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Removal and destruction of high concentrations of gaseous toluene in a two-phase partitioning bioreactor by *Alcaligenes xylosoxidans*

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Abstract

A two-phase bioreactor consisting of hexadecane dispersed in an aqueous, cell-containing medium (organic fraction = 0.33) was used to trap toluene vapours from an air stream. The affinity for toluene by the solvent resulted in high efficiency of removal and transfer to the aqueous phase based on equilibrium transfer. The system was readily able to handle a loading capacity of 748 mg l^{-1} h^{-1} at a toluene degradation efficiency of greater than 98%.

Introduction

The removal and destruction of toxic volatile, organic compounds (VOCs) from air streams is important for safety as well as environmental reasons. Chemical treatment methods such as thermal oxidation and incineration are often too expensive to treat emissions from high volume, low concentration sources (Deshusses *et al.* 1999, Abumaizar *et al.* 1997). Cheaper biological solutions have been sought over the past decade, and biofiltration has emerged as a primary method to treat high volume/low concentration emissions in a cost-effective manner (Deshusses 1997).

Biofilters are tubular reactors filled with porous and inert packing materials onto which microbial populations develop as they degrade VOCs from applied air streams. Although biofilters are often very effective, particularly at low applied (< 5 mg l^{-1}) VOC concentrations, they can suffer from a variety of operational difficulties such as biomass overgrowth (leading to excessive pressure drops and channelling), are sensitive to surges in VOC loadings, and because of their plug flow nature, generally do not make effective use of the entire reactor volume since as little as 4% of the total bacterial population (i.e. at the inlet) may be responsible for as much as 65% of pollutant degradation (Deshusses 1997, McNevin & Barford 2000).

As an alternative, two-phase partitioning bioreactors (TPPBs) have been used in conjunction with an absorption column containing an organic solvent to scrub benzene from an air stream. The organic solvent in the scrubber trapped the benzene and was circulated to the TPPB, where benzene was transferred from the immiscible solvent to the cells in the aqueous phase (Yeom *et al.* 2001, Yeom & Daugulis 2001a) and the regenerated solvent was recirculated back to the absorber. More recently, via process compression, a preliminary demonstration has been provided for the removal of benzene from a gas stream directly by the liquid contents of a TPPB without an absorption column (Davidson & Daugulis 2003) by a simplified single-stage configuration.

In the present work, we show that toluene can be removed in a single stage TPPB system (i.e. the solvent in the TPPB acts as a scrubber) at high VOC loadings and removal efficiencies. The maximum toluene elimination capacity of such a system, although not reached in this study, appears to be much higher than is possible in biofilters.

Materials and methods

Organism, media, cultivation conditions, and solvent selection

Alcaligenes xylosoxidans was grown as previously described (Yeom & Daugulis 2001b), and hexadecane, the scrubbing/delivery solvent, was selected as described in earlier work (Yeom & Daugulis 2001a).

Reactor setup

A New Brunswick Scientific BioFlo III bioreactor with 2 l aqueous medium and 1 l hexadecane was used in this work (organic fraction = 0.33). During the fermentation, the reactor was controlled at 30 °C, pH 6.6, with an agitation speed of 800 rpm, creating a complete dispersion between the aqueous and organic phases. The air/toluene delivery system consisted of an Erlenmeyer flask containing 2 l of toluene and maintained at 30 °C, through which compressed air was blown, and sent to the sparger in the bioreactor. Additional O_2 was provided to the cells via aeration, also through the sparger. The system was operated to maintain the cell concentration within a band of approximately 1.5–5 g l^{-1} by periodic medium exchanges in which about half the reactor volume was removed by a peristaltic pump, and replaced with fresh medium and solvent in the original phase ratio (2:1 v/v).

Sampling and analysis

Mixed liquid samples were periodically withdrawn and centrifuged to separate the cells, aqueous phase and organic phase. Organic liquid samples were then injected into an FID GC (DB Wax Column) and the remaining liquid suctioned off. The biomass was then washed with water, re-suspended, and the turbidity measured at 600 nm. Inlet and outlet gas samples were taken at the same time as liquid samples through use of a gas tight 250 μl syringe, and analyzed by GC.

Results and discussion

Figure 1 shows the inlet and outlet gas, as well as the organic phase toluene concentrations. Note that the cells had previously been grown in batch culture (data not shown) to a concentration of 2.7 g l^{-1} by adding liquid toluene directly to the solvent phase before VOC addition at time zero. Figure 2 shows the frequency

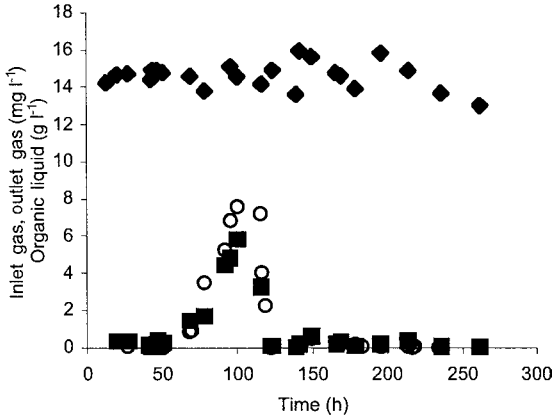

Fig. 1. Mixed inlet gas toluene concentration (diamonds), outlet gas toluene concentration (squares) and organic phase toluene concentration (circles) during long-term operation of the single-stage TPPB system.

and impact of medium exchanges undertaken with the intention of keeping the cell concentration within the desired cell concentration band. This experiment (Figures 1 and 2) was operated in order to: provide an initial indication of the stable performance characteristics of the system (for circa 48 h), followed by an imposed upset of the system (pH shock) for circa 48 h to investigate the extent of performance deterioration as well as rapidity of recovery, and ending with a return to stable operation.

At all times, toluene was delivered to the TPPB at a gas flow-rate of 33 l h^{-1}, and a toluene concentration of 68 mg l^{-1}. Aeration was provided separately at a rate of 120 l h^{-1}, and thus the 'mixed' toluene concentration could also be calculated as 14.6 mg l^{-1}, and the total gas flow as 153 l h^{-1}. Note that the aeration rate is an independently controllable variable, and could have been adjusted to a lower rate (as long as oxygen did not become limiting) which would have increased the 'mixed' inlet toluene concentration. In any event, the toluene loading was 748 mg l^{-1} h^{-1} (based on the 3 l working volume) and the response of the system to this loading was extremely rapid and stable (Figure 1), with exit gas toluene concentrations being decreased to less than 0.4 mg l^{-1}, and organic toluene concentrations below 150 mg l^{-1} within a few hours of initiating VOC addition. The response dynamics of this system appear to be substantially faster than those observed for biofilter systems, whose performance is hindered by multiple diffusional paths and plug flow behaviour. The imposed pH shock (setting the pH to 4.4 from 6.6, and returning it to 6.6 after circa 48 h), as expected, resulted in an increase in the organic phase toluene

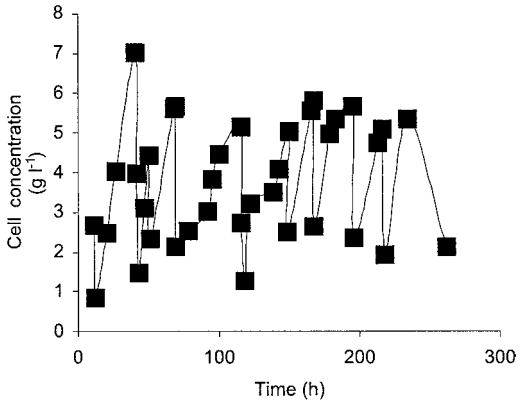

Fig. 2. Cell concentration during TPPB operation. Increases are due to cell growth on toluene, and decreases are due to medium exchanges to maintain the cells in the concentration band of circa 1.5–5 g l^{-1}.

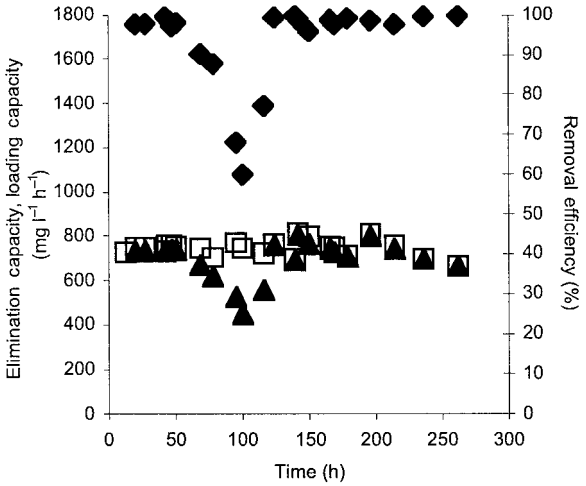

Fig. 3. Toluene loading (squares), elimination capacity (triangles) and removal efficiency (diamonds) during TPPB operation.

concentration (to about 7500 mg l^{-1}) and exit gas concentration (to circa 5.8 mg l^{-1}). However, the system very quickly recovered once the higher pH had been restored, to essentially the same stable performance values of exit gas and organic phase toluene concentrations. From Figure 2 it can be seen that the cell growth during this upset period was slowed, but not halted.

Figure 3 shows the toluene loading, elimination capacity, and treatment efficiency over the course of 262 h of operation. Excluding the imposed upset, the treatment efficiency was 99% at a toluene loading of 748 mg l^{-1} h^{-1}, and represents a substantial improvement over previous performance of biofilters on toluene (258 mg l^{-1} h^{-1} at 95% removal by Auria *et al.* 2001).

This very strong performance by the TPPB system can be attributed to several factors. First, the solvent has a very high affinity for toluene (in fact toluene is infinitely soluble in hexadecane) providing a very effective means of VOC capture. This is in contrast to biofilters which often have difficulty absorbing hydrophobic VOCs by the small water layers associated with the packing and microbial film layers. Second, the solvent provides a 'sink' for the VOC which can substantially act as a buffer (because of the high partition coefficient of toluene between hexadecane and water) to accommodate higher VOC loadings as they may occur, and also deliver VOCs already contained in the organic phase even during periods of low VOC application. Third, the system is completely mixed, allowing the total solvent volume to scrub the VOC, and the total reactor volume (i.e. all of the cells in the reactor) to be effective in degradation. Again, this is in contrast to biofilters that often to do not equally and effectively use their entire reactor volume for VOC removal and degradation. Moreover, the very rapid recovery from the imposed system upset is a further reflection of a reactor that is not spatially segregated.

Estimates of cell yields and specific substrate utilization rates (mg toluene per g cells per h) were also made. Without consideration of the upset period, a cell yield of 0.186 g g^{-1} was calculated, which is consistent with previous work in our laboratory with this organism growing on toluene. The specific toluene utilization rate was more difficult to calculate due to the dynamic nature of the value of the cell concentration. Nevertheless, we feel that the most meaningful estimate is the one determined at the *minimum* cell concentration; that is, the value which gives the *largest* value for specific substrate utilization rate. Accordingly, by taking the average of the cell concentrations at the minimum points in Figure 2 over the 8 non-upset periods (1.95 g cells per l), and a substrate feeding rate of 748 mg l^{-1} h^{-1}, a specific toluene utilization rate of 384 mg toluene g^{-1} cells h^{-1} is obtained. The minimum average cell concentration readily handled the applied toluene load (i.e. 1.96 g l^{-1} cells were able to degrade 748 mg toluene l^{-1} h^{-1}), and therefore higher cell concentrations would also be able to consume the same feeding rate (as can be seen in Figure 2). Accordingly, the *maximum* specific substrate utilization rate of *Alcaligenes xylosoxidans* is *at least* 384 mg toluene g^{-1} cells h^{-1}, which suggests that the loading to the system could be even higher than the one used, while maintaining near-complete substrate utilization. The fact that all of the cells are involved

in toluene degradation (by virtue of the system being well-mixed), and because of the relative ease of operating at cell concentrations significantly higher than 1.95 g l^{-1} (Figure 2), it is apparent that significantly higher loadings could be used, the value determined by the product of the cell concentration and the maximum substrate utilization rate. It is possible that some other factor could begin to limit the process (e.g. oxygen), although during the course of the reported experiment, the dissolved oxygen concentration did not fall below 60% of saturation.

Conclusion

This work has shown that single-stage TPPBs can be used to remove and degrade high concentrations of VOCs from air streams at extremely high loadings and high efficiencies. The effectiveness of these processes is due largely to the presence of the solvent which can act to buffer the system from substrate surges, and periods of low substrate addition, and by the fact that the system is well-mixed. Significantly, this latter feature results in the entire reactor volumes being used for both absorption of the VOC (organic phase) as well as for its degradation (aqueous phase), a situation that is not true for biofilters. The substrate loading to these TPPB systems will be determined (assuming no other limitations) by the maximum substrate utilization rate (which, in this application, is greater than 384 mg toluene g^{-1} cells h^{-1}), and the cell concentration in the system, which can readily be maintained at levels of several g l^{-1} (i.e. at 5 g l^{-1} cells the elimination capacity should be *at least* about 2000 mg l^{-1} h^{-1}).

We are currently examining the effects of varying solvent phase ratios, and mixtures of VOCs in the feed on TPPB performance, as well as seeking to determine the maximum specific substrate utilization rate of this organism in order to operate at maximum loading.

Acknowledgement

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