm.

<table>
<thead>
<tr>
<th>Previous drying level (%RH)</th>
<th>Time for complete toluene consumption (h)</th>
<th>Oxygen consumption specific rate (g O₂ m⁻³ mg protein⁻¹ h⁻¹)</th>
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<td><strong>100% RH Atmosphere</strong></td>
<td></td>
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<tr>
<td>100</td>
<td>21</td>
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<td>97.4</td>
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<td>83.9</td>
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<tr>
<td>32.0</td>
<td>33</td>
<td>0.419</td>
</tr>
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</table>

NC: Not consumed.


