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Research article

Toluene biodegradation in an algal-bacterial airlift photobioreactor: Influence of the biomass concentration and of the presence of an organic phase

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ABSTRACT

The potential of algal-bacterial symbiosis for off-gas abatement was investigated for the first time by comparatively evaluating the performance of a bacterial (CB) and an algal-bacterial (PB) airlift bioreactors during the treatment of a 6 g m^{-3} toluene laden air emission. The influence of biomass concentration and of the addition of a non-aqueous phase was also investigated. A poor and fluctuating performance was recorded during the initial stages of the experiment, which was attributed to the low biomass concentration present in both reactors and to the accumulation of toxic metabolites. In this sense, an increase in the dilution rate from 0.23 to 0.45 d^{-1} and in biomass concentration from ~1 to ~5 g L^{-1} resulted in elimination capacities (ECs) of 300 g m^{-3} h^{-1} (corresponding to removal efficiencies ~ 90%). Microalgae activity allowed for a reduction in the emitted CO_2 and an increase in dissolved O_2 concentration in the PB. However, excess biomass growth over 11 g L^{-1} hindered light penetration and severely decreased photosynthetic activity. The addition of silicone oil at 20% (on a volume basis) stabilized system performance, leading to dissolved O_2 concentrations of 7 mg L^{-1} and steady ECs of 320 g m^{-3} h^{-1} in the PB. The ECs here recorded were considerably higher than those previously reported in toluene-degrading bioreactors. Finally, microbial population analysis by DGGE-sequencing demonstrated the differential specialization of the microbial community in both reactors, likely resulting in different toluene degradation pathways and metabolites production.

1. Introduction

The amount of toluene used in the chemical industry has noticeably increased over the last decades, leading to a concomitant increase in the atmospheric emissions of this aromatic pollutant (EURAR-T, 2003; WHO, 2000). The technology selected for toluene removal depends on many factors such as toluene concentration, process conditions or economical aspects. Under specific situations, toluene recovery is economically feasible and physical-chemical technologies such as activated carbon are implemented. However, the application of physical-chemical technologies (adsorption, thermal incineration or UV oxidation) for toluene abatement commonly generates hazardous by-products and/or wastes and entails high investment and operating costs, particularly when treating diluted polluted emissions (Harding et al., 2003). In this context, bioremediation based on bacterial or fungal activity has arisen as a cost-effective and environmentally sustainable alternative to conventional physical-chemical methods.

In particular, pneumatically agitated suspended-growth systems such as airlift bioreactors have demonstrated a cost-effective toluene abatement performance while avoiding typical operational problems encountered in packed-bed bioreactors: excessive biomass growth, flooding, channeling, pressure drop build-up or formation of anaerobic or dry zones (Vergara-Fernández et al., 2008). Nevertheless, airlift bioreactors for the treatment of toluene still face severe limitations such as (i) a limited pollutant mass transfer from the gas to the liquid phase due to its high Henry constant (Henry's law constant at 25 °C (H) = C_{aq} C_g^{-1} = 3.7, where C_{aq} and C_g are the concentration in the aqueous and gas phase, respectively) (Sander, 2014), which results in a low pollutant bioavailability, (ii) microbial inhibition at high toluene inlet concentrations, and (iii) O_2 limitation when treating high toluene loads. An enhanced oxygen supply to the microbial community in the reactor by increasing the turbulence of the culture broth might

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Abbreviation

C_{aq}	Concentration in the aqueous phase, g L^{-1}
C_g	Concentration in the gas phase, g L^{-1}
CB	Bacterial airlift bioreactor, –
DO	Dissolved oxygen concentration, mg L^{-1}
EC	Elimination capacity, g m^{-3} h^{-1}
H	Henry's law constant, dimensionless
MSM	Mineral salt medium, –
NAP	Non-aqueous phase, –
PB	Algal-bacterial airlift bioreactor, –
PCR	Polymerase chain reaction, –
RE	Removal efficiency, %
TN	Total nitrogen concentration, mg L^{-1}
TOC	Total organic carbon concentration, mg L^{-1}
TSS	Total suspended solids, g L^{-1}
VOC	Volatile organic compound, –

be problematic since intensive mechanical aeration in bioreactors is highly costly and might cause volatilization and re-dispersion of the toluene present in the aqueous phase (Muñoz et al., 2005).

Photosynthetic oxygenation in algal-bacterial photobioreactors constitutes an efficient alternative to conventional aeration methods. In this process, the oxygen photosynthetically produced by microalgae in the presence of light and CO_2 is used by heterotrophic bacteria to *in situ* oxidize the organic pollutant, producing in return the CO_2 needed for microalgae photosynthesis. Unlike bacterial processes where the mineralization of organic pollutants releases CO_2 and H_2O as main end-products, microalgal processes are able to fix and recover carbon and other nutrients as valuable biomass besides furnishing O_2 to the heterotrophic community. Moreover, some microalgae are capable of biotransforming xenobiotic organic contaminants (Muñoz and Guieysse, 2006; Semple et al., 1999). Therefore, microalgae may boost the biodegradation of toluene either by directly biotransforming the pollutant or by enhancing the degradation potential of the microbial community present in the bioreactor. Besides, additional O_2 supply by photosynthetic activity might prevent oxygen limitation and accelerate the bacterial degradation of toluene (Semple et al., 1999). Up to date, most studies on toluene biodegradation were based on the activity of bacteria and fungi. On the contrary, microalgae-supported biodegradation of aromatic contaminants has been scarcely investigated, and the catabolic pathways of biodegradation of aromatic compounds in microalgae are still largely unknown. Thus, despite the potential benefits of microalgae-based off-gas treatment, there is no single study comparatively evaluating the performance of algal-bacterial and bacterial bioreactors for the treatment of volatile organic compounds (VOCs).

On the other hand, the limited mass transfer of toluene to the liquid phase might be overcome by the addition to the bioreactor of a non-aqueous phase (NAP) with high affinity for this pollutant, resulting in a so-called two-liquid phase partitioning bioreactor. Two-phase bioreactors have been successfully applied to treat both high and low VOC-laden gas emissions, improving the performance of biological processes (Lebrero et al., 2013; Muñoz et al., 2012). The NAP not only supports an enhanced mass transfer of the target pollutant and O_2 from the gas phase to the microorganisms, but also acts as a buffer against surges in pollutant or metabolite concentrations that might be potentially toxic to the microbial community (Lebrero et al., 2015).

This work was devised to comparatively evaluate a bacterial and an algal-bacterial airlift bioreactor treating toluene at a high loading rate. For this purpose, two identical airlift bioreactors were operated under dark and light conditions, respectively, and continuously fed with a toluene-laden air stream. The influence of biomass concentration on the toluene removal performance was investigated by implementing an operating strategy based on decoupling the hydraulic retention time from the solids retention time. Moreover, the inhibition resulting from metabolites accumulation was assessed and controlled by modifying the liquid dilution rate. The influence of the addition of a non-aqueous phase in order to overcome mass transfer limitations was also studied in the last operating stage. Finally, the differential specialization of the microbial communities in both bioreactors was determined by molecular techniques.

2. Materials and methods

2.1. Microorganisms and culture conditions

Both bioreactors were inoculated with a mixture of *Chlorella sorokiniana* (0.5 L), activated sludge from Valladolid wastewater treatment plant (0.1 L) and a toluene acclimated *Pseudomonas putida* DSM- 6899 culture (0.35 L) (DSMZ, Leibniz-Institut, Germany) to an initial total suspended solids concentration (TSS) of 0.79 g L^{-1}. The mineral salt medium (MSM) used throughout the entire experiment was composed of (g L^{-1}): KNO_3 1.25, $CaCl_2 \cdot H_2O$ 0.1105, H_3BO_3 0.1142, $FeSO_4 \cdot 7H_2O$ 0.0498, $ZnSO_4 \cdot 7H_2O$ 0.0882, $MnCl_2 \cdot 4H_2O$ 0.0144, MoO_3 0.0071, $CuCl_2 \cdot 2H_2O$ 0.0157, $CoCl_2.2H_2O$ 0.0049, EDTA ($C_{10}H_{16}N_2O_8$) 0.5, KH_2PO_4 0.6247 and K_2HPO_4 1.3251.

2.2. Experimental set-up

The experimental plant consisted of two identical internal-loop airlift glass bioreactors with a total volume of 2.5 L and a working volume of 2.2 L, operated in parallel in an air-conditioned room at a constant temperature of 25 °C (Fig. 1). The inner tube had a diameter of 0.055 m and a height of 0.295 m, while the external cylinder diameter and height were 0.09 m and 0.42 m, respectively. A porous metallic diffuser, with an average pore diameter of 2 μm, was placed at the bottom of the inner tube to promote gas distribution. The air-lift photobioreactor (PB) was continuously illuminated with LED lamps arranged concentrically at an average intensity of 330.5 μmol m^{-2} s^{-1} (within the optimum range for the photosynthetic apparatus of microalgae, Muñoz and Guieysse, 2006), while the control air-lift (CB) was covered with an opaque structure to prevent diffuse light penetration.

The toluene-contaminated stream was obtained by sparging ambient air (flow controller, Aalborg, USA) in a reservoir containing liquid toluene kept at a constant temperature of 25 ± 2 °C. The toluene-laden stream was then diluted with ambient air in a mixing chamber and subsequently divided into two identical streams (flow controller, Aalborg, USA) to feed both air-lift reactors. The toluene inlet concentration was maintained at 6.2 ± 0.7 g m^{-3} and the reactors were operated at an empty bed residence time of ~1.1 min (corresponding to an inlet load of 369 ± 45 g m^{-3} h^{-1}).

2.3. Operational procedure

During the first 6 days of operation, 0.5 L of the culture broth were daily removed from each bioreactor and replaced by fresh MSM. From day 6–26, the exchange of culture medium was increased to 1 L in order to avoid metabolite accumulation, which resulted in low TSS concentrations in both reactors. Therefore, 0.5 L of the 1 L culture broth daily replaced were centrifuged and the biomass returned to the corresponding reactor from day 26 on. This strategy allowed controlling the biomass concentration and