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Continuous operation of membrane bioreactor treating toluene vapors by *Burkholderia vietnamiensis* G4

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Abstract

A laboratory-scale biofilm membrane bioreactor inoculated with *Burkholderia vietnamiensis* G4 was examined to treat toluene vapors in a waste gas stream. The gas feed side and nutrient solution were separated by a composite membrane consisting of a porous polyacrylonitrile (PAN) support layer coated with a very thin $(0.3 \mu m)$ dense polydimethylsiloxane (PDMS) top layer. After inoculation, a biofilm developed on the dense layer. The biofilm membrane bioreactor was operated continuously at different residence times (28–2 s) and loading rates (1.2–26.7 kg m−³ d−1), with inlet toluene concentrations ranging from 0.21 to 4.1 g m⁻³. The overall performance of the membrane bioreactor was evaluated over a period of 165 days. Removal efficiencies ranging from 78% to 99% and elimination capacities from 4.2 to 14.4 kg m−³ d−¹ were observed after start-up period depending on the mode of operation. A maximum elimination capacity of 14.4 kg m⁻³ d⁻¹ was observed at a loading rate of 17.4 kg m⁻³ d⁻¹. Overall, the results illustrate that biofilm membrane reactors can potentially be more effective than conventional biofilters and biotrickling filters for the treatment of air pollutants such as toluene.

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Keywords: Membrane bioreactor; Waste gas; Biofilm; Biodegradation

1. Introduction

Volatile organic compounds (VOCs) are commonly found in air emissions from wastewater treatment plants, motor vehicles, gasoline storage facilities and transportation, dry cleaning, and other industrial sources. Several physical–chemical techniques including condensation, incineration, absorption/stripping, adsorption, catalytic combustion, and activated carbon adsorption [\[1–5\],](#page-6-0) or a combination of these techniques have been used to treat VOCs in waste gas streams [\[4–6\].](#page-6-0) Each of these techniques has some drawbacks. Condensation requires concentrated waste streams [\[1\],](#page-6-0) thermal incineration is costly due to high fuel prices to treat dilute air streams [\[7\], w](#page-6-0)hile adsorption and absorption processes commonly convert the air pollutant to another form or phase, i.e., air contaminant to water contaminant.

In contrast, biological treatment methods result in total destruction of the compounds rather than physical transfer of the contaminant to another phase and also offer the potential for low cost implementation [\[8\].](#page-6-0) Biological methods for treating contaminated air are usually divided into four categories: biofilter, biotrickling filters, bioscrubbers, and membrane bioreactors [\[9\].](#page-6-0) Biological treatment is advantageous compared to physical/chemical treatments when the VOCs are biodegradable and the concentration is low. These advantages include low capital and operating cost, low energy requirement, and the absence of waste products that require further treatment or disposal [\[10–12\].](#page-6-0)

Biofiltration has been widely studied for the control of biodegradable and odorous VOCs in air. Biofilter can also treat fluctuating concentration of VOCs provided with an extra physical unit operation to buffer the pollutant load [\[13\].](#page-6-0) However, studies and field application of these systems have been limited to inlet VOC loading rates of less than $50 \text{ g m}^{-3} \text{ h}^{-1}$ [\[11\].](#page-6-0) At high VOC loading rates, microbial growth results in the clogging of media pore spaces with microbial biomass. This causes channelling in the packed bed, which consequently results in deterioration of the unit performance. Finally, the system fails due to high head losses across the bed. In addition, these systems are of limited use where degradation results in the accumulation of acidic compounds[\[12,14\]. M](#page-6-0)oreover, control of humidity and

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moisture contents of the packing materials is a difficult task in biofiltration processes [\[15\].](#page-6-0)

In a membrane bioreactor for waste gases (MBRWG), liquid phase and waste gas remain separated by a membrane and are subsequently degraded by the microorganisms in the biofilm attached to the membrane surface. Membrane materials can be dense, microporous, porous or composite. A conceptual diagram of a membrane bioreactor is shown in [Fig. 1.](#page-2-0)

Kumar et al. [\[16\]](#page-6-0) conducted a review of developments concerning membrane bioreactor systems for waste gas treatment. Several bench-scale studies have demonstrated the value of dense phase membrane bioreactors [\[17–19\],](#page-6-0) while others have focused on the removal of contaminants from air using a porous membrane module [\[20–21\]. I](#page-6-0)n a composite membrane bioreactor, the porous layer is used as support, while the thin dense layer prevents microbial growth through the membrane [\[22\].](#page-6-0)

Prior studies on toluene biotreatment have highlighted challenges in obtaining effective toluene treatment. The volumetric degradation rates of toluene were often too low for the process to be practical. Usually, this was due to low activity of the culture or the system became biokinetically and/or mass transfer limited over a period of time [\[16\].](#page-6-0) So far MBRWG for toluene removal have been seeded by pure culture (*Pseudomonas putida*) or by bacterial consortia enriched from activated sludge as biofilm or suspended cells [\[16\].](#page-6-0) Biological treatment of VOCs in air depends on the ability of certain microorganisms to metabolise these VOCs and use them as their sole source of carbon and energy producing carbon dioxide, water vapor, and biomass [\[23\].](#page-6-0) Thus, a microbially engineered bioreactor system that could effectively treat toluene over an extended period of time would be desirable. The *Burkholderia cepecia* complex members possess considerable biotechnological potential as agents of bioremediation [\[24\].](#page-6-0) *B*. *cepecia* G4 proficiently degraded toluene in a foamed emulsion bioreactor [\[25\]. I](#page-6-0)t is expected that *Burkholderia vietnamiensis* G4, a member of genus *Burkholderia* can proficiently degrade toluene in a MBRWG.

Regarding the membranes, higher and constant removal have been obtained with nonporous membranes such as polydimethylsiloxane (PDMS) in comparison to hydrophobic microporous membranes [\[16\].](#page-6-0) In previous work [\[22\]](#page-6-0) a composite membrane, consisting of a porous polyvinyldine fluoride (PVDF, $210 \mu m$) coated with a layer of 1-2.5 μ m of PDMS, was incorporated in a MBRWG for toluene removal. Composites with a thin coating layer of only $0.3 \mu m$ of PDMS over a polyacrylnitrile (PAN, 185 µm) support are commercially available. On the basis of high permeability of thin-film composites [\[26\],](#page-6-0) it is expected that their incorporation in MBR further can improve the overall reactor operation.

The aim of the present study is to evaluate the long-term performance of a MBR treating gaseous toluene by *B*. *vietnamiensis* G4 under various operating conditions. A comparison between present and prior study on MBRWG for toluene removal was also made.

2. Materials and methods

2.1. Lab-scale membrane bioreactor set-up

A MBRWG was set-up as shown in [Fig. 1.](#page-2-0) A commercially available PDMS/PAN composite membrane (GKSS, Germany, 40 cm² effective membrane area) was used, consisting of PDMS as the hydrophobic dense top layer with a thickness of $0.3 \mu m$ and PAN as the hydrophobic support layer material with a thickness of $185 \mu m$. The membrane was incorporated into a Perspex reactor module. Through one compartment, mineral medium was recirculated at the dense membrane side at a flow rate of $75 \text{ cm}^3 \text{ min}^{-1}$ by a peristaltic pump (3) (Masterflex, Cole Parmer). A full description of the experimental set-up can be found in De Bo et al. [\[26\].](#page-6-0) For all the experiments described herein, the MBR was rinsed with ethanol, and the mineral medium and heat resistant reactor parts were

Fig. 1. Experimental set-up of the membrane bioreactor. Legends: (1) magnetic stirrer; (2) nutrient solution; (3) peristaltic pump; (4) pulse dampener; (5) membrane bioreactor; (6) isothermal chamber; (7) biofilm attached on membrane.

autoclaved prior to the experiments. This ensured that *B. vietnamiensis* G4 remained the dominant organism in the system. The mineral medium (MM) used for MBR consisted of 1 g L⁻¹ KH₂PO₄, 1gL⁻¹ K₂HPO₄, 1gL⁻¹ KNO₃, 1gL⁻¹ NaCl, $0.2 g L^{-1}$ MgSO₄, $26 mg L^{-1}$ CaCl₂·2H₂O, $5.2 mg L^{-1}$ EDTA Na₄ (H₂O)₂, 1.5 mg L⁻¹ FeCl₂·4H₂O, 0.1 mg L⁻¹ MnCl₂·2H₂O, 0.012 mg L⁻¹ CoCl₂·6H₂O, 0.07 mg L⁻¹ ZnCl₂, 0.06 mg L⁻¹ H₃BO₃, 0.025 mg L⁻¹ NiCl₂·6H₂O, 0.025 mg L⁻¹ NaMo₄·2H₂O, 0.015 mg L⁻¹ CuCl₂·2H₂O. Between the pump and the module, a pulse dampener (4) (Cole Parmer) was placed. The MM was magnetically stirred at 400 rpm (IKA RCT basic, IKA labortechnik).

Through the other compartment, contaminated air passed along the porous membrane side in counter-current with the liquid stream. Gas flow rates between 17 and 240 cm³ min⁻¹ were selected, corresponding with gas residence times between 28 and 2 s. The air stream was loaded with toluene through a dynamic vapor generating system. In this system, liquid toluene (Aldrich; 99.8% pure) was kept in a reservoir, placed in a thermostatic water bath at 30.0 ± 0.5 °C. The headspace of the reservoir was connected with the main air flow through a diffusion capillary. Using Stephan's law, the toluene diffusion could be calculated [\[27\]. T](#page-6-0)oluene loaded air (20 cm³ min⁻¹; 1.0 g TOL m⁻³) passed through the 250 cm^3 bubble column (containing 230 cm^3 of mineral medium and 20 cm3 of *B*. *vietnamiensis* G4 suspension) until 99% of the toluene was removed from the air stream.

2.2. Analytical methods

Gas phase toluene concentration was measured using a Varian 3700 gas chromatograph (Varian Associates Inc.) coupled with FID detector. Gas samples were taken directly in triplicate with a 1 mL Vici gas syringe. The residual standard deviation on the measurements was less than 10%. Liquid phase toluene concentrations were determined by taking 1 mL water samples with a plastic syringe (BD plastipak). The samples were brought into a 4.5 ml vial with a Teflon®-lined Mininert® screw cap and placed in a thermostatic bath at 30.0 ◦C. After 2 h, 1 mL of the gas phase was sampled and injected into the gas chromatograph. Response factors for GC measurements were determined by analyzing external standard prepared as headspace concentrations. Henry's coefficient was used to calculate TOL concentration in mineral medium according to Dewulf et al. [\[28\]. C](#page-6-0)ell dry weight was determined gravimetrically [\[29\]. T](#page-6-0)he pH was measured with a Jenway 3310 apparatus, equipped with a Hanna Instruments electrode.

3. Results and discussion

3.1. Transport of toluene through the membrane reactor

The transport of toluene through the membrane was determined for gas residence times ranging between 2 and 28 s under two different conditions. First, air was supplied at both sides of the membrane (gas/gas). Toluene loaded air $(0.9 \text{ g TOL m}^{-3})$ passed at the porous side of the membrane, while clean air passed along the dense side. The clean air flow rate was set constant at $75 \text{ cm}^3 \text{ min}^{-1}$. A mass balance was made over the module and showed toluene recovery of 98–99%. Next, tap water passed along the dense side of the membrane (gas/liquid), at the same flow rate as for clean air. In contrast with other experiments carried out in this study, the liquid phase was not recirculated. Parameters were determined after steady state conditions were attained, approximately after 15–20 h. To check accuracy of the parameters, a mass balance was calculated over the reactor. A clear difference in results between a gas/gas and gas/liquid mode

Fig. 2. Transport through the membrane reactor.

Fig. 3. Toluene batch biodegradation by *Burkholderia vietnamiensis* G4: (\triangle) toluene conc. $(g m^{-3})$; (●) control (no microorganism).

mass transfer coefficient was 0.003 m s⁻¹ (± 0.0004 m s⁻¹) calculated from Eq (1).

3.3. Batch degradation of TOL

Pure microbial culture of *B*. *vietnamiensis* G4 was selected on the basis of literature search for the biodegradation of selected compounds and was obtained from BCCM/LMG Laboratory of Microbiology, Ghent University, Belgium. Batch biodegradation tests were performed in 118 mL penicillin bottles, sealed with Teflon-lined Mininert valves (Alltech Associates) and incubated in a thermostatic bath at 30.0 ± 0.1 °C. The decrease of the gaseous TOL concentration (0.36 g m⁻³) as a function of time (i.e. the progress curve), was determined for *B*. *vietnamiensis* G4 suspension and a control (no microorganisms) shown in Fig. 3. The volume of cell suspension was 10.0 mL. The penicillin bottles were mixed by means of a magnetic stirrer (IKA Labotechnik) at 400 rpm. Sampling of the headspace was started 30 min after the injection of TOL and lasted 11 h. The kinetic parameters were determined according to Amor et al. [\[30\]](#page-6-0) and fitted in the Monod's equation [\[31\]. D](#page-6-0)uring the batch biodegradation of TOL a μ_{max} of 0.4 h⁻¹ and K_s of 0.21 g m⁻³ was observed. This observed value of μ_{max} is comparable with others reported in literature.

Fig. 4. Performance of membrane bioreactor under different operating conditions: (\bullet) loading rates; (\Diamond) residence time (s); (\blacksquare) mineral medium replacement; (\triangle) removal efficiency.

3.2. Abiotic mass transfer

Mass flux was calculated from the gas inlet, outlet and membrane surface area. The overall mass transfer coefficient was calculated from the ratio of mass flux and the logarithm of the mean driving force. The overall mass transfer resistance can be modeled as the sum of the gas $(1/k_g)$, membrane $(1/k_m)$, and liquid $(1/k_1)$ resistances, shown in Eq. (1) .

$$
\frac{1}{K_{\text{ov}}} = \frac{1}{k_{\text{g}}H} + \frac{1}{k_{\text{m}}H} + \frac{1}{k_1} \tag{1}
$$

Under gas/gas condition the membrane mass transfer, overall mass transfer, and gas phase mass transfer coefficient were 0.004 m s^{-1} (±0.0005 m s⁻¹), 0.0028 m s⁻¹ (±0.0007 m s⁻¹) and 0.016 m s^{-1} ($\pm 0.007 \text{ m s}^{-1}$), respectively. The liquid phase

3.4. Membrane bioreactor performance

The reactor was seeded with the *B*. *vietnamiensis* G4, which had been grown in a mineral medium with toluene as a sole carbon and energy source. During the operation period of 165 days, toluene loading rate, gas residence time, and removal efficiency of toluene are shown in [Fig. 4, a](#page-3-0)ir flow rates and toluene feeding controlled by mass flow regulator determined the gas residence time and toluene loading rate in the membrane bioreactor.

The performance of the membrane bioreactor was evaluated by the following performance parameters: toluene loading rate, removal efficiency, and the elimination capacity. The definitions of these parameters are set out below:

$$
Load = \frac{Q \times C_{\text{in}}}{V}
$$
 (2)

$$
RE = \frac{C_{\text{in}} - C_{\text{out}}}{C_{\text{out}}} \times 100
$$
 (3)

$$
EC = Q \times \frac{C_{\text{in}} - C_{\text{out}}}{V} \tag{4}
$$

3.5. Membrane bioreactor start-up (period I: 1–44 days)

In this membrane bioreactor, TOL loaded air diffuses through the porous side of the membrane and subsequently degraded by the microorganisms in the biofilm attached to the dense membrane. After 2 days, >60% TOL removal was observed. The microbial suspension was replaced by fresh MM manually, and thus all non-adhering cells were removed. During the first 43 days, the gas residence time (τ) was set at 11 s. Toluene removal efficiency increased and reached 74% with an average loading rate of 7.2 kg m−³ d−1. During the first 23 days, a 30% decrease in removal efficiency was observed. This is may be due to the mechanical problems (leakage at the liquid side and shutdown of peristaltic pump) in the reactor set-up. On day 35, a removal efficiency of 74% was observed and was in the same order during next 36–43 days. The pH of the liquid phase was always between 7.2 and 7.5. However, on day 44, by increasing the residence time to 28 s, consequently decreasing the average loading rate to $1.02 \text{ kg m}^{-3} \text{ d}^{-1}$, a removal efficiency of 99% was observed.

Fig. 5. Toluene maximum removal efficiency at different residence time.

3.6. Influence of loading rate and gas residence time on the reactor performance

After period I, different periods (II–VIII) were established with decreasing residence time of 28 s (period II), 24 s (period III), $20 s$ (period IV), $15 s$ (period V), $10 s$ (period VI), 5 s (period VII), and 2 s (period VIII). During each of these periods the MBRWG was subjected to a range of load conditions to determine the removal characteristics through the unit. TOL inlet concentrations (C_{in}) were changed between 0.21 and 4.10 g m^{-3} . The mass loading rate (LR) was increased from 0.67 to 26.7 kg m⁻³ d⁻¹. During period II (44–51 d) at LR of 0.84–1.88 kg m⁻³ d⁻¹ $(\tau = 28 \text{ s})$ a removal efficiency of 99% was observed. During period III (52–84 d) at LR of 1.89–14.4 kg m⁻³ d⁻¹ $(\tau = 24 \text{ s})$ removal efficiency reached 99%. During period IV (85–109 d) at LR of 4.1–13.87 kg m⁻³ d⁻¹ (τ = 20 s) removal efficiency decreased to 86%. During period V (110–126 d) at LR of 4 to $16.68 \text{ kg m}^{-3} \text{ d}^{-1}$ ($\tau = 15 \text{ s}$) removal efficiency decreased to 86%. During period VI (127–140 d) at LR of 6.9–15.52 kg m⁻³ d⁻¹ (τ = 15 s) removal efficiency dropped to 78%. During period VII (141–151 d) at LR of 3.66–16.41 kg m⁻³ d⁻¹ (τ = 5 s) removal efficiency was 78%. During period VIII (152–165 d) at LR of 14.6–26.35 kg m⁻³ d⁻¹ $(\tau = 5 s)$ removal efficiency was dropped to 60%. As shown in [Fig. 4,](#page-3-0) the removal efficiency decreased as gas residence was decreased. For a gas residence time longer than 5 s, the removal efficiency was always >78%. When gas residence time was reduced to 2 s, the removal efficiency decreased to 60%, which is probably due to mass transfer limitation. After changing the concentrations and/or the gas residence time, removal efficiency and elimination capacity became stable after 20–24 h. Each setting was kept constant for 4–5 days to be sure that reactor performance was stable over time. Overview of the results plotted in [Fig. 4](#page-3-0) demonstrates that the removal efficiency depends on both the gas residence time and the loading rates. The removal efficiency was maintained at 78% for an inlet load of $16.7 \text{ kg m}^{-3} \text{ d}^{-1}$ at a gas residence time of 5 s, but declined at higher loads. Gas residence time is an important parameter in a MBRWG operation because it must be sufficiently long to obtain a high removal efficiency of pollutants. The relationship of toluene maximum removal efficiency

Fig. 6. Average elimination capacity (EC) for TOL as a function of loading rate, operate at a residence time of 24, 20, 10 and 2 s. The straight line represents 100% removal efficiency, while dotted lines are best fits of data.

with shortened gas residence time at maximum loading condition can be expressed as the curve shown in [Fig. 5.](#page-4-0) When gas residence time was reduced from 28 to 2 s removal efficiency decreased from 99% to 60%. The lower removal efficiency obtained at decreasing gas residence time can be explained by change to a mass transfer rate controlled biosystem. The flux of toluene into the biofilm declines at decreasing residence time, due to lower inlet concentration gradients over the membrane surface. However, when TOL load was beyond 22 kg m⁻³ d⁻¹ the elimination capacity of TOL did not further increase with an increase of loading rate, indicating that TOL biodegradation rate followed zero-order kinetics (change of the MBRWG to a bio-kinetic limited system) in this concentration range, in agreement with "the operating regimes" of the bioreactors proposed by Cox and Deshusses [\[32\].](#page-6-0) This may be due to the organism activity might be restrained at high concentration of TOL.

3.7. Elimination capacity

Elimination capacity (EC) is one important parameter to evaluate the MBR performance. The performance of a membrane bioreactor under different operational parameters can be summarized by plotting the EC against the LR. It can be seen from [Fig. 4](#page-3-0) that >90% removal efficiency was obtained at organic loading rate up to $14.4 \text{ kg m}^{-3} \text{ d}^{-1}$ ($\tau = 20 \text{ s}$). At LR of 22.0 kg m^{-3} d⁻¹ (τ = 2 s), removal efficiency decreased to 30%. There was a trend of increasing elimination capacity with increasing inlet loading and then reaching a constant level, which was named as maximum elimination capacity [\(Fig. 6\).](#page-4-0)

4. Comparison of the performance of various membrane bioreactors for toluene removal

In Table 1 entries include reactor design, operation and performance parameters, observed range of toluene, reactor dimensions, types of membrane, and inoculum type. Compared to a flat and capillary membrane configuration, hollow fibres have large specific gas-membrane contact area. Because of the large range in these specific membrane areas used in membrane bioreactor experiments, data on mass loading rate, LR, and elimination capacity, EC, should be compared per unit of available (specific) membrane area. Volumetric ECs suggest that a flat membrane configuration is inferior to hollow fibres. However, on the basis of the available membrane area, data are in the same order of magnitude. As can be seen in Table 1, per unit of membrane area, $EC_{m,max}$ amounts 28.8 g toluene m⁻² d⁻¹, is the highest compared with other membrane bioreactors in the same range of loading rates. Only England and Fitch reported higher elimination capacity [\[18\], b](#page-6-0)ut at loading rates that were more than 100 times larger than the loadings applied in this study. Differences in removal percentage between the current study and prior studies may be attributed to differences in compound mass transfer in membranes, air flow rates, membrane surface areas, and/or biofilm composition [\[16\].](#page-6-0)

elimination capacity.

^a Gas residence time in lumen. Gas residence time in lumen.

ء

c

Gas residence time in shell and lumen.

Gas residence time in shell and lumen

Pores are water-filled.

Pores are water-filled.

5. Conclusions

The results presented, herein, clearly demonstrate that toluene can be effectively treated in a MBRWG. Depending on the conditions, high elimination rate or high removal percentage of toluene was obtained. This study demonstrates the stability and good reactor performance of a composite membrane (PDMS/PAN) bioreactor inoculated with *B*. *vietnamiensis* G4 for treatment of toluene contaminated air. However, inoculated MBRWG cannot be sure whether it remained pure. The bioreactor performance was affected by the gas residence time and inlet concentration. Lowering the gas residence time at a constant loading rate resulted in lower reactor performance. A TOL maximum elimination capacity of $14.4 \text{ kg m}^{-3} \text{ d}^{-1}$ was observed, which is the highest degradation reported in the literature for similar loading rates to those used in the experiments. Compared to other MBRWG for toluene removal present study shows that use of *B. vietnamiensis* G4 is a good option for the treatment of toluene.

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