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Addressing biofilter limitations: A two-phase partitioning bioreactor process for the treatment of benzene and toluene contaminated gas streams

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Abstract

A two-phase partitioning bioreactor (TPPB) achieved simultaneous and continuous removal and degradation of benzene and toluene from an air stream. The aqueous- organic system utilized n-hexadecane as the organic phase, and the organism *Alcaligenes xylosoxidans* Y234 in the aqueous phase to achieve the degradation of benzene and toluene. The system, which operates as a well-mixed dispersion and is therefore resistant to substrate surges, was first shown to be capable of utilizing toluene while operating at a loading capacity of 235 g m^{-3} h^{-1} with an elimination capacity of 233 g m^{-3} h^{-1}. It was also determined that to characterize TPPB performance in terms of elimination capacity the definition of elimination capacity must be extended to include the cell mass present, a readily controllable variable given the nature of the system. Based on this criterion, it was estimated that for a cell concentration of 1 g l^{-1} present in the TPPB, the potential maximum toluene elimination capacity is 1290 g m^{-3} h^{-1} which is substantially higher than any toluene elimination capacity achieved by biofilters at a high removal efficiency. If no other factor were to limit the system, elimination capacities could be many times higher still, and are dependent on maintaining desired cell concentrations above 1 g l^{-1}. The TPPB was then operated at nominal loading capacities of 63 g m^{-3} h^{-1} (benzene) and 51 g m^{-3} h^{-1} (toluene) at a removal efficiency greater than 99% to demonstrated the applicability of this system in dealing with two chemical species simultaneously. TPPB systems therefore have been shown to be effective at removing gaseous organic contaminants at high removal efficiencies while also possessing desirable operating features, such as providing and maintaining high cell concentrations throughout the reactor, and a capacity to effectively deal with high contaminant loadings.

Introduction

Traditional treatment methods for air pollution such as thermal oxidation and incineration are often too expensive to treat emissions from high volume, low concentration sources when trying to meet the demands of current air quality regulations (Deshusses et al. 1999; Abumaizar et al. 1997). Cheaper biological solutions have been sought over the past decade and biofiltration has emerged as a prime candidate to treat these high volume/low concentration emissions of VOCs (volatile organic compounds) in a cost-effective manner. As has recently been noted, however (Deshusses et al. 1999), this technology can be fraught with operational difficulties such as bed plugging or poisoning, packing acidification, nutrient shortages, etc., and has low performance potential especially when applied to compounds with high Henry's Law coefficients, since resulting concentrations in the biofilm are too low to sustain a high degradation rate (Deshusses & Johnson 2000).

The application of Two-Phase Partitioning Bioreactors (TPPBs) to the treatment of VOCs is a technology that inherently has fewer problems while being able to potentially exceed the performance of biofilters. In a TPPB an immiscible organic phase acts as a

reservoir for toxic substrates that are delivered to the cell-containing aqueous phase at concentrations determined by equilibrium partitioning. TPPB research has been applied primarily to the treatment of liquid phase pollutants (Ortega-Calvo et al. 1995; Collins & Daugulis 1996; 1997a, b; 1999a, b; Munro & Daugulis 1997; Marcoux et al. 2000; Guieysse et al. 2001; Janikowski et al. 2002) but recent work (Yeom et al. 2000; Yeom & Daugulis 2001) has utilized a TPPB as part of a system to treat gas phase benzene. In these reports a TPPB was used in conjunction with an absorption column containing an organic solvent to scrub the gas stream of the pollutant. The organic solvent in the scrubber trapped the benzene and was circulated to the TPPB where benzene was transferred from the solvent to the cells in the aqueous phase.

More recently, via process compression (i.e. a reduction in processing units from one to two, with a concomitant decrease in ancillary equipment requirements, e.g. pumps), 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 current work this process concept has been extended to the removal of toluene, and estimates of the maximum system elimination capacity were made. This was followed by the application of the system to a gas stream containing a mixture of benzene and toluene to demonstrate that multiple substrates could also be dealt with using this configuration.

Materials and methods

Organism and growth conditions

Alcaligenes xylosoxidans Y234 was obtained from Dr. S.H. Yeom, Department of Chemical Engineering, Seoul National University. It is capable of degrading benzene, toluene and xylene as sole substrates (Yeom 1998). Stock cultures were maintained on agar plates with 20 g/l Bacto-Agar along with the following nutrient concentrations (per liter): 7 g $(NH_4)_2SO_4$, 0.75 g $MgSO_4{\cdot}7H_2$, 6.6 g K_2HPO_4, 8.42 g KH_2PO_4, 2 g sodium benzoate and 0.08 ml of a trace element solution containing (per liter) 16.2 g $FeCl_3{\cdot}6H_2O$, 9.44 g $CaHPO_4$, 0.15 g $CuSO_45H_2O$ and 40 g citric acid.

Liquid medium for innoculum purposes was prepared with the same nutrient compositions as the agar plates. Six 125 Erlenmeyer flasks with 50 ml medium were inoculated with Y234 and were grown on a gyratory shaker at 30 °C at 150 rpm for 24 h prior to reactor innoculation. A 24 h period of batch fermentation with liquid toluene (two 6 ml aliquots) added to the organic phase (n-hexadecane, selected as described in Yeom & Daugulis 2001)) took place prior to the continuous gas feed mode of operation so that a substantial concentration of cells would be present.

Chemicals

The benzene, toluene and all salts used in the medium formulation were obtained from Fisher Scientific, Ottawa, ON. The n-hexadecane was obtained from Alfa Aesar of Ward Hill MA, USA.

Reactor setup

The TPPB system is comprised of an aqueous phase growth medium and an immiscible and biocompatible organic solvent that are intimately contacted by agitation and aeration, to the point of being a complete dispersion. A full description of the TPPB arrangement, along with a detailed diagram, are shown in Daugulis (2001), and a schematic of the two-stage absorption column/TPPB system used previously are in Yeom and Daugulis (2001). Continuous gas feed fermentation was carried out in a 2 l New Brunswick Scientific BioFlo bioreactor. The bioreactor was maintained at 30 °C and automatically controlled to pH of 6.6 with 2M KOH. Dissolved oxygen was monitored with a galvanic oxygen electrode. To maintain the reactor contents as a well-mixed dispersion, the reactor was agitated at 800 rpm, and the oxygen requirements of the cells were provide by separate aeration to the reactor via the hollow stirrer shaft, and integrated aeration holes below the impellors. The selected solvent, n-hexadecane (Yeom & Daugulis 2001) was added as a 340 ml aliquot, and the aqueous volume was maintained at approximately 660 ml for a total liquid volume of 1 l.

Nutrients were periodically supplied in a concentrated 50 ml feeding containing the following amounts of nutrients at the beginning of the fermentation and at each medium exchange (described below): 7 g $(NH_4)_2SO_4$, 0.75 g $MgSO_4{\cdot}7H_2O$, 2 g KH_2PO_4 and 0.08 ml of the trace element solution.

Controlled delivery of benzene and toluene gas was achieved by passing compressed air through the headspace of a sealed 2 l flask containing 1.5 l toluene, or 1.5 l toluene and benzene. The flask was maintained at the desired temperature (30 °C) by a water bath

and gas was delivered to the bottom of the bioreactor through an open glass tube. Keeping the temperature of the flask above ambient allowed more VOC to be volatilized for a given gas flowrate. During experiments treating benzene and toluene simultaneously, the flask was maintained at 40 °C with 1180 ml of toluene and 320 ml of benzene. This ratio and temperature were selected to deliver approximately equal amounts of each VOC to the bioreactor at loading capacities of 63 g m^{-3} h^{-1} of benzene and 51 g m^{-3} h^{-1} of toluene. Note that volumetric rates here, and throughout, refer to the total liquid volume in the reactor of 1 l.

Treatment of a gas stream contaminated with toluene

The bioreactor was prepared as described above and operated with a loading capacity of 235 g m^{-3} h^{-1} for 60 hours. The toluene gas flow was 1.58 l h^{-1}, and the air flow providing aeration was 24.6 l h^{-1}. Cell concentration was controlled within a concentration band of approximately between 1 and 3 g l^{-1} by periodic medium and solvent exchanges that replaced about half the reactor liquid contents with fresh n-hexadecane (170 ml), water (300 ml) and a 50 ml nutrient feeding.

Determination of maximum system performance

The bioreactor was prepared as described above and operated with a loading capacity of 230 g m^{-3} h^{-1} and allowed to reach stable operation. The cell concentration was then reduced through 4 serial medium exchanges to the point where it could no longer consume the mass load of benzene. This was followed by an accumulation of toluene in the organic phase, and a subsequent decrease in organic phase concentration once the bacterial population had grown to a sufficiently high level to degrade the imposed loading capacity. The cell concentration at the point of inflection from increasing to decreasing toluene concentration was determined for this loading capacity and used to calculate the specific rate of toluene consumption (i.e. the mass of toluene consumed per mass of cells per hour). This experimental protocol was repeated twice.

Treatment of a gas stream contaminated with benzene and toluene

The TPPB system was prepared as described above and the bacteria were grown under batch conditions on toluene prior to the introduction of the benzene and toluene gas stream. The gas stream was supplied at a flow rate of 0.6 l h^{-1} with benzene and toluene concentrations of 105 g m^{-3} and 84.5 g m^{-3}, and oxygen was supplied via an airflow of 60 l h^{-1}. Cell concentration was controlled as described above with periodic medium and solvent exchanges.

Analytical and sampling procedures

Organic phase and gas phase benzene concentrations were quantified directly using a Varian 3400 gas chromatograph with an FID detector and a DB Wax column with peak integration performed by the Waters Millenium[32] software package. Concentrations of benzene in the aqueous phase were determined by mass balance based on the organic phase concentration and the n-hexadecane/water distribution coefficient. Since the two liquid phases in TPPBs are in equilibrium as has been previously demonstrated (Vrionis et al. 2002) this indirect method of estimating aqueous phase concentrations is valid.

Gas samples of 100 μl were directly injected into the G.C. Liquid samples from the bioreactor were 8–10 ml and were spun down at 3400 rpm and 4 °C for 45 s to separate the phases. The organic phase was directly sampled from the top of the centrifuge tube (0.2 μl volumes were injected into the GC) and then aspirated to leave only the aqueous phase. The cells were washed to remove any remaining solvent and salts. The original aqueous volume was restored with fresh water and the optical density determined with a Biochrom Ultrospec 3000 UV/Visible Spectrophotometer. The actual cell concentration (g l^{-1}) was then determined from a previously prepared calibration curve relating optical density to cell dry weight.

Results

Continuous treatment of a toluene gas stream

The toluene delivery system supplied a gas stream with an average concentration of 152 g m^{-3} at a flow rate of 1.58 l h^{-1} that produced a loading capacity (normalized to total liquid volume) of 235 g m^{-3} h^{-1}. Additional air supplied to the reactor was controlled at a flow of 24.6 l h^{-1}. If the total mass load of toluene were considered relative to the total airflow rate then the inlet concentration could be considered to be 9.2 g m^{-3}. This hypothetical 'mixed' inlet concentration along with the actual inlet and outlet

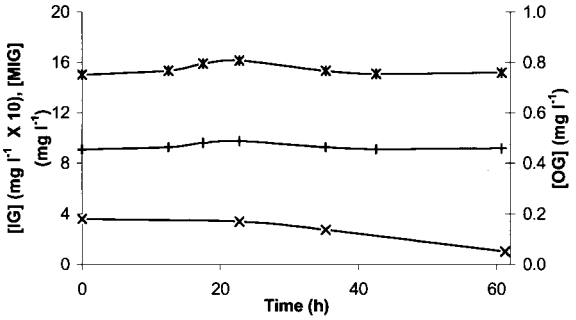

Figure 1. Gas phase concentrations during delivery of toluene at a loading capacity of 235 g m^{-3} h^{-1}. *, inlet gas concentration [IG]; +, mixed inlet gas concentration [MIG]; ×, outlet gas concentration [OG].

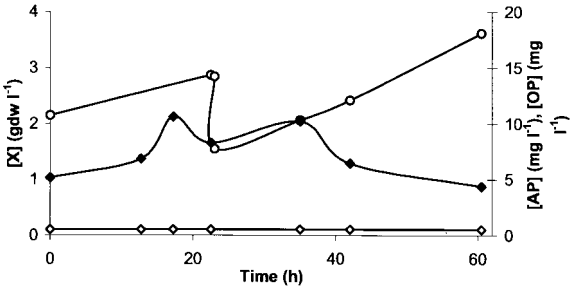

Figure 2. Liquid phase concentrations during delivery of toluene at a loading capacity of 235 g m^{-3} h^{-1}. ◇, aqueous phase concentration [AP]; ○, cell concentration [X]; ♦, organic phase concentration [OP], DO > 60% at all times.

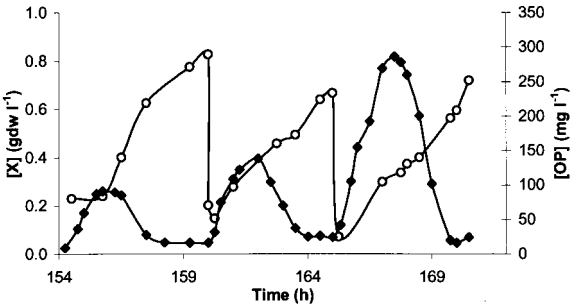

Figure 3. Toluene and biomass concentrations during system perturbation caused by biomass removal at a toluene loading capacity of 235 g m^{-3} h^{-1}. ○, cell concentration [X]; ♦, organic phase concentration [OP].

stream concentrations is shown in Figure 1. The system achieved a stable average removal efficiency of 99% and an elimination capacity of 233 g m^{-3} h^{-1}. The elimination capacity is the product of the removal efficiency and the loading capacity and is an expression of the mass of toluene degraded per unit volume of the reactor per hour.

Figure 2 shows the toluene concentration in the organic and aqueous phases as well as the cell concentration. The organic phase concentration remained low throughout the fermentation and fluctuated between 5 and 10 mg l^{-1} (note that these values are consistent with outlet gas concentration and the equilibrium relationship between these 2 phases). The aqueous phase concentration, also quite low, remained less than 0.6 mg l^{-1} throughout the fermentation. The cell concentration was controlled between 1 and 3 g l^{-1} by a medium exchange at 24 h. From the data shown in Figure 2 a cell yield of 0.17 g g^{-1} was calculated. Microbial yields are notoriously difficult to determine in TPPB systems due to the presence of an organic phase that often attracts bacterial cells, and this value is lower than that (0.24 g g^{-1}) estimated by Yeom (1998) for a single phase system. Oxygen limitation did not occur, as the measured dissolved oxygen concentration never decreased below 60% of saturation.

Determination of maximum system performance

To determine the maximum toluene elimination capacity, estimates were made of the exact mass of cells required to handle a loading capacity of 230 g m^{-3} h^{-1}. After several hours of operation to stabilize the delivery of toluene at 230 g m^{-3} h^{-1} (data not shown) at a high removal efficiency, cells were removed through several sequential medium exchanges to the point where there were no longer enough to handle the toluene load to the system. Figure 3 shows the results of this deliberate system upset caused by removal of bacteria, and recovery of the system as the bacteria grew back to a concentration that could degrade the imposed toluene load. The cell concentration present at each inflection point in the organic phase (i.e., from increasing to decreasing concentration) was measured and used to calculate the specific toluene utilization rate for the bacteria. Cell concentrations of 0.24, 0.27, and 0.32 g l^{-1} were measured and an average toluene utilization rate of 1.29 g g^{-1} h^{-1} was calculated. This value is very close to the value of 1.32 g g^{-1} h^{-1} determined by Yeom (1998), who had originally isolated and characterized this organism. A value of 1.29 g g^{-1} h^{-1} suggests that the system could theoretically operate at an elimination capacity of 1290 g m^{-3} h^{-1} with a cell concentration of 1 g l^{-1}.

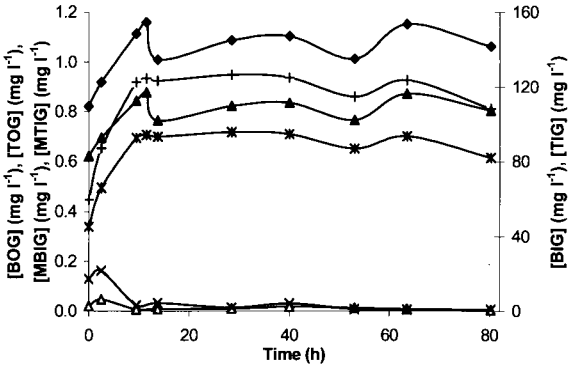

Figure 4. Gas phase concentrations during operation with loading capacities of 54 g m^{-3} h^{-1} benzene and 43 g m^{-3} h^{-1} toluene. ▲, benzene inlet gas concentration [BIG]; ♦, benzene mixed inlet gas concentration [MBIG]; △, benzene outlet gas concentration [BOG]; *, toluene inlet gas concentration [TIG]; +, toluene mixed inlet gas concentration [MTIG]; ×, toluene outlet gas concentration [TOG].

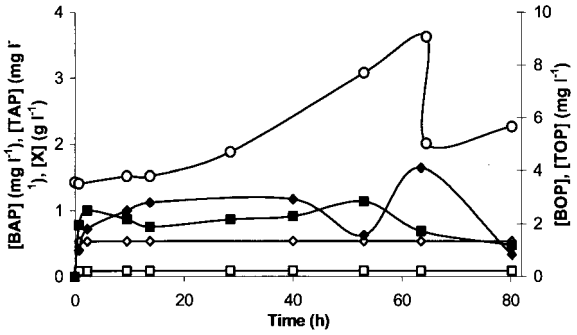

Figure 5. Liquid phase concentrations during operation with loading capacities of 54 g m^{-3} h^{-1} benzene and 43 g m^{-3} h^{-1} toluene. □, aqueous phase benzene concentration [BAP]]; ◇, aqueous phase toluene concentration [TAP]; ○, cell concentration [X]; ■, organic phase benzene concentration [BOP]; ♦, organic phase toluene concentration [TOP]; DO > 60% at all times.

Treatment of a gas stream contaminated with benzene and toluene

The gas stream was supplied at a flow rate of 0.6 l h^{-1} with benzene and toluene concentrations of 105 g m^{-3} and 84.5 g m^{-3}, respectively. The air stream used to provide oxygenation for the reactor had a flow rate of 60 l h^{-1} resulting in 'mixed' inlet gas stream concentration of 1 g m^{-3} and 0.84 g m^{-3} for benzene and toluene, respectively. Figure 4 shows the inlet, outlet and 'mixed' gas stream concentrations during the experiment.

At this flow rate and concentration, benzene was delivered at a loading capacity of 63 g m^{-3} h^{-1} and toluene at 51 g m^{-3} h^{-1}. The system was able to maintain removal efficiencies near 100% for both chemical species with elimination capacities of 62.4 g m^{-3} h^{-1} for benzene and 47.9 g m^{-3} h^{-1} for toluene. The benzene removal efficiency was maintained at 99% throughout the fermentation, and at 94% for toluene (98% if the first 3 hours are excluded).

During the 80 h of the fermentation, cell concentrations were controlled in a concentration band between 2 and 4 g l^{-1} by medium exchange. In Figure 5 the cell growth trends are displayed along with the organic and aqueous phase substrate concentrations. Similar trends are seen here as were observed during the treatment of the toluene contaminated gas stream shown in Figure 2, and by Davidson and Daugulis (2003) during the removal and degradation of benzene from a gas stream. The organic phase concentrations exhibited minor fluctuations between 1 and 3 mg l^{-1} and the aqueous phase concentrations remained below 0.5 mg l^{-1} throughout the fermentation.

Discussion

The inlet gas concentration of 152 g m^{-3} (Figure 1) for this continuous treatment process is 25 times greater than any treated by biofilters, and even the 'mixed' gas stream concentration of 9.2 g m^{-3} is substantial compared to the highest concentrations being dealt with by biofilters: 1–6 g m^{-3} (Auria et al. 2001; Cox et al. 2000; Deshusses & Johnson 2000; Matteau & Ramsay 1997). The additional aeration was provided to ensure that the system was not oxygen limited, in order to allow us to determine the inherent (i.e. non-oxygen limited) performance of the TPPB. Moreover, the air stream providing oxygen to the cells was supplied separately to the system, and was able to maintain dissolved oxygen levels above 60% of saturation at all times. Obviously, this is an independently controllable operating feature of TPPBs being used to degrade VOCs, and by reducing the air flow (while still maintaining dissolved oxygen above, say, 20% saturation) a higher mixed VOC concentration than 9.2 g m^{-3} would be calculated. In order for biofilters ***or*** TPPBs not to be oxygen limited essentially the same amount of aeration (and indeed all non-carbon nutrients) will need to be provided to both systems, as the cells will need the equivalent amounts of stoichiometric oxygen to provide the same amount of VOC degradation. Providing aeration to the TPPB system may perhaps be viewed as a manipulation or 'conditioning' of the feed stream, analogous to the

humidification step required for biofilters, but not for TPPBs.

Biofilters have great difficulty in dealing with high inlet VOC concentrations because of the potential for microorganisms to be inhibited or killed at the biofilters inlet (Deshusses 1997; McNevin & Barford 2000). This is a serious problem for biofilters since most of the active degraders are near the inlet, and as little as 4% of the total bacterial population may be responsible for as much as 65 % of pollutant degradation (Deshusses 1997; McNevin & Barford 2000). Biomass overgrowth problems could worsen at the inlet since there would be more substrate available for growth leading to clogging and channeling. These physical problems experienced by biofilters are not present in a TPPB since the system is comprised of liquid phases that are completely dispersed and are well mixed, and the bacteria are protected from inhibitory effects of the VOC by the favorable equilibrium partitioning.

The demonstrated toluene elimination capacity of 233 g m^{-3} h^{-1} for this system is comparable to the highest reported value in the literature with a high removal efficiency: 258 g m^{-3} h^{-1} at 95% removal (Auria et al. 2001). A second experiment determined that this TPPB system has the potential to operate at a maximum toluene elimination capacity of 1290 g m^{-3} h^{-1} at a cell concentration of 1 g l^{-1} provided that no mass transfer (e.g. oxygen) limitations exist. This is about 5 times higher than the highest elimination capacity reported by biofilters and furthermore, since operation at higher cell concentrations can be achieved (as evidenced by the ease of maintaining cells within a 1–3 g l^{-1} band), potential elimination capacities can be substantially higher still.

The final experiment illustrated the utility of this TPPB system by treating a mixed gas stream contaminated with benzene and toluene. Recent preliminary work with this system degrading benzene demonstrated a benzene elimination capacity of 133 g m^{-3} h^{-1} and produced an estimated maximum benzene elimination capacity of 570 g m^{-3} h^{-1} at 1 g l^{-1} cells (Davidson & Daugulis 2003). Along with the impressive elimination capacities reported here, this system has the potential to out-perform biofilters, given that the cell concentration can be manipulated, and controlled, to provide extremely high VOC removal rates, while not experiencing the operational problems seen during biofilter operation. Ultimately, economic considerations will be important in determining which type of VOC biotreatment process will be used in a particular application. Biofilters have been shown over many years to be an economic means of treating low concentration, high volume contaminated air streams, and one of their key features is simplicity. TPPBs, although they are able to treat low and high VOC gas streams, are substantially impervious to fluctuations in VOC loading, effectively utilize the entire reactor volume equally, and have higher elimination capacities (i.e. smaller units to treat the same mass loading), are arguably more complex systems. We are continuing to develop TPPB systems to treat VOCs via both experimental and dynamic modeling work, focusing on optimum solvent phase ratios, effect of solvent on enhancing oxygen transfer, and controlling biomass concentrations through nutrient dosing.

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