Gas-phase toluene biodegradation by Burkholderia vietnamiensis G4 *in a biofilm membrane reactor*

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ABSTRACT

A laboratory-scale biofilm membrane bioreactor inoculated with *Burkholderia Vietnamiensis* G4 was examined to treat toluene vapors from a synthetic 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 μm) 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 (28-5 sec) and loading rates (1.2-17.7 kg m^{-3} d^{-1}), with an inlet toluene concentrations ranging from 0.21-4.1 g m^{-3}. The overall performance of the membrane bioreactor was evaluated over a period of 151 days. Removal efficiencies ranging from 78-99% and elimination capacities ranging from 4.2-14.4 kg m^{-3} d^{-1} were observed depending on the mode of operations. 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.

1 INTRODUCTION

Biological methods for treating contaminated air are usually divided into four categories: biofilter, biotrickling filters, bioscrubbers, and membrane bioreactors. 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 (Wu *et al*., 1999; Zilli *et al*., 2000).

Biofiltration has been widely studied for the control of biodegradable and odorous VOCs in air. However, studies and field application of these systems have been limited to inlet VOC loading rates of less than 50 g m^{-3} h^{-1} (Wu *et al*., 1999). 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 (Zilli *et al*., 2000; Ergas *et al*., 1995). Moreover, control of humidity and moisture contents of the packing materials is a difficult task in biofiltration processes (Sun *et al*., 2002).

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. A conceptual diagram of a membrane bioreactor is shown in Figure 1.

Kumar *et al*. (2007) conducted a review of developments concerning MBRWG. Several bench-scale studies have demonstrated the value of dense phase membrane bioreactors (Attawayet *et al*., 2001; Ficth *et al*., 2003; Freitas dos Santos *et al*., 2003), while others have focused on the removal of contaminants from air using a porous membrane module (Ergas *et al*., 1999; Keskiner and Ergas 2000). In a composite membrane bioreactor, the porous layer is used as support, while the thin dense layer prevents microbial growth through the membrane (Van Langenhove *et al*., 2004).

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 biokinetic and/or mass transfer limited over a period of time (Kumar *et al*., 2007). 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 (Kumar *et al*., 2007). 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 (Mutafov *et al*., 2004). Thus, a microbially engineered bioreactor system that could effectively treat toluene over extended period of time would be desirable. The *Burkholderia cepecia* complex members possess considerable biotechnological potential as agents of bioremediation (O'Sullivan and Mahenthiralingam, 2005). *Burkholderia cepecia* G4 proficiently degraded toluene in a foamed emulsion bioreactor (Kan and Deshusses, 2005). It is expected that *Burkholderia Vietnamiensis* G4, a member of genus *Burkholderia* can proficiently degrade toluene in a MBRWG.

The aim of present study was to evaluate the long-term performance of a MBRWG treating toluene vapors by *Burkholderia 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

MBRWG was set up as shown in Figure 1. Commercially available PDMS/PAN composite membrane (GKSS, Germany, 40 cm^2 effective membrane area) was used, consisting of PDMS as hydrophobic dense top layer with a thickness of 0.3 μm and PAN as hydrophobic support layer material with a thickness of 185 μm. The membrane was incorporated into the Perspex reactor module. Through one compartment, mineral medium was recirculated at the dense membrane side at a flow rate of 75 cm^3 min^{-1} by a peristaltic pump (2) (Masterflex, Cole Parmer). For all the experiments described herein, the MBR was rinsed with ethanol, and the mineral medium and heat resistant reactor parts were autoclaved prior to the experiments. This ensured that *Burkholderia Vietnamiensis* G4 remained the dominant organism in the system.

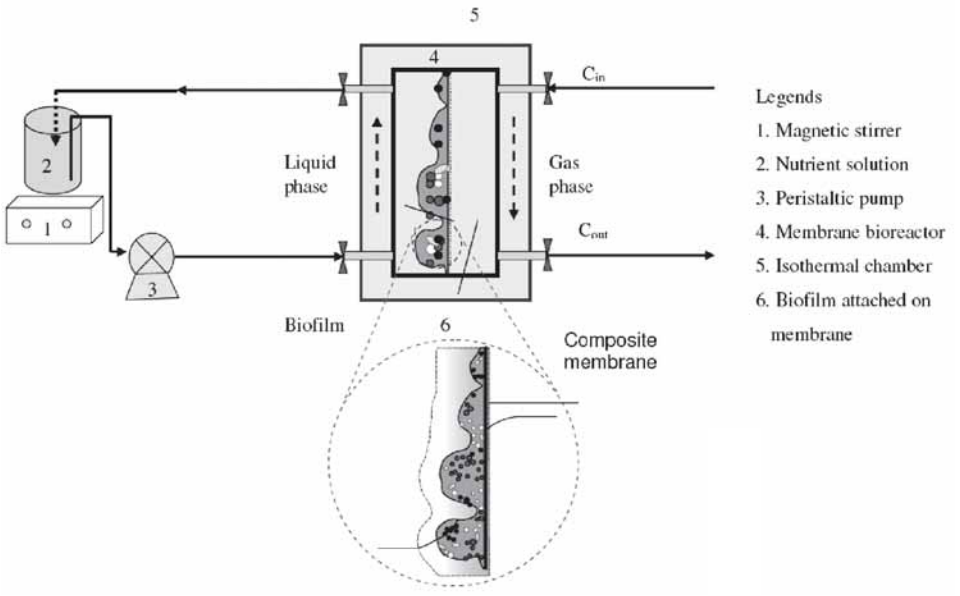

Figure 1. Experimental set-up of the membrane bioreactor.

The mineral medium (MM) used for MBR consisted of 1 g L^{-1} KH_2PO_4, 1 g L^{-1} K_2HPO_4, 1 g L^{-1} KNO_3, 1 g L^{-1} NaCl, 0.2 g L^{-1} $MgSO_4$, 26 mg L^{-1} $CaCl_2.2H_2O$, 5.2 mg L^{-1} EDTA Na_4 $(H_2O)_2$, 1.5 mg L^{-1} $FeCl_2$, $4H_2O$, 0.1 mg L^{-1} $MnCl_2$. $2H_2O$, 0.012 mg L^{-1} $CoCl_2.6H_2O$, 0.07 mg L^{-1} $ZnCl_2$, 0.06 mg L^{-1} H_3BO_3, 0.025 mg L^{-1} $NiCl_2$ $6H_2O$, 0.025 mg L^{-1} $NaMo_4.2H_2O$, 0.015 mg L^{-1} $CuCl_2.2H_2O$. Between the pump and the module (4), a pulse dampener (3) (Cole Parmer) was placed. The MM was magnetically stirred at 400 rpm (1) (IKA RCT basic, IKA labortechnik).

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 in triplicate with a 1 mL Vici gas syringe. The residual standard deviation on the measurements were less than 10%. Water 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 hours, 1 mL of the gas phase was sampled and injected into the gas chromatograph. Cell dry weight was determined gravimetrically (APHA, 1980). The pH was measured with a Jenway 3310 apparatus, equipped with a Hanna Instruments electrode.

3 RESULTS AND DISCUSSION

3.1 Membrane bioreactor performance

The reactor was seeded with the *Burkholderia Vietnamiensis* G4*,* which had been grown in a mineral medium with toluene as a sole carbon and energy source. During the operation period of 151 days, toluene loading rate, gas residence time, and removal efficiency of toluene are shown in Figure 2, air 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, elimination capacity. The definitions of these parameters are set out below:

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

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

$$EC = Q \times \frac{C_{in} - C_{out}}{V} \quad (4)$$

3.2 Membrane bioreactor start-up (period I: 1 – 43 days)

In membrane bioreactor, composite membrane (PDMS/PAN) was incorporated in the Perspex reactor module, TOL loaded air and mineral medium remain separated by the composite membrane. The inoculum was recirculated along the dense side of the membrane, while 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 two days, more than 60 % TOL removal was obtained. The microbial suspension was replaced by fresh MM, 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 start-up period water condensation at the feed side was observed but after a period of 15 days it was no longer observed. This may be due to the development of biofilm growth (visible) on the dense side. During period of 22-34 days, a 30% decrease in removal efficiency was observed, but after replacement of mineral medium (day 23, 35) it could recover to 74 % removal efficiency. However, after increasing the gas residence time to 28 s consequently decreasing TOL average loading rate to 1.2 kg m^{-3} d^{-1} could recover to 99 % TOL removal efficiency. This could be explained by biomass growth and enzyme production is necessary for TOL removal.

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

After period I (start-up), different periods (II to VIII) were established with decreasing residence time from 28 s (period II), 24 s (period III), 20 s (period IV), 15 s (period V), 10 s (period VI), and 5 s (period VII).

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 to 4.10 TOL g m^{-3}. The gas residence time was switched between 28 s and 5 s. Consequently, the mass loading rate (LR) was increased from to 0.67 to 17.7 kg m^{-3} d^{-1}. At day 44 gas residence time was increased from 11 to 28 s. During period II (44-51 d) at LR of 0.84 to 1.88 kg m^{-3} d^{-1} at τ = 28 s removal efficiency was 99%. During period III (52-84 d) at LR of 1.89 to 14.4 kg m^{-3} d^{-1} at a τ = 24 s removal efficiency reached 99%. During period IV (85-109 d) at LR of 4.1 to 13.87 kg m^{-3} d^{-1} at τ = 20 s removal efficiency decreased to 86%. During period V (110-126 d) at LR of 4 to 16.68 kg m^{-3} d^{-1} at τ = 15 s removal efficiency dropped to 86%. During period VI (127-140 d) at LR of 6.9 to 15.52 kg m^{-3} d^{-1} at τ = 10 s removal efficiency of 78% was observed. During period VII (141-151 d) at LR of 3.66 to 16.41 kg m^{-3} d^{-1} at τ = 5 s removal efficiency was 78%. As shown

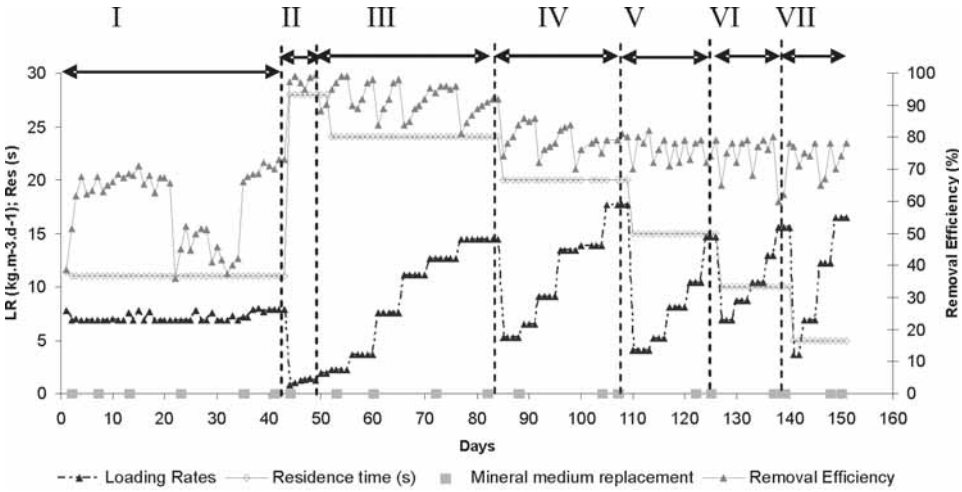

Figure 2. Performance of membrane bioreactor under different operating conditions.

in Figure 4, the TOL removal efficiency decreased as gas residence was decreased. For a gas residence time longer than 5 s, the removal efficiency was always >78 %. 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 Figure 2 demonstrates that the removal efficiency depends on both the gas residence time and the inlet concentration. The removal efficiency was maintained at 78 % for an inlet load of 16.7 kg m^{-3} d^{-1} at a gas residence time of 5 s, but declined at higher loads. It appears that growth of micro-organisms based on dry matter determination (data not shown) is inhibited at higher toluene loading rates. The result obtained during the present study is compared and discussed with prior studies in Table 1.

3.4 Elimination capacity

Elimination capacity (EC) is one important parameter to evaluate the MBR performance. The performance of membrane bioreactor under different operational parameters can be summarized by plotting the EC against the LR. It can be seen from Figure 3 that > 90 % removal efficiency was obtained at organic loading rate up to 14.4 kg m^{-3} d^{-1} (τ = 20 s). At LR of 16.4 kg m^{-3} d^{-1} (τ = 5 s), removal efficiency decreased to 20%. 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 (Figure 3).

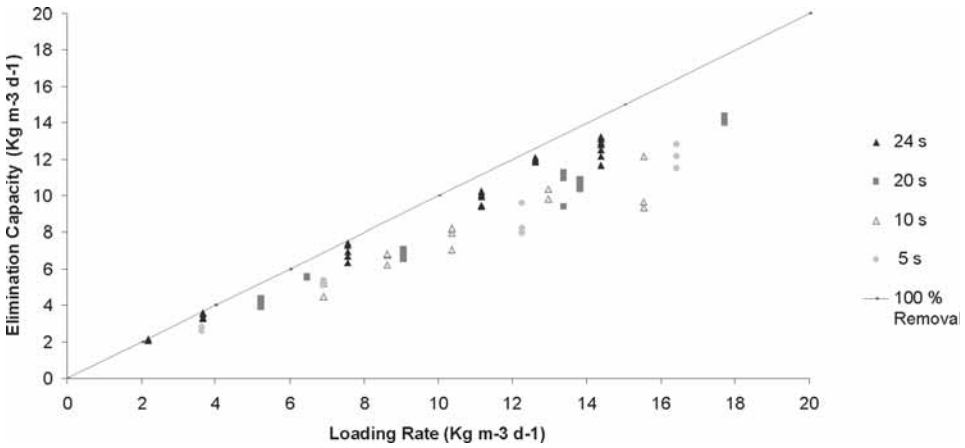

Figure 3. Average elimination capacity (EC) for TOL as a function of loading rate, operate at a residence time of 24, 20, 10 and 5s. The straight line represents 100% removal efficiency, while dotted lines are best fits of data.

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 capillar 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 the Table 1, per unit of membrane area, $EC_{m,\,max}$ amounts 28.8 g toluene $m^{-2}\,d^{-1}$, is the highest than obtained with other membrane bioreactors in the same range of loading rates. Only England and Fitch (2002) reported higher elimination capacity, but 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 (Kumar *et al.*, 2007).

Table 1.
Comparison of the performance of various gas-phase membrane bioreactors for the treatment of toluene.

Reactor set-up						Reactor performance			
	Inoculum (co-substrate);		*Configuration,*	*a*	*τ*	$EC_{m,\,max}$	LR_m	*η*	Ref.
Days	*b = biofilm, s = suspend. cells*		*type, material*	$m^2\ m^{-3}$	*s*	$g\ m^{-2}\ d^{-1}$		%	
90	*Pseudomonas putida* Tol1A	b	HF, P, PE	10256	0.8 - 4.2	1.6	1.6	97	1
< 1	*Pseudomonas* GJ40	s	F, P, PP	500	1.6 - 9.6	2.8	8.1	35	2
120	Activated sludge	b	HF, P, PP	20000	0.9 - 1.8	3.0	8.6	35	3
168	Activated sludge	b	C, P, PSf*	2622	16 / 32	3.9	4.7	84	4
n.r.	n.r.	b	C, NP, PDMS	n.r.	n.r.	16	84	20	5
150	*Pseudomonas putida* A1	b	HF, PE	n.r.	0.5 – 1.3	n.r.	n.r.	86	6
339	*Pseudomonas putida* TVA8	b	CM,PDMS/PVDF	500	8 - 24	19	23	84	7
37	Activated sludge	b	T, NP, PDMS	558	1.0	144	720	20	8
165	*Burkholderia Vietnamiemsis* G4	b	CM, PDMS/PAN	500	5-28	28.8	35.4	82	This work

Configurations: HF: hollow fibre (ID < 0.5 mm), C: capillary (0.5 mm < ID < 10 mm), T: tubular (ID > 10 mm), SW:spiral-wound, F = flat membrane

Membrane type: P : porous, NP : nonporous, CM : composite membrane, Membrane polymer: PP: polypropylene, PSf : polysulfone, PE : polyethylene, PDMS : polydimethylsiloxane; * indicates pores are water-filled,

PVDF : polyvinylidenefluoride, Zrf : zirfon, n.r. : not reported or not sufficient data to calculate

Notations : a: specific membrane area (m^2 membrane per m^3 air volume); LR: volumetric loading rate; LR_m : loading rate per unit of available membrane area; η: removal efficiency; EC_{max} :maximum volumetric elimination capacity.

[1] Ergas *et al*., 1997; [2] Parvatiyar *et al*., 1996a; [3] Ergas *et al*., 1999; [4] Parvatiyar *et al*., 1996b; [5] Reiser *et al*., 1994; [6] Dong *et al*., 2005; [7] Jacobs *et al*., 2004 ; [8] England and Fitch, (2002).

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. Following conclusions can be drawn based on this study:

1) This study demonstrates the stability and good reactor performance of a composite membrane (PDMS/PAN) bioreactor for treatment of toluene contaminated air. The bioreactor was inoculated with *Burkholderia*

vietnamiensis G4. 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 kg m^{-3} d^{-1} was observed, which is the highest degradation reported in the literature for similar loading rates to those used in the experiments.

2) In the beginning water condensation at the feed side was observed but after a period of 15 days it was no longer observed. It may be due to the development of biofilm growth on the dense side.
3) During period II, increasing the residence time from 11 to 28 s gives 99% removal efficiency at TOL LR of 1.2 kg m^{-3} d^{-1}.
4) Compared to other MBRWG for toluene removal present study shows that use of *Burkholderia vietnamiensis* G4 is a good option for the treatment of toluene.

6 ACKNOWLEDGEMENTS

The author acknowledges the Ghent University, Gent, Belgium for providing Special Research Grant (BOF) for doctoral research.

References

APHA. (1980) Standard methods for the examination of water and waste water, 15th ed.; American Public Health Association: Washington, DC.

Attaway, H., Gooding, C. and Schmidt, M. (2001) Biodegradation of BTEX vapors in a silicone membrane bioreactor system. *J. Ind. Microbiol. Biotechnol*. 26: 316-325.

Dong, K. and Jim, Heonki. (2005) Degradation of toluene vapor in a hydrophobic polyethylene hollow fiber membrane bioreactor with *Pseudomonas putida*. *Proc. Biochem*. 40: 2015-2020.

England, E. and Fitch, M. (2002) Heat transfer and toluene removal in bench-scale membrane bioreactors. Proceedings of the Air and Waste Management Association Conference, Maryland, United States, 23-27.

Ergas, S.J. and McGrath, M.S. (1997) Membane bioreactor for control of volatile organic compound emissions. *J. Environ. Eng*.-ASCE 123 (6): 593-598.

Ergas, S.J., Schroeder, E. D., Chang, D.P.Y. and Morton, R. (1995) Control of VOC emissions from a POTW using a compost biofilter. *Water Env. Fed.* 67: 816-821.

Ergas, S.J., Shumway, L, Fitch, M.W. and Neemann, J.J. (1999) Membrane process for biological treatment of contaminated gas streams. *Biotechnol. Bioeng*. 63 (4): 431-441.

Fitch, M., Neemann, J. and England, E. (2003) Mass transfer and benzene removal from air using latex rubber tubing and a hollow fiber membrane module. *Appl. Biochem. Biotechnol.* 104: 199-214.

Freitas dos Santos, L., Hommerich, U. and Livingston, A. (1995) Dichloroethane removal from gas stream by an extractive membrane reactor. *Biotechnol. Prog*. 11: 194-201.

Jacobs, P., De Bo, I., Demeestere, K., Verstraete, W. and Van Langenhove, H. (2004) Toluene removal from waste air using a flat composite membrane bioreactors. *Biotechnol. Bioeng*. 85: 68-77.

Kan E. and Deshusses M.A. (2005) Continuous operation of foamed emulsion bioreactor treating toluene vapors. *Biotechnol. Bioeng*. 92: 364-371.

Keskiner, Y. and Ergas, S. (2000) Hollow fiber membrane bioreactor for aqueous and gas phase ammonia removal by nitrification. *Hazard Ind. Wastes*. 32: 867-876.

Kumar, A., Dewulf, J. and Van Langenhove, H. (2007) Membrane based biological waste gas treatment. *Chem. Eng. J.* doi:10.1016/j.cej.2007.06.006 (in press).

Mutafov, S., Angelova, B., Schmauder, H.P., Avramova, T. and Boyadijieva, L. (2004) Stoichiometry of microbial continuous-flow purification of toluene contaminated air. *Appl. Microbiol. Biotechnol*. 65: 222-234.

O'Sullivan, L.A. and Mahenthiralingam, E. (2005) Biotechnological potential within the genus *Burkholderia*. *Lett. Appl. Microbiol.* 41: 8-11.

Parvatiyar M.G., Govind R. and Bishop D.F. (1996a) Biodegradation of toluene in a membrane biofilter. *J. Membr. Sci.* 115: 121-127.

Parvatiyar, M.G., Govind, R. and Bishop, D.F. (1996b) Treatment of trichloroethylene in a membrane biofilter. *Biotechnol. Bioeng*. 50: 57-64.

Reiser, M., Fischer, K. and Engesser, K.H. (1994) Kombination aus Biowascher-und Biomembranverfahren zur reinigung von Abluuft und hydrophilen und hydrofoben Inhaltsstoffen, VDI Berichte, 1104 103.

Sun, Y., Quan, Y., Chen, J., Yang, F., Xue, D. and Liu, Y. (2002) Toluene vapor degradation and microbial community in biofilter at various moisture content. *Process. Biochem*. 38: 109-113.

Van Langenhove, H., De Bo, I., Jacobs, P., Demeestere, K. and Dewulf, J. (2004) A membrane bioreactor for the removal of dimethyl sulphide and toluene from waste air. *Water Sci. Technol.* 50: 215-224.

Wu, G., Conti, B., Leroux, A., Brzeinski, R., Viel, G. and Hetiz, M. (1999) A high performance biofilter for VOC emission control. *J. Air Waste Manage. Assoc*. 49: 185-192.

Zilli, M., Del Borghi, A. and Converti, A. (2000) Toluene vapour removal in a laboratory-scale biofilter. *Appl. Microbiol. Biotechnol*. 54: 248-254.