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The treatment of gaseous benzene by two-phase partitioning bioreactors: a high performance alternative to the use of biofilters

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Abstract A 2-l (1-l working volume) two-phase partitioning bioreactor (TPPB) was used as an integrated scrubber/bioreactor in which the removal and destruction of benzene from a gas stream was achieved by the reactor's organic/aqueous liquid contents. The organic solvent used to trap benzene was *n*-hexadecane, and degradation of benzene was achieved in the aqueous phase using the bacterium *Alcaligenes xylosoxidans* Y234. A gas stream with a benzene concentration of 340 mg l⁻¹ at a flow rate of 0.414 l h⁻¹ was delivered to the system at a loading capacity of 140 g m⁻³ h⁻¹, and an elimination capacity of 133 g m⁻³ h⁻¹ was achieved (the volume in this term is the total liquid volume of the TPPB). This elimination capacity is between 3 and 13 times greater than any benzene elimination achieved by biofiltration, a competing biological air treatment strategy. It was also determined that the evaluation of TPPB performance in terms of elimination capacity should include the cell mass present in the system, as this is a readily controllable quantity. A specific benzene utilization rate of 0.57 g benzene (g cells)⁻¹ h⁻¹ was experimentally determined in a bioreactor with a cell concentration that varied dynamically between 0.2 and 1 g l⁻¹. If it assumed that this specific benzene utilization rate (0.57 g g⁻¹ h⁻¹) is independent of cell concentration, then a TPPB operated at high cell concentrations could potentially achieve elimination capacities several hundred times greater than those obtained with biofilters.

Introduction

Industrial air pollution has come under increasing scrutiny in recent years as more demanding regulations and restrictions are being placed on industrial air emissions (Deshusses et al. 1999). Traditional treatment methods

such as thermal oxidation and incineration are too expensive in many cases to treat emissions from high volume, low concentration sources (Abumaizar et al. 1997; Deshusses et al. 1999). Cheaper biological solutions have been sought over the past decade and biofiltration has emerged as a prime candidate to treat high volume/low concentration emissions in a cost-effective manner. This technology, however, is fraught with operational difficulties from biomass overgrowth and deterioration of the filter bed support medium, and it is susceptible to surges and/or high concentrations of volatile organic compounds (VOCs) in the feed stream.

The application of two phase partitioning bioreactors (TPPBs) to VOC treatment is a technology that inherently has fewer problems while being able to potentially exceed the performance of biofilters. TPPBs rely on the use of an immiscible organic phase that acts as a reservoir for toxic or insoluble substrates that are delivered to the cell-containing aqueous phase at sub-inhibitory concentrations determined by thermodynamic equilibrium partitioning. Past work with TPPBs has focussed primarily on the treatment of liquid phase pollutants (Ortega-Calvo et al. 1995; Collins and Daugulis 1996, 1997a, b, 1999a, b; Munro and Daugulis 1997; Marcoux et al. 2000; Guieysse et al. 2001; Janikowski et al. 2002). More recent work experimented with the removal and degradation of airborne VOCs from gas streams with a two-stage system comprising an absorption column, using an organic solvent as a scrubbing liquid, and an associated bioreactor. Once trapped by the organic solvent in the scrubber, the VOC substrate was sent to the TPPB system where it was transferred, on demand, from the solvent to the cells in the aqueous phase, and the regenerated solvent was recirculated back to the absorber (Yeom et al. 2000; Yeom and Daugulis 2001).

In the present work, the removal of benzene from a gas stream was achieved directly by the liquid contents of a TPPB, without the need for an absorption column. This demonstration of concept of the single-stage process was first confirmed, and estimates of the maximum system performance were also undertaken. The performance was

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shown to greatly exceed the performance of biofilters, without the attendant problems associated with these conventional systems.

Materials and methods

Organism and growth conditions

Alcaligenes xylosoxidans Y234, obtained from Dr. S.H. Yeom, Department of Chemical Engineering, Seoul National University, is capable of degrading benzene, toluene and xylene as sole substrates (Yeom 1998). Stock cultures were maintained on agar plates with 20 g l⁻¹ bacto-agar along with the following nutrient concentrations: 7 g l⁻¹ (NH₄)₂SO₄, 0.75 g l⁻¹ MgSO₄·7H₂O, 6.6 g l⁻¹ K₂HPO₄, 8.42 g l⁻¹ KH₂PO₄, 2 g l⁻¹ sodium benzoate and 0.08 ml of a trace element solution containing 16.2 g l⁻¹ FeCl₃·6H₂O, 9.44 g l⁻¹ CaHPO₄, 0.15 g l⁻¹ CuSO₄·5H₂O and 40 g l⁻¹ citric acid.

Liquid medium for inoculum purposes was prepared with the same nutrient compositions as the agar plates. Six 125-ml Erlenmeyer flasks with 50 ml liquid bacterial culture were incubated on a gyratory shaker at 30°C at 150 rpm for 24 h prior to reactor inoculation. A 24-h period of batch bioreactor fermentation with liquid benzene added to the organic phase took place prior to the continuous gas feed mode of operation so that a substantial concentration of cells would be present.

Chemicals

The benzene and all salts used in the medium formulation were obtained from Fisher Scientific, Ottawa, Ontario, Canada. The *n*-hexadecane was obtained from Alfa Aesar of Ward Hill, Mass.

Reactor setup

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 6.6 with 2 M 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 aeration to the reactor at 24.6 l h⁻¹. The selected solvent, *n*-hexadecane, (Yeom and 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 supplied in a concentrated 50 ml bolus containing the following amounts of nutrients at the beginning of the fermentation and at each medium exchange (described below): 7 g (NH₄)₂SO₄, 0.75 g MgSO₄·7H₂O, 2 g KH₂PO₄ and 0.08 ml of the trace element solution.

Controlled delivery of benzene gas was achieved by passing compressed air through the headspace of a sealed 2-l flask filled with 1.5 l of benzene that was maintained at 30°C by a water bath and was delivered to the bottom of the bioreactor through an open glass tube. Benzene enriched gas was delivered at a rate of 140 mg h⁻¹ (loading capacity of 140 g m⁻³ h⁻¹) during continuous gas feed experiments and at a rate of 158 mg h⁻¹ (loading capacity of 158 g m⁻³ h⁻¹) during experiments to estimate maximum performance.

Continuous gas feed experiment

The bioreactor was prepared as described above and operated with a loading capacity of 140 g m⁻³ h⁻¹ for 140 h. Cell concentration was controlled within a concentration band of approximately between 2 and 4 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 bolus.

Maximum performance estimate

The bioreactor was prepared as described above and operated with a loading capacity of 158 g m⁻³ h⁻¹ and allowed to reach stable operation. The cell concentration was reduced through four serial medium exchanges to the point where it could no longer consume the mass load of benzene. This was followed by an accumulation of benzene 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 transition from increasing to decreasing benzene concentration was determined for this loading capacity and used to calculate the specific rate of benzene consumption (i.e. the mass of benzene consumed per mass of cells per hour). This experimental protocol was repeated twice.

Analytical and sampling procedures

Organic phase and gas phase benzene concentrations were quantified directly using a Varian 3400 gas chromatograph (GC) with a flame ionization detector, and a J and W Scientific DB-5 capillary column with peak integration performed by the Waters Millennium³² 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.

Gas samples of 100 μl were directly injected into the GC. Liquid samples from the bioreactor were 8–10 ml and were centrifuged at 3,400 rpm 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 cell concentration determined using a Biochrom Ultrospec 3000 UV/Visible spectrophotometer.

Results

Continuous treatment of a benzene gas stream

The benzene delivery system was stabilized to provide a constant flow rate of 0.414 l h⁻¹ at a benzene concentration of 340 mg l⁻¹ for a total mass delivery of 140 mg h⁻¹. The mass delivery rate was normalized with respect to total liquid volume and expressed as a loading capacity of 140 g m⁻³ h⁻¹. Figure 1 shows the inlet and outlet gas concentrations along with the hypothetical concentration of the inlet gas if the total air input were considered (make up aeration stream for oxygen delivery and air stream from benzene delivery system). In considering the inlet concentration in this manner, the benzene concentration would be 5.6 mg l⁻¹ instead of 340 mg l⁻¹. The outlet gas had an average concentration of 0.27 mg l⁻¹ which was a 95% reduction compared with the "mixed" inlet gas and a 99.9% reduction in concentration compared with the actual inlet gas concentration.

Figure 2 shows the loading and elimination capacities for this system along with removal efficiency. The elimination capacity is the product of the removal efficiency and the loading capacity and is an expression of the mass of benzene degraded per unit volume of the reactor per hour. The average removal efficiency was 95% and the corresponding elimination capacity was

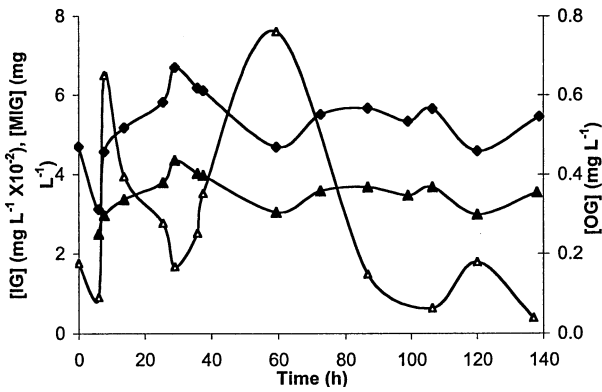


Fig. 1 Gas phase concentrations during delivery of benzene at a loading capacity of $140 \text{ g m}^{-3} \text{ h}^{-1}$. \blacktriangle Inlet gas concentration ($[IG]$), \blacklozenge mixed inlet gas concentration ($[MIG]$), \triangle outlet gas concentration ($[OG]$)

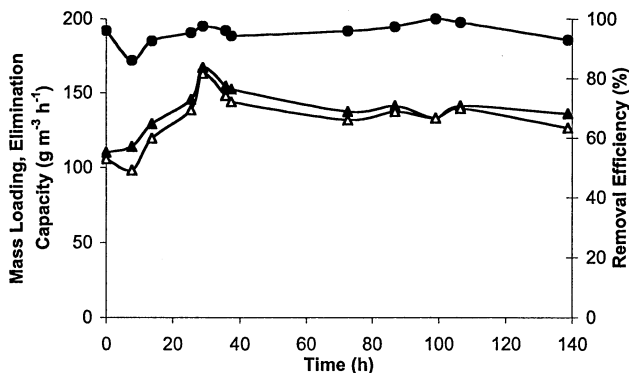


Fig. 2 System performance during delivery of benzene at a loading capacity of $140 \text{ g m}^{-3} \text{ h}^{-1}$. \triangle Elimination capacity, \blacktriangle loading capacity, \bullet removal efficiency

$133 \text{ g m}^{-3} \text{ h}^{-1}$. At all times during the fermentation, the dissolved oxygen was in excess of 40% of saturation.

Figure 3 shows the concentrations of benzene and cells in the liquid portion of the reactor. Having been operated in batch mode for 24 h prior to the introduction of the gas stream, no lag phase is present (data not shown). The organic phase concentration remained low throughout the fermentation and fluctuated between 2 and 5 mg l^{-1} and the aqueous phase concentration was also quite low and was less than 0.1 mg l^{-1} throughout the fermentation. The cell concentration was controlled between 2 and 4 g l^{-1} by periodic medium exchanges that occurred approximately every 20 h. It was also possible to estimate the cell yield coefficient from these data and it was determined to be $0.56 \text{ g cells (g benzene)}^{-1}$.

Maximum performance estimate

To characterize the maximum potential performance of the system, the minimum mass of bacteria required to handle a specific loading capacity was determined. Stable operation with enough bacteria to degrade a constant

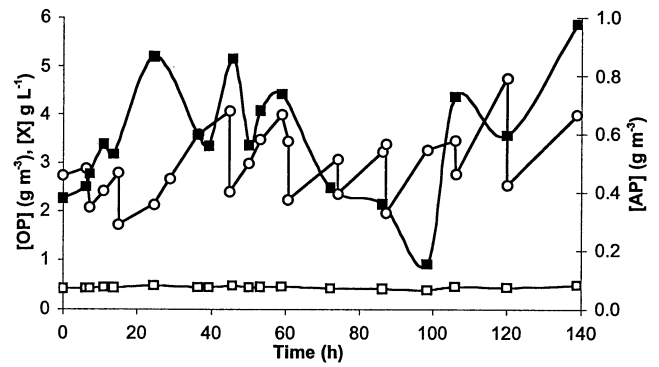


Fig. 3 Liquid phase concentrations during delivery of benzene at a loading capacity of $140 \text{ g m}^{-3} \text{ h}^{-1}$. \square Aqueous phase concentration ($[AP]$), \circ cell concentration ($[X]$), \blacksquare organic phase concentration ($[OP]$). Dissolved oxygen concentration was $>40\%$ at all times

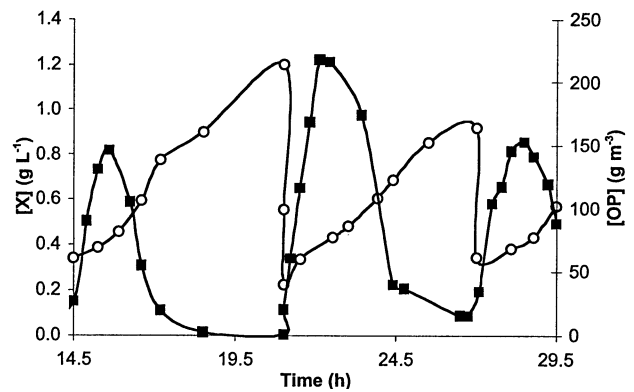


Fig. 4 Benzene and biomass concentrations during system perturbation caused by biomass removal at a benzene loading capacity of $158 \text{ g m}^{-3} \text{ h}^{-1}$. \circ $[X]$, \blacksquare $[OP]$

loading capacity of $158 \text{ g m}^{-3} \text{ h}^{-1}$ was first established. Bacteria were then removed to the point where there were no longer enough to handle the loading capacity. Figure 4 illustrates 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 loading. At each transition (maximum) point in the organic phase concentration, cell masses of 0.27, 0.27 and 0.28 g (i.e. approximately 0.41 g l^{-1} in 660 ml) were measured. From this an average specific benzene utilization rate of $0.57 \text{ g g}^{-1} \text{ h}^{-1}$ was calculated which suggests that at a bioreactor cell concentration of 1 g l^{-1} an elimination capacity of $570 \text{ g m}^{-3} \text{ h}^{-1}$ should be achievable.

Discussion

The inlet gas concentration of 340 mg l^{-1} for this continuous treatment process is far in excess of any treated by biofilters (Oh and Bartha 1997; Zhou et al. 1998; Zhu et al. 1998; Deshusses and Johnson 2000). If

the inlet concentration of pollutant VOCs to a biofilter were too high, the bacteria at the inlet could be inhibited or killed (Deshusses 1997; McNevin and Barford 2000). Biomass overgrowth problems worsen at the inlet at higher mass loads, and clogging and channelling may occur and decrease performance. These physical problems are not present in a TPPB since the system is well mixed, and the bacteria are protected from any inhibitory effects of the VOC by mixing, and the favourable equilibrium partitioning that keeps the aqueous phase concentration at sub-inhibitory levels. Control of biomass was easily exercised by the periodic medium exchanges. For biofilters there is no analogous process that is as simple for controlling cell growth.

The claim could be made that this TPPB system was handling highly concentrated streams only through dilution with excess air and that the true inlet gas concentration was 5.6 mg l^{-1} . However, the air stream providing oxygen to the cells was supplied separately to the system, and was provided at a rate that maintained dissolved oxygen levels above 40% of saturation at all times. Clearly, this is an independently controllable operating feature of TPPB systems 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 would be calculated. The additional feature of 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.

In addition to VOC feed concentration, the calculated loading capacity, elimination capacity and removal efficiency are also essential in defining performance. The elimination capacity of $133 \text{ g m}^{-3} \text{ h}^{-1}$ for this system is between 3 and 13 times greater than any reported for biofilters degrading benzene ($10\text{--}50 \text{ g m}^{-3} \text{ h}^{-1}$; Oh and Bartha 1997; Zhou et al. 1998; Zhu et al. 1998; Deshusses and Johnson 2000). The high removal efficiency of 95% is a very important benchmark to have achieved. Deshusses and Johnson (2000) describe 95% removal efficiency as being the minimum acceptable level for adequate treatment and the loading capacity at this point is given the special name of the "critical loading capacity". This TPPB system was shown to operate at high benzene elimination capacity compared with current biofilters and it had a critical loading capacity that was also in excess of biofilters.

Past TPPB work with gas phase benzene in two-stage absorber/bioreactor systems has produced higher elimination capacities but with unacceptably low removal efficiencies: $252 \text{ g m}^{-3} \text{ h}^{-1}$ with 75% removal (Yeom and Daugulis 2001), and $696 \text{ g m}^{-3} \text{ h}^{-1}$ with 87% removal (Yeom et al. 2000; Yeom and Daugulis 2001).

None of this work, however, nor the continuous gas feed treatment described above, gives any indication of the potential ultimate performance of TPPBs. It was determined in a second experiment that with a cell concentration of 1 g l^{-1} this TPPB system using *A.*

xylooxidans Y234 has the potential to operate at a maximum elimination capacity of $570 \text{ g m}^{-3} \text{ h}^{-1}$ provided that no mass transfer (e.g. oxygen) limitations exist. Furthermore, since higher cell concentrations can be readily obtained (as shown by the ease of maintaining cells within a $2\text{--}4 \text{ g l}^{-1}$ band), potential elimination capacities can be substantially higher. The definition of elimination capacity for TPPBs, however, should take into account the mass of cells present in the system and, by this standard, the elimination capacity for benzene by this system is $570 \text{ g benzene (g cells)}^{-1} \text{ m}^{-3} \text{ h}^{-1}$.

Therefore, TPPBs have the potential to operate at elimination capacities hundreds of times greater than those of biofilters. Two operating features of TPPBs for VOC removal are particularly responsible for this performance: the fact that these systems are well mixed, and the ability to maintain and control high, active cell concentrations at desired levels. In the case of biofilters, cell concentrations are invariably concentrated at the inlet, with much lower biomass levels along the biofilter length. Thus not only are cell concentrations non-uniform, difficult to determine, and virtually impossible to control in biofilters, the reactor volumes are not optimally used and the inlet is sensitive to toxicity effects. In contrast, the elimination of concentration gradients (VOC and microbial) contribute to TPPBs being able to handle VOC concentrations that are too high for biofilters, and to handle the same benzene mass loads as biofilters currently deal with, but with a reactor a fraction of the size.

The results presented show that the process compression of the two-unit absorber/bioreactor system into a single unit is effective, and provides significant simplification in operation. The TPPB system also shows the potential to dramatically out-perform biofilters treating benzene with reactors that could be much smaller.

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