

# Development of a Novel Bioreactor System for Treatment of Gaseous Benzene

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**Abstract:** A novel, continuous bioreactor system combining a bubble column (absorption section) and a two-phase bioreactor (degradation section) has been designed to treat a gas stream containing benzene. The bubble column contained hexadecane as an absorbent for benzene, and was systemically chosen considering physical, biological, environmental, operational, and economic factors. This solvent has infinite solubility for benzene and very low volatility. After absorbing benzene in the bubble column, the hexadecane served as the organic phase of the two-phase partitioning bioreactor, transferring benzene into the aqueous phase where it was degraded by *Alcaligenes xylooxidans* Y234. The hexadecane was then continuously recirculated back to the absorber section for the removal of additional benzene. All mass transfer and biodegradation characteristics in this system were investigated prior to operation of the integrated unit, and these included: the mass transfer rate of benzene in the absorption column; the mass transfer rate of benzene from the organic phase into the aqueous phase in the two-phase bioreactor; the stripping rate of benzene out of the two-phase bioreactor, etc. All of these parameters were incorporated into model equations, which were used to investigate the effects of operating conditions on the performance of the system. Finally, two experiments were conducted to show the feasibility of this system. Based on an aqueous bioreactor volume of 1 L, when the inlet gas flow and gaseous benzene concentration were 120 L/h and 4.2 mg/L, respectively, the benzene removal efficiency was 75% at steady state. This process is believed to be very practical for the treatment of high concentrations of gaseous pollutants, and represents an alternative to the use of biofilters. © 2000 John Wiley & Sons, Inc. *Biotechnol Bioeng* 72: 156–165, 2001.

**Keywords:** benzene; biodegradation; partitioning bioreactor

## INTRODUCTION

Volatile aromatic compounds, such as benzene, toluene, and xylene (BTX), are major products of the petroleum and fine chemical industries and among the most frequently used

organic solvents (Chang et al., 1993; Oh et al., 1994; Yeom and Yoo, 1999). However, because they are suspected to be carcinogens and can produce offensive odors, their release to the environment is strictly controlled and they are classified as priority environmental pollutants by the U.S. Environmental Protection Agency (EPA, 1986). A variety of methods have been suggested to treat these materials. Physical or chemical methods such as incineration, chlorination, ozonation, and combustion are expensive, requiring elaborate equipment and substantial amounts of additional fuel. Adsorption onto activated carbon is also costly and the saturated carbon may be a hazardous waste, requiring either regeneration or transportation to a hazardous-waste landfill (Hodge and Devinsky, 1995). Biological treatment using microorganisms has the potential of not producing secondary effluent problems. Also, in general, biological treatment processes may be the most cost-effective technique for treating aqueous waste streams containing organic compounds (Thayer, 1991).

BTX compounds may be present as contaminants in solid, liquid, and gaseous streams, and gas-phase removal of volatile compounds has received considerable attention of late. Biofilters, considered to be one of the most promising methods for the treatment of volatile compounds, consist of a packed column with biologically active materials such as immobilized cells and compost (Ottengraf, 1986). The pollutants contained in the air stream are aerobically degraded in a biofilter column. Biofilters, however, can treat only low concentrations of volatile pollutants (Hodge and Devinsky, 1995). Maintaining proper temperature, pH, and moisture are also challenging problems (Shareefdeen and Baltzis, 1994).

Yeom and Yoo (1999) recently suggested a hybrid bioreactor comprised of a bubble column bioreactor in series with a biofilter. When a high concentration of gaseous pollutant was fed to the hybrid bioreactor, much of it was degraded in the bubble column bioreactor section with some being stripped by aeration and subsequently degraded in the biofilter. This hybrid system had additional flexibility relative to a simple biofilter, because, by manipulating opera-

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tion variables, such as air flow rate and residence time of wastewater, it was possible to share the pollutant burden between the two sections. Even though it had several advantages in the treatment of volatile pollutants, it still had the same limitations associated with biofilters as noted previously.

We have recently been examining the potential of two-phase biocatalysis for bioremediation applications in which a second, distinct organic phase is employed for the “delivery” of inhibitory substrates to a cell-containing aqueous phase. In this scheme, very large amounts of xenobiotic substrate can be dissolved in a hydrophobic organic phase, and can then partition at an appropriately low concentration by means of equilibrium to cells. Such a system is self-regulating in the sense that the metabolic activity of the cells determines the rate of transfer of the xenobiotics, and the processing concept has been successfully applied to the degradation of very large amounts of phenol (Collins and Daugulis, 1997), pentachlorophenol (Munro and Daugulis, 1996), and BTX (Collins and Daugulis, 1999a). We have also been able to show that the organic phase can be used to efficiently (i.e., >99%) recover spilled BTX from soil, and then be used in the two-phase system to completely degrade the contaminant species (Collins and Daugulis, 1999b).

In this study, we combined an absorption column with the two-phase bioreactor to remove and degrade benzene from a gas stream. In the absorption column, hexadecane (which was selected because of its favorable physical properties for capturing benzene) was used to scrub a gas stream containing 4.2 mg/L benzene. The solvent, which also had to meet various biological criteria for use in such a system, was then pumped to the two-phase bioreactor where *Alcaligenes xylosoxidans* Y234 in the aqueous phase consumed the contaminant. The hexadecane was then recirculated back to the stripper for reuse. Prior to experimentally demonstrating the effectiveness of this concept, it was necessary to quantify the mass transfer rates between the various phases as well as microbial kinetic coefficients. These were used in a mathematical model to identify appropriate operating conditions (e.g., concentrations, flow rates) to be used. Two experiments were then undertaken to show the validity of this process concept.

## MATERIALS AND METHODS

### Microorganism

*Alcaligenes xylosoxidans* Y234 isolated from oil-contaminated soil was used in this study. It can degrade benzene, toluene, and phenol (Yeom et al., 1998; Yeom and Daugulis, 1999b).

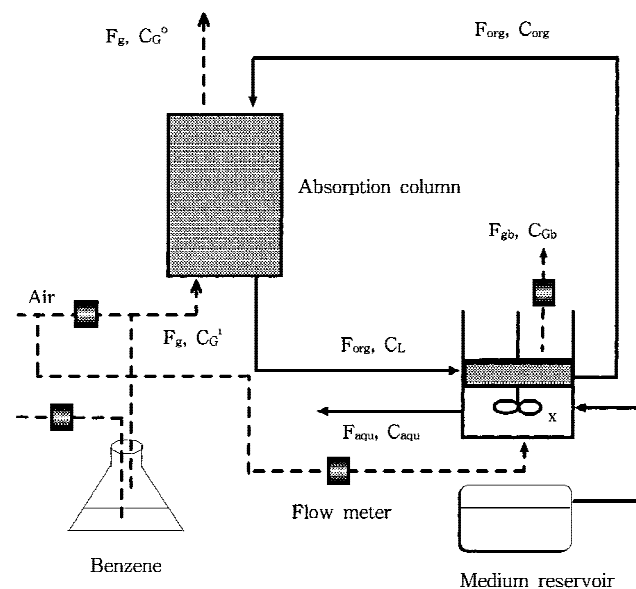
### Media

To eliminate any microbial adaptation period in the concept demonstration, the *A. xylosoxidans* Y234 was preadapted to

benzene as follows. The cells were precultured at 30°C in a 125-mL flask containing 50 mL of medium [10 g/L glucose, 5.0 g/L yeast extract, 5.0 g/L (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 5.0 g/L KH<sub>2</sub>PO<sub>4</sub>, and 1.0 g/L MgSO<sub>4</sub> · 7H<sub>2</sub>O]. After the cells were harvested, they were resuspended in distilled water and then centrifuged twice. The cells were then transferred into a 160-mL serum bottle containing 30 mL of medium. The medium composition was: 5.0 g/L K<sub>2</sub>HPO<sub>4</sub>, 4.5 g/L KH<sub>2</sub>PO<sub>4</sub>, 2.0 g/L (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 0.3 g/L MgSO<sub>4</sub> · 7H<sub>2</sub>O, and 200 μL trace elements. The trace elements consisted of 16.2 g/L FeCl<sub>3</sub> · 6H<sub>2</sub>O, 9.44 g/L CaHPO<sub>4</sub>, 0.15 g/L CuSO<sub>4</sub> · 5H<sub>2</sub>O, and 40.0 g/L citric acid. Three microliters of benzene were put into the bottle as a sole carbon and energy source and the liquid benzene concentration was assumed to be 42.8 mg/L using Henry’s constant (Yeom and Yoo, 1999) and was measured to be 40 mg/L. The bottle was sealed with a butyl-rubber septum and aluminum crimp cap. After 20-h incubation, the cells were fully adapted to benzene and inoculated into the aqueous phase of the two-phase bioreactor. During continuous operation of the combined absorber/bioreactor system, the mineral medium composition was 5.5 g/L (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 1.0 g/L KH<sub>2</sub>PO<sub>4</sub>, 0.75 g/L MgSO<sub>4</sub> · 7H<sub>2</sub>O, and 1 mL/L of trace element solution.

### Absorber/Bioreactor Configuration

A schematic diagram of the integrated system is shown in Figure 1. The central feature of this arrangement consisted of a 2-L New Brunswick Scientific Bioflo fermentor with a 1-L aqueous volume and a 500-mL organic volume, as previously described (Collins and Daugulis, 1997). In addition, a means of generating a constant composition benzene-in-air stream was required, and this was achieved by manipu-



**Figure 1.** Schematic diagram of the novel bioreactor system for the treatment of gaseous benzene. Solid line: liquid flow; dashed line: gas flow.

lating the ratio of the air flow rate to a 1-L flask containing pure benzene, and a make-up air flow stream. The system also made use of a glass cylindrical absorption column (with an inner diameter of 6.5 cm and a working volume of 1.0 L) to scrub the benzene gas stream with the solvent. A sintered glass sparger was used to introduce the gas stream into the absorber. The solvent was circulated in a closed loop in this system, with benzene being picked up in the absorber, and being "delivered" to the cells in the two-phase bioreactor, with subsequent return to the absorber. The entire system was maintained at 30°C by means of water baths, heating coils, and internal temperature control on the fermentor.

### Solvent Selection

The strategy and procedure for solvent selection in two-phase partitioning bioreactors has been described previously (Collins and Daugulis, 1997; Yeom and Daugulis, 1999b), and considers physical, biological, environmental, operational, and economic factors. In the present application, hexadecane was chosen as the best solvent for the treatment of benzene in this configuration.

### Analytical Methods

To measure the benzene concentration in the absorption column containing hexadecane, a 3-mL liquid sample was taken and mixed with the same volume of distilled water. The mixed solution was shaken vigorously and left for 4 h. Two microliters of the aqueous phase (lower phase) was taken and injected into a Perkin-Elmer gas chromatograph. The peak area was compared with a previously prepared calibration curve. The benzene concentration in the organic phase was estimated from the measured aqueous phase concentration by using a distribution coefficient of 140.1, which was previously determined. The benzene concentration in the organic phase (hexadecane) in the two-phase bioreactor was measured in the same way. In order to measure the benzene concentration in the aqueous phase of the two-phase bioreactor, aqueous samples were taken and centrifuged to remove cells. Two microliters of cell-free aqueous phase was injected directly into the GC. Because phase separation in the bioreactor was extremely good, samples could be taken directly from each phase without stopping stirring or aeration. To measure the gas phase benzene concentration leaving the absorption column and the bioreactor, 250  $\mu$ L of gas phase was withdrawn with a gas-tight syringe and injected into the GC. The resulting area was also compared with a calibration curve of gaseous benzene to determine the actual benzene concentration. The operation conditions of the GC in both the cases (liquid and gas benzene) were: 250°C injection temperature; 50°C oven temperature; and 200°C detection temperature. The cell concentration was measured by optical density using a Brinkman PC600 colorimeter at 640 nm and a previously prepared calibration curve.

## RESULTS AND DISCUSSION

### Solvent Selection

One of the most important aspects in the development of this system was selecting an appropriate solvent. As mentioned in a previous study, the solvent must be biocompatible, nonbiodegradable, nontoxic, and inexpensive, and have a high partition coefficient and selectivity as well as low volatility and density (Yeom and Daugulis, 1999a). By means of a systematic solvent selection strategy, as previously described (Collins and Daugulis, 1997; Yeom and Daugulis, 1999b), hexadecane was chosen as the solvent for the treatment of benzene in this two-phase system.

### Mass Transfer Measurements in the Absorption Column

A bubble column was used in this study to remove benzene from air by contacting with hexadecane. The liquid benzene concentration along the column length (using three sampling ports) with continuous gas injection was investigated to examine the fluid dynamics in the bubble column. We found that there was no benzene concentration gradient (i.e., that the column contents were well-mixed) along the column length as long as the air flow was maintained above 30 L/h (1061.0 cm/h of superficial gas velocity) in the experimental configuration (data not shown).

Because there is no reaction in the column (and as long as the air flow to the column is above 30 L/h) the equation below can be used to describe the mass transfer of benzene from the gas stream to the liquid phase (Hecht et al., 1995):

$$\frac{dC_L}{dt} = k_L a_{\text{gas}} (C^* - C_L) \quad (1)$$

where  $C_L$  is the hexadecane benzene concentration,  $k_L a_{\text{gas}}$  is the mass transfer coefficient of benzene into the liquid phase, and  $C^*$  is the saturated organic phase benzene concentration, which is in equilibrium with the benzene in the bulk gas phase. In this equation, the driving force for mass transfer is the concentration difference between the saturated benzene concentration and the actual benzene concentration in the column. If both values,  $k_L a_{\text{gas}}$  and  $C^*$ , are available, the liquid phase benzene concentration in the column can be predicted with respect to operating conditions such as gas flow rate and inlet benzene concentration. In the bubble column, the mass transfer coefficient ( $k_L a_{\text{gas}}$ ) is known to be dependent on the physical properties of the solvent and solute, superficial gas flow rate, and the dimensions of the column, whereas the saturated benzene concentration ( $C^*$ ) is dependent on the gaseous benzene concentration and solvent properties (Akita and Yoshida, 1973). If both values,  $k_L a_{\text{gas}}$  and  $C^*$ , are not available in the literature, they can be measured experimentally. The saturation concentration of benzene in the hexadecane that was in equilibrium with various gas phase benzene concentrations was determined experimentally. Gaseous benzene was fed to the

bubble column at various concentrations and the liquid phase benzene concentration was measured. At each gas phase benzene concentration when the concentration of benzene in the hexadecane reached a constant value, the system had reached equilibrium. Figure 2 shows the linear dependency of  $C^*$  on the gaseous benzene concentration ( $R^2 = 0.988$ ).

Next, the  $k_L a_{\text{gas}}$  values were determined. By integrating Eq. (1), we can get Eq. (2):

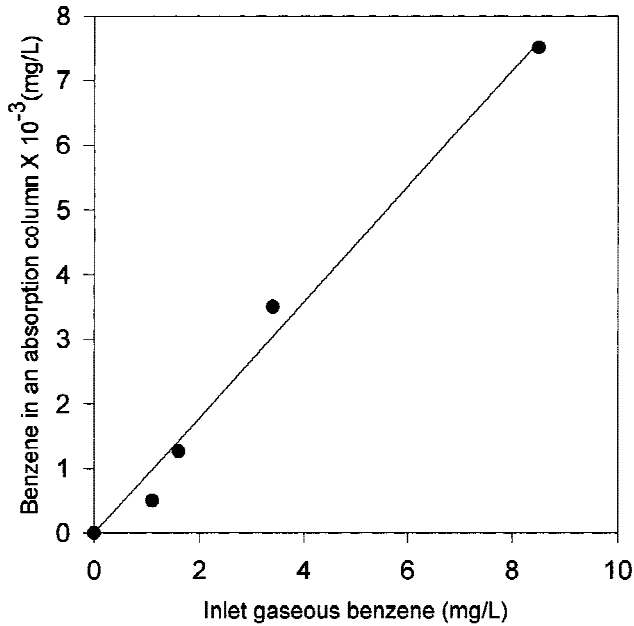
$$\ln(C^* - C_L) - \ln C^* = k_L a_{\text{gas}} t \quad (2)$$

and by plotting  $\ln(C^* - C_L)$  versus  $t$ ,  $k_L a_{\text{gas}}$  was determined from the slope (Shuler and Kargi, 1992). As shown in Table I, it can be seen that the effect of influent gaseous benzene concentration on  $k_L a_{\text{gas}}$  is almost negligible compared to that of the gas flow rate. The correlation of  $k_L a_{\text{gas}}$  with gas flow rate ( $R^2 = 0.986$ ) is:

$$k_L a_{\text{gas}} = 3.802 \times 10^{-5} v_G \quad (3)$$

where,  $v_G$  is the superficial gas flow rate. These results are consistent with others reports (Deckwer and Schumpe, 1993; Hecht et al., 1995), which noted that  $k_L a_{\text{gas}}$  is proportional only to the superficial flow rate as long as the radius of the bioreactor and the physical properties of the solution do not change. With  $C^*$  and  $k_L a_{\text{gas}}$  now correlated with the influent gaseous benzene concentration and gas flow rate, respectively, it is possible to predict the behavior of benzene absorption into hexadecane from the gas stream over a range of gas flow rates and gaseous benzene concentrations. However, Eq. (1) is valid only on the condition that the liquid phase is well mixed.

In this system, it is desirable to minimize the exit benzene concentration from the absorber to get high removal effi-



**Figure 2.** Equilibrium correlation between gaseous benzene concentration with benzene in hexadecane.

**Table I.** Effect of inlet gas flow rate and benzene concentration on the mass transfer coefficient of benzene from the gas stream into hexadecane in the absorption column.

Gas flow rate (L/h)	Inlet benzene (mg/L)	
	3.4	8.5
60	0.076	0.059
120	0.124	0.127

ciency. To do this we needed to formulate an equation that would predict the exit benzene concentration from the column with respect to absorber operating conditions. Although the liquid phase in the absorption column was well mixed, it could not necessarily be said that the gas phase was also well mixed. For the gas phase in bubble columns, a plug-flow type equation is often applied to predict the exit gas concentrations as previously noted by Hecht et al. (1995). On the basis of a plug-flow assumption, the equation to determine the benzene concentration in the exit gas stream from simple mass balance is:

$$v_G \frac{dC_G}{dz} = k_L a_{\text{gas}} (C^* - C_L) \quad (4)$$

From the linear correlation of the equilibrium benzene concentration with gaseous benzene as shown in Fig. 2, it is possible to have:

$$C^* = f C_G \quad (5)$$

where,  $f$  is a proportionality constant similar to Henry's constant and was found to be  $0.912 \times 10^3$  from the slope in Fig. 2. By using this relationship in equation (4) and integrating, the following equation is obtained:

$$C_G^o = C_G^i e^{-St} + \frac{C_L}{f} (1 - e^{-St})$$

where  $C_G^i$  are  $C_G^o$  are inlet and outlet gaseous benzene concentration.  $St$  is the Stanton number and is defined as:

$$ST = \frac{f k_L a_{\text{gas}} L}{v_G} \quad (7)$$

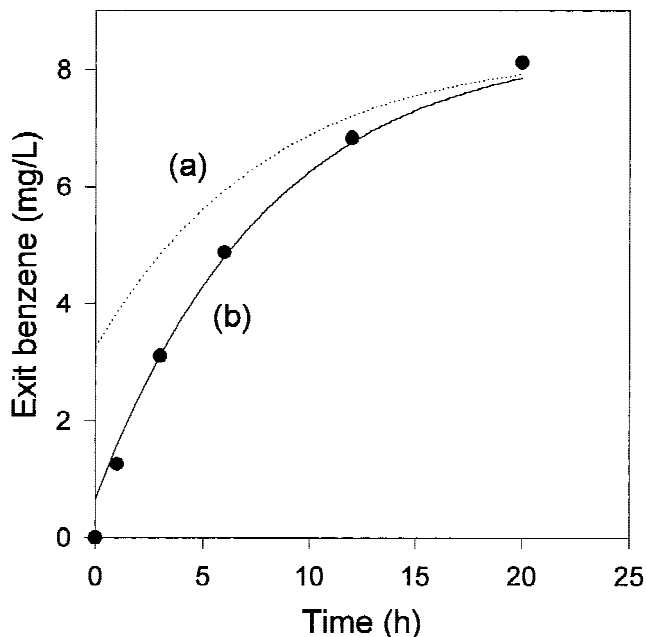
where  $L$  is the length of the column containing the hexadecane. The experimental data were compared with Eq. (6), and Figure 3 (curve *a*) shows that this equation does not match the actual exit benzene concentration well. As an alternative approach, a completely mixed gas phase was assumed and a mass balance on gaseous benzene was set up to predict exit benzene concentration as shown:

$$F_g C_G^i - F_g C_G^o = V_{\text{bub}} k_L a_{\text{gas}} (C^* - C_L) \quad (8)$$

where,  $V_{\text{bub}}$  is the working volume of the absorption column. By arranging, we can get the following equation.

$$C_G^o = C_G^i - \frac{V_{\text{bub}} k_L a_{\text{gas}} (C^* - C_L)}{F_g} \quad (9)$$



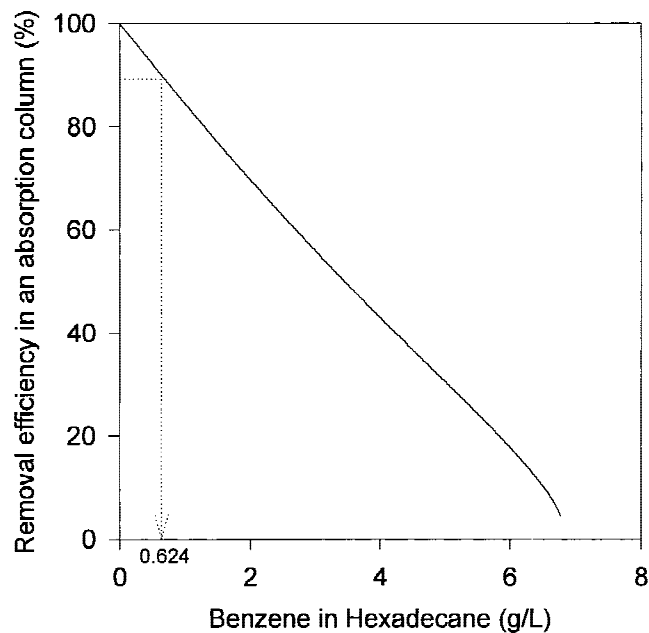


**Figure 3.** Exit benzene concentration from the absorption column as predicted by plug-flow (curve *a*) and well-mixed (curve *b*) equations for the gas phase. (●) Experimental data.

As shown in Figure 3 (curve *b*), even though there is some discrepancy at time zero, Eq. (9) predicts the exit benzene concentration very well. As seen earlier, as the benzene concentration in the hexadecane increases, so does the exit benzene concentration in the exit gas stream. Thus, it was necessary to determine the value of the benzene concentration in the hexadecane that would ensure a desirable level of removal efficiency in the column. This was done by plotting liquid benzene concentration versus exit gas benzene concentration. For example, in the situation in which the influent benzene concentration and gas flow rate are 8.5 mg/L and 120 L/h, respectively, if we are to remove >90% of the benzene, the benzene concentration in the absorption column should be <0.624 g/L, as shown in Figure 4.

### Mass Transfer Measurements in Two-Phase Partitioning Bioreactor

The mass transfer rate of benzene from the organic phase to the aqueous phase in the two-phase partitioning bioreactor,  $k_L a_{liq}$ , was measured with respect to aeration and agitation rates. In consideration of possible oxygen limitation by the cells, higher agitation rates and aeration rates are preferred. Also, high agitation rates will enhance the mass transfer rate of benzene between the two phases. However, high agitation rates may cause an emulsion and high aeration will also cause more stripping of benzene from the two-phase bioreactor. In examining the effect of agitation on phase stability we found that the phases remained distinct at up to 500 rpm. As for the limit of the aeration rate, we had found that there was no oxygen limitation by the cells with aeration of 250 mL/min (0.25 vvm on the basis of aqueous phase volume)



**Figure 4.** Benzene concentration in hexadecane in the absorption column that provides 90% removal at an inlet benzene concentration of 8.5 mg/L and a gas flow rate of 120 L/h.

in batch operation when the cell concentration reached 1700 mg/L (Yeom and Daugulis 1999b). In the case of higher cell concentrations, aeration would have to be increased. In these measurements, therefore, the aeration rate was fixed at 0 mL/min (0 vvm), 250 mL/min (0.25 vvm), or 500 mL/min (0.5 vvm).

The mass transfer of benzene from the organic phase to the aqueous phase can be determined by:

$$\frac{dC_{\text{aqu}}}{dt} = k_L a_{liq} (C_{\text{org}} - m_s C_{\text{aqu}}) \quad (10)$$

where  $C_{\text{aqu}}$  and  $C_{\text{org}}$  are the benzene concentrations in the aqueous and organic phases, respectively, and  $m_s$  is the distribution coefficient of benzene between the aqueous and organic (hexadecane) phases. The driving force for mass transfer is the difference between the benzene concentration in the organic phase and the corresponding equilibrium benzene concentration in the aqueous phase. To obtain an accurate  $k_L a_{liq}$ , it is necessary to measure the benzene concentration in both liquid phases and in the gas phase leaving the bioreactor. However, if the initial benzene concentration is high enough and the total experimentation time is short enough, the benzene concentration in the organic phase can be assumed to be constant. Thus, it is possible to integrate Eq. (10) and by plotting  $\ln(C_{\text{org}} - m_s C_{\text{aqu}})$  versus  $t$ , the slope provides  $k_L a_{liq}$ , which is the mass transfer coefficient in Eq. (10). The experimental results are shown in Table II. Because it was impossible to take samples more frequently than every 5 s,  $k_L a_{liq}$  at 500 rpm could not be measured directly (the transfer rate was so high that much shorter sampling times would be required), but was estimated by linear extrapolation of the data at 0, 200, and 350 rpm (the

**Table II.** Effect of aeration and agitation rates on the mass transfer coefficient of benzene from organic phase to aqueous phase in the two-phase partitioning bioreactor.

Aeration (vvm)	Agitation (rpm)			
	0	200	350	500 <sup>a</sup>
0	0.072	1.11	4.50	10.15
0.25	0.088	1.03	4.04	9.01
0.50	0.061	0.89	2.81	5.83

<sup>a</sup>extrapolated value.

speed at which a sampling rate of 5 s was used). Although the value of  $k_L a_{liq}$  is thought to be dependent on both agitation and aeration rates in the two-phase bioreactor, the agitation rate, of the two, appeared to be the most influential factor on the mass transfer rate. The  $k_L a_{liq}$  increased with an increase in the agitation rate but decreased with an increase in aeration rate, except for the case of 0 rpm. Because benzene has high volatility, it can be stripped from the aqueous phase, which is consistent with the  $k_L a_{liq}$  being inversely proportional to the aeration rate. The measured values here are likely the summation of the mass transfer rate of benzene into the aqueous phase by agitation and stripping by aeration.

By aerating the bioreactor it was anticipated that some benzene would be continuously stripped out of the two-phase system, and it is desirable to minimize this loss. We already obtained parameters for the stripping of benzene from the absorption column as described earlier, but according to Akita and Yoshida (1973), the mass transfer rate is dependent on the properties of the bioreactor contents and on the diameter of the reactor. In addition, the bioreactor was being agitated. By measuring the benzene concentration in the organic phase at 350 rpm and various air flow rates, the coefficient for benzene stripping from the organic phase ( $k_L a_{gas2}$ ) was calculated using Eq. (2). The resulting correlation between  $k_L a_{gas2}$  with superficial air flow rate is:

$$k_L a_{gas2} = 647 \times 10^{-4} v_G \quad (11)$$

By comparing the benzene stripping coefficient from an aqueous phase ( $0.708 \text{ h}^{-1}$  at  $530.52 \text{ cm/h}$  and  $1.074 \text{ h}^{-1}$  at  $1061.03 \text{ cm/h}$ ) (Yeom and Yoo, 1999), it can be seen that the benzene stripping coefficient in this study is only one-seventh at the same superficial air flow rate, which means that the ability of hexadecane to retain benzene is seven times higher than that of water.

### Mathematical Formulation for Simulation of the System

Mathematical model equations were formulated in this study to determine the effect of each operating parameter on the performance of the system. The simulation study also helped to determine which parameter is most critical in continuous operation and to optimizing the system for high removal efficiency. Based on the experimental system

shown in Figure 1 and on the knowledge that the liquid phase in the absorption column was well mixed, the absorption column can be described by Eq. (12). In the case of the bioreactor, benzene is stripped out by aeration by the driving force of  $(C_{org} - C_{bubble}^*)$ .  $C_{bubble}^*$  is the liquid benzene concentration in the organic phase equilibrated with the benzene concentration in the inlet air bubbles. Because this benzene concentration is negligible compared with the benzene concentration in the organic phase, the final balance on benzene in the organic phase of the bioreactor can be described by Eq. (13). The benzene in the aqueous phase is degraded by the microorganisms as well as being diluted by an aqueous feed rate, as shown in Eq. (14).  $C_{org}$  here already includes benzene being stripped by air [as per Eq. (13)]. The microorganism balance, with cells growing on benzene, but being diluted by the aqueous feed rate, is as described in Eq. (15):

$$\frac{dC_L}{dt} = k_L a_{gas} (C^* - C_L) + F_{org} \frac{(C_{org} - C_L)}{V_{bub}} \quad (12)$$

$$\frac{dC_{org}}{dt} = F_{org} \frac{(C_L - C_{org})}{V_{org}} - 2k_L a_{liq} (C_{org} - m_s C_{aqu}) - k_L a_{gas2} C_{org} \quad (13)$$

$$\frac{dC_{aqu}}{dt} = k_L a_{liq} (C_{org} - m_s C_{aqu}) - \frac{1}{Y} \frac{\mu_{max} C_{aqu}}{K_s + C_{aqu}} X - \frac{F_{aqu}}{V_{aqu}} C_{aqu} \quad (14)$$

$$\frac{dX}{dt} = \frac{\mu_{max} C_{aqu}}{K_s + C_{aqu}} X - \frac{F_{aqu}}{V_{aqu}} X \quad (15)$$

where  $F_{org}$  is the hexadecane circulation rate (HCR) and  $F_{aqu}$  is the aqueous feed rate of new medium (AFR).  $V_{bub}$ ,  $V_{aqu}$ , and  $V_{org}$  are the working volumes of the bubble column, aqueous phase, and organic phase in the two-phase bioreactor, respectively. The operating parameters of the system are listed in Table III. The biological terms in the Monod equation,  $\mu_{max}$  (maximum specific growth rate) and  $K_s$  (half-saturation constant), were calculated from previous batch experiments (data not shown). If the inlet gas flow rate, inlet gas benzene concentration, and agitation and aeration rates are fixed, there are only two remaining operating variables: the hexadecane circulation rate (HCR,  $F_{org}$ ) and the aqueous feed rate (AFR,  $F_{aqu}$ ). The removal efficiency

**Table III.** List of operating parameters for the simulation of the novel bioreactor system.

Parameter	Value
$k_L a_{gas}$ (1/h)	Eq. (3)
$k_L a_{gas2}$ (1/h)	Eq. (II)
$k_L a_{liq}$ (1/h)	Table II
$C^*$ (mg/L)	Eq. (5)
$m_s$ (-)	140.08
$\mu_{max}$ (1/h)	0.58
$Y$ (-)	0.46
$K_s$ (mg/L)	12.47

at steady-state can therefore be calculated by considering input and output amounts of benzene:

Removal efficiency (%) =

$$\left(1 - \frac{F_g C_G^o + F_{gb} C_{Gb} + F_{aqu} C_{aqu}}{F_g C_G^i}\right) \times 100 \quad (16)$$

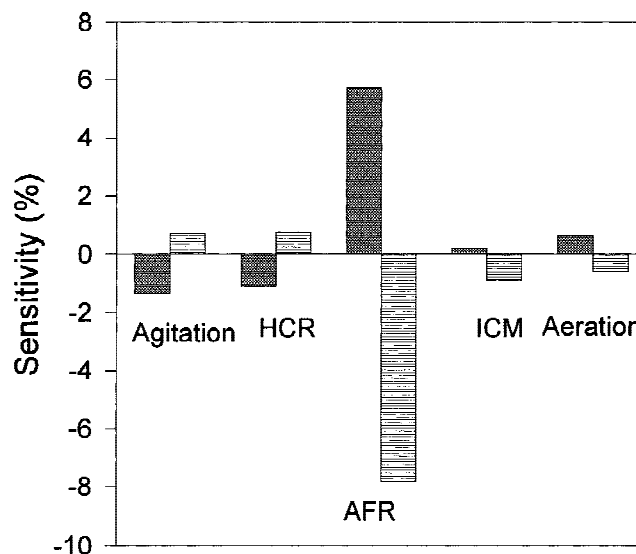
where  $F_{gb}$  and  $C_{Gb}$  are outlet gas flow rate and exit gas benzene concentration from the two-phase bioreactor. If all of the variables in Eq. (16) are measured, the removal efficiency of the entire system can be calculated. Alternatively, it is also possible to estimate the removal efficiency through simulation. We can replace the terms relating to the exit gaseous benzene concentration ( $F_g C_G^o$ ,  $F_{gb} C_{Gb}$ ) with mass transfer terms from Eqs. (8) and (13). Now, Eq. (16) can be rearranged as:

Removal efficiency (%) =

$$\left(\frac{V_{bub} k_L a_{gas} (C^* - C_L) - V_{org} k_L a_{gas2} C_{org} - F_{aqu} C_{aqu}}{F_g C_G^i}\right) \times 100 \quad (17)$$

If the inlet gas flow rate and gaseous benzene concentration are given,  $C^*$ ,  $k_L a_{gas}$  and  $k_L a_{gas2}$  can be determined from the correlations mentioned earlier. The values of  $V_{bub}$ ,  $V_{org}$ , and  $F_{aqu}$  are given as operating parameters and then the remaining values ( $C_L$ ,  $C_{org}$ , and  $C_{aqu}$ ) are given from simulation results. It should be noted that the removal efficiency includes the removal of benzene both by absorption and biodegradation at transient state, but only by biodegradation at steady state.

The performance of the integrated system can be characterized by the removal efficiency of benzene at steady state. This was investigated with respect to the various operating parameters, and a sensitivity analysis was performed to evaluate which ones were most critical to the removal efficiency, as shown in Figure 5. Parameter sensitivity was determined as the difference in removal efficiency at the steady state when the values of the parameters were changed by +20% and -20%. Five parameters were considered: the agitation rate (350 rpm); aeration rate (0.3 vvm); HCR (2.0 L/h); AFR (0.2 L/h); and initial cell concentration (100 mg/L). The initial cell concentration induced the least change in the removal efficiency, with an impact of only 1.10%. As for aeration, which provides oxygen to the cells but also strips benzene from the bioreactor, its effect on the removal efficiency was thought to be dependent on benzene concentration in the organic phase. In an earlier study we found that a high benzene concentration of 14,000 mg/L in the organic phase caused a stripping rate of almost 240 mg/h, with a concomitant reduction in removal efficiency of 30.5% (Yeom and Daugulis, 1999b). However, in the present study, the highest benzene concentration was as low as 2000 mg/L in the organic phase in the two-phase bioreactor, and the sensitivity of aeration was found to be very low (1.22%). HCR, which delivers benzene from the absorption

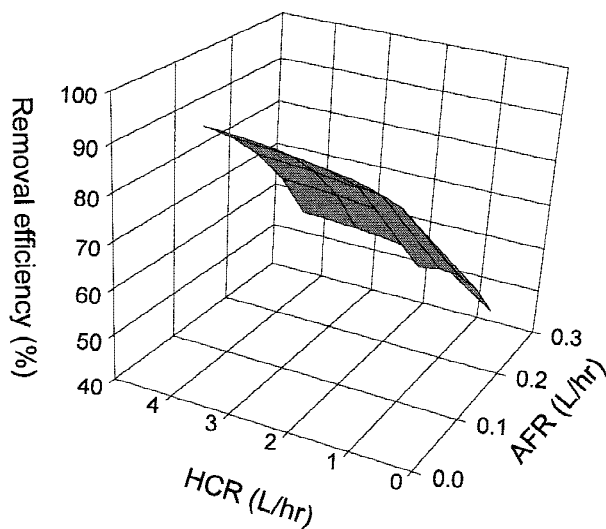


**Figure 5.** Sensitivity analysis of operational parameters on the removal efficiency of benzene. Screened bars: +20% variation; horizontally lined bars: -20% variation.

column to the two-phase bioreactor, was thought to be a very important operating parameter, but actually showed very low sensitivity over the experimental range studies. Agitation rate, which determines the mass transfer rate of benzene from the organic to the aqueous phases in the two-phase bioreactor, also showed low sensitivity, which suggests that mass transfer is not a rate-determining step in the two-phase system. Unlike the parameters mentioned earlier, AFR showed a significant 13.5% sensitivity of removal efficiency.

Next, we attempted to identify appropriate operating conditions for the treatment of a 120-L/h gas flow rate (well above the flow rate that induces well mixedness in the absorber) and with 4.2 mg/L of gaseous benzene. Because the usual retention time of gas flow in biofilters is in the range of 0.5 to 1.0 min, and the pollutant concentration is around 1.0 mg/L, we set the same retention time and a pollutant concentration at four times higher. Aeration and agitation rates were fixed at 0.25 vvm and 350 rpm, respectively. Because HCR and AFR were thought to be the most critical operating parameters from the sensitivity analysis, their influences on the removal efficiency of benzene were investigated by simulation. As shown in Figure 6, the removal efficiency increased with an increase in HCR or a decrease in AFR. Theoretically, it is possible to get >99% removal efficiency with extremely high HCR and low AFR; however, the system may be limited by a high cell concentration, which can cause oxygen limitation. In a previous study, it was found that there was no oxygen limitation with 0.25 vvm aeration and a cell concentration of 1700 mg/L. With this in mind, the optimal conditions were determined by grid search to be:

- Hexadecane circulation rate = 3.0 L/h.
- Aqueous flow rate = 0.12 L/h.



**Figure 6.** Effects of hexadecane circulation rate and aqueous phase flow rate on the removal efficiency of benzene.

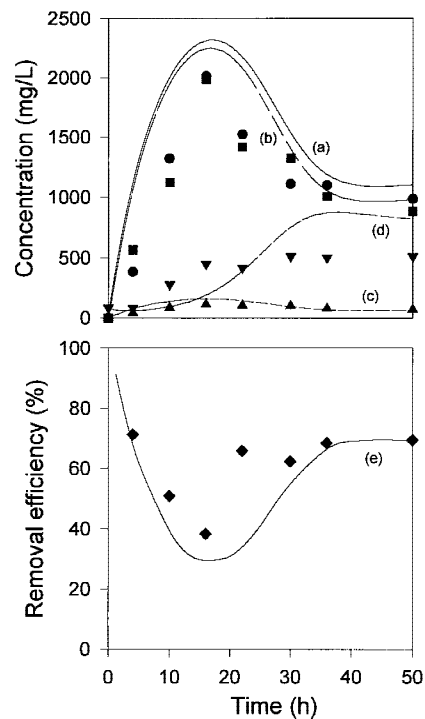
- Aeration rate = 0.25 vvm.
- Agitation rate = 350 rpm.

With these operating conditions, the predicted benzene concentration in the absorption column and in the organic and aqueous phases in the two-phase bioreactor at steady state were predicted to be 707.7, 562.0, and 3.25 mg/L, respectively. The final cell concentration was predicted to be 1645.2 mg/L.

### Verification Experiments

Two experiments were conducted to demonstrate the feasibility of the system, and to compare the results to the earlier predictions. Also, we wanted to show that the simulation exercise was useful in predicting optimal conditions and thus high removal efficiency. In both experiments, the influent gas flow rate and benzene concentration were set to 120 L/h and 4.2 mg/L, respectively.

In the first (nonoptimal) experiment, the aeration rate in the two-phase bioreactor was chosen arbitrarily as 500 mL/min (0.5 vvm on the basis of aqueous phase volume) and HCR and AFR were set at 3.0 and 0.2 L/h, respectively. The initial cell concentration was 85 mg/L. Figure 7 shows both the simulation and experimental results. The cells grew without a lag period, and up to around 15 h of operation, the benzene concentration in both the absorption column and the organic phase in the two-phase bioreactor increased. This was attributable to both the low biodegradation rate of benzene due to low cell concentration and the low benzene stripping due to low benzene concentration. As the benzene concentration increased, the exit benzene concentration also increased, and thus the removal efficiency decreased as shown in Figure 7. When the cell concentration and benzene concentration in the absorption column and organic phase reached 450, 2014, and 1985 mg/L, respectively, after 16 h

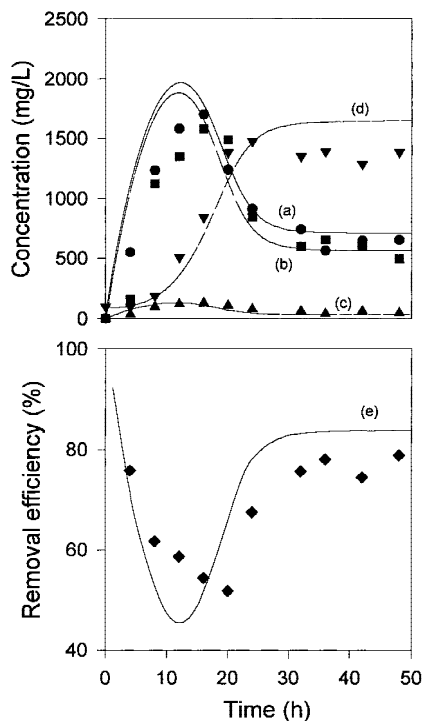


**Figure 7.** Operation of integrated system with arbitrarily chosen operating conditions: (a) benzene in absorption column (●); (b) benzene in organic phase (■); (c) benzene in an aqueous phase (▲); (d) cell concentration (▼); and (e) removal efficiency of benzene (◆). Lines represent simulation results.

of operation, the benzene concentration started to decrease due to higher biodegradation rate (high cell concentration), and the removal efficiency began to increase. After 30 h of operation, the system entered steady state, in which the benzene concentration and cell concentration in the system did not change. The expected cell concentration at steady state was 821 mg/L, but the actual concentration was 512 mg/L (37.6% lower). During the exponential phase of cell growth, the cells produced foam. Most of the foam stayed at the top of organic phase, but some also formed at the interface between the aqueous and organic phases. By means of microscopic examination it was determined that the foam contained a significant amount of cells, and cell growth was also observed on the walls of the bioreactor, as reported in a previous study (Yeom and Daugulis, 1999b). The removal efficiency of benzene at steady state from both the simulation and the experiment was about 69%.

In the second experiment, the optimal operating conditions were adopted from the simulation. The aeration rate was decreased to 0.25 vvm and the AFR was also decreased to 0.12 L/h. The other conditions were the same as those in the first experiment. During the early stages of the experiment the benzene concentration in the absorption column and the two-phase bioreactor increased due to the low cell concentration, as was also seen in the first experiment. After 15 h of operation, when the cells reached 840 mg/L, and the benzene concentrations in both the absorption column and the organic phase in the two-phase bioreactor reached 1700





**Figure 8.** Operation of integrated system under predicted optimal conditions: (a) benzene in the absorption column (●); (b) benzene in the organic phase (■); (c) benzene in the aqueous phase (▲); (d) cell concentration (▼); and (e) removal efficiency of benzene (◆). Lines represent simulation results.

mg/L and 1580 mg/L respectively, the benzene concentration started to decrease. After 30 h of operation, the system had reached steady state. Because the AFR was set lower than in the first experiment, the cell concentration in the aqueous phase was higher (1380 mg/L) and this led to a higher removal efficiency. During the exponential growth phase, the cells again produced foam, which resulted in a reduction in the effective aqueous phase cell concentration. The predicted removal efficiency in this experiment was 84% and the actual measured value was 75%.

The removal efficiency could be increased further by an increase in the cell concentration; however, the higher cell concentration would require high aeration and agitation rates, which could cause more benzene to be stripped. In this case, a condenser at the top of the bubble column and two-phase bioreactor could address this problem.

## CONCLUSION

In this study, a novel bioreactor system combining a bubble column and a two-phase bioreactor was used to remove gaseous benzene. Even though a relatively high concentration of benzene was injected into the system, it was absorbed in the bubble column containing hexadecane. The absorbed benzene was transferred to the two-phase bioreactor and partitioned to the aqueous phase where it was degraded by *Alcaligenes xylosoxidans* Y234. We found that

the mass transfer rate was much higher than the biodegradation rate, which means that biodegradation is the rate-limiting step. If the cell concentration were increased in the system, the benzene removal efficiency could be increased. The observed lower cell concentration (relative to the simulations) was due to entrainment and foaming, and is likely the major cause of the discrepancy between the predicted and actual performance of the system. A higher cell concentration would require higher agitation and aeration rates, which could potentially break the stability of the two-phase system and produce excessive foam. Using pure oxygen is an effective alternative approach (Collins and Daugulis, 1999b), but is relatively costly.

In future work, addressing the foaming issue will be one of the main challenges. We also plan to treat very high gaseous benzene concentrations (e.g., 20 mg/L) using the two-phase system, which is substantially higher than has been seen with biofilters. In addition, we will be examining the effectiveness of this system to handle gaseous mixtures (e.g., BTX) of volatile organics. Finally, using a counter-current packed-bed column instead of a bubble column should be a better choice in the design of the system in terms of achieving more efficient gas absorption.

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## NOMENCLATURE

$C^*$	liquid benzene concentration equilibrated with input gaseous benzene concentration in the absorption column (mg/L)
$C_{\text{aqu}}$	benzene concentration in aqueous phase in the two-phase bioreactor (mg/L)
$C_{\text{bubble}}^*$	liquid benzene concentration in the organic phase equilibrated with the benzene concentration in the inlet air bubbles into organic phase (mg/L)
$C_L$	liquid benzene concentration in the absorption column (mg/L)
$C_G$	gaseous benzene concentration in an absorption column (mg/L)
$C_{GB}$	exit gas benzene concentration from the two-phase bioreactor (mg/L)
$C_g^i$	benzene concentration into the absorption column (mg/L)
$C_g^o$	exit benzene concentration out of the absorption column (mg/L)
$C_{\text{org}}$	benzene concentration in the organic phase of two-phase bioreactor section (mg/L)
$f$	proportionality constant between gaseous benzene and equilibrated benzene in hexadecane (–)
$F_{\text{aqu}}$	aqueous feed rate of new medium (L/h)
$F_g$	gas flow rate into the absorption column (L/h)
$F_{gb}$	outlet gas flow rate from the two-phase bioreactor (L/h)
$F_{\text{org}}$	hexadecane circulation rate (L/h)
$k_L a_{\text{gas}}$	mass transfer coefficient of benzene in the absorption column (1/h)
$k_L a_{\text{gas}2}$	mass transfer coefficient of benzene out of the organic phase in the two-phase bioreactor section (1/h)
$k_L a_{\text{liq}}$	mass transfer coefficient of benzene from organic to aqueous phases in a two-phase bioreactor (1/h)
$K_s$	half-saturation constant in a Monod equation (mg/L)
$L$	length of absorption column containing hexadecane (cm)
$m_s$	partition coefficient of benzene between water and hexadecane (–)
$r$	reaction rate (mg/L · h)

$s$  substrate concentration (mg/L)  
 $t$  time (h)  
 $V_{\text{ad}}$  working volume of aqueous phase (L)  
 $V_{\text{bb}}$  working volume of absorption column section (L)  
 $V_{\text{og}}$  working volume of organic phase (L)  
 $X$  cell concentration in aqueous phase (mg/L)  
 $Y$  cell yield coefficient on benzene (-)  
 $Z$  axial coordinate (cm)

#### Greek letters

$\mu_{\text{max}}$  maximum specific growth rate (1/h)  
 $v_G$  superficial gas flow rate (cm/h)

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