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Analysis of a Simple Biodegradation Process for the Removal of Volatile Organic Chemicals from Wastewater Based on a Gas Stripping Principle

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A simple biodegradation system consisting of an air stripping tank and a bioreactor was proposed for the treatment of volatile organic chemicals in wastewater. Toluene was used as a model of volatile organic chemicals. An aqueous solution of toluene and a basic mineral medium were placed in the air stripping tank and bioreactor, respectively. Toluene was stripped by supplying compressed air into the stripping tank through a sparger, and the stripped toluene was degraded by *Pseudomonas putida* mt-2 (ATCC 33015) in the bioreactor under aerobic conditions. The effect of the air stripping rate on bacterial growth was examined. A quantitative relationship was found between the air flow rate in the air stripping tank (Q_a) and the stripping rate constant. During cultivation, the bacterial cells grew by utilizing toluene as the sole carbon source, and reached their maximum cell concentration (X_m) at the stationary phase. X_m showed a gradual decrease with increase in Q_a from 1.8 to 7.2 *l*/h, indicating a decrease in the rate of toluene degradation with increasing Q_a. The X_m at $Q_a=1.8$ *l*/h was the highest among the experiments under different values of Q_a, which was almost twice that at $Q_a=7.2$ *l*/h. Mathematical analysis taking the growth kinetics and mass transfer of toluene into consideration satisfactorily explained the system performance.

[**Key words:** biodegradation, bioreactor, gas stripping, toluene, volatile organic chemicals, wastewater]

Artificial organic chemicals have been utilized widely in industry and agriculture. Environmental pollution caused by the use of such chemicals has attracted much attention in recent years. Aromatic compounds such as toluene, benzene, nitrophenol and nitrotoluene, have been of considerable industrial importance as solvents and are the main raw materials used for the production of dyes, pesticides and explosives. These compounds are present in abundance in the environment. Biological treatment is expected to be effective for the treatment of industrial wastewater containing these chemicals. However, many organic compounds are highly toxic to microorganisms, and thus are difficult to degrade microbiologically unless the microbes are acclimated. In a mixed culture exposed to hazardous organic chemicals, microbes that are capable of degrading the chemicals grow, while those without the degrading capability die out. Therefore, a pure culture with a high capacity for degrading the hazardous chemicals and microbes selected by acclimation to the chemicals can be applied for industrial wastewater treatment from the practical viewpoint. Even though many aromatic compounds can be degraded by pure cultures and acclimated mixed cultures under aerobic conditions achieved by aeration (1, 2), due to the poor water solubility and high volatility of chemicals such as toluene and benzene, air stripping of the pollutants by aeration (3) not only reduces the rate of biodegradation, but also results in air pollution. Moreover, many of the point-source wastewaters exhibit extremes in pH, high salt concentrations, and/or contain carried-over catalysts (4), which are difficult to treat directly with microorganisms. An effective biological treatment system is thus needed for the removal of volatile organic chemicals at the source.

For the removal of volatile organic compounds contained in waste gases, biofiltration systems consisting of beds of biologically active material have been developed (5, 6), where operating conditions such as the moisture content of the bed and the transfer rate of pollutants are important. In biological wastewater treatment processes, volatile organic chemicals always undergo air stripping while being degraded (3, 7). From these reports, it is expected that for effective treatment of volatile organic chemicals in industrial wastewater at the source, a treatment process consisting of a gas stripping apparatus where the volatile chemicals are stripped by air or gas flow, and a bioreactor where the stripped volatile chemicals are degraded by microbes, is a promising idea. This process is considered to have the following advantages: (i) the aromatic pollutants produced by gas stripping can be degraded biologically in a separate bioreactor and thus factors such as the pH and salt concentration of the industrial wastewater will not affect the microorganisms; (ii) the aromatic pollutants can be degraded effectively by microbes which are in pure culture or acclimated under well-designed culture conditions; and (iii) operation is simple and the cost is low.

The present research was aimed at analyzing the performance of such a simple biodegradation system consisting of an air stripping tank followed in series by a bioreactor, using toluene as the model aromatic pollutant and *Pseudomonas putida* mt-2 (ATCC 33015) as the degrader. This microorganism utilizes toluene as the sole carbon source for its growth. The effect of the air stripping rate on bacterial growth and toluene degradation was examined experimentally and analyzed mathematically by taking the mass transfer of toluene and biodegradation kinetics into consideration.

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MATERIALS AND METHODS

Microorganism and medium Bacterium, *Pseudomonas putida* mt-2 (ATCC 33015) purchased from the Japan Collection of Microorganisms was used as the toluene degrader. This bacterium carries the well-known TOL plasmid which encodes the enzymes required for the degradation of toluene and xylene (8). The composition of the basal medium was as follows (in milligrams per *l*): $(NH_4)_2SO_4$, 2500; K_2HPO_4, 1400; $Na_2HPO_4 \cdot 12H_2O$, 3600; $MgSO_4 \cdot 7H_2O$, 2500; $CaCl_2 \cdot 2H_2O$, 16.0; $FeSO_4 \cdot 7H_2O$, 2.0. An aqueous solution of toluene prepared by dissolving toluene in pure water to about saturation concentration (approximately 500 mg/*l*) was used.

Experimental apparatus and procedures Bacterial cells were transferred from glycerol stock (1.0 ml) sorted at −80°C to a 100-ml vial containing 10 ml medium with toluene, which was a mixture of the aqueous solution of toluene and the basal medium at a volume ratio of 1 : 1. The bottle was sealed completely and precultured for 3 d in an incubator at 28°C and shaken at 130 rpm. The precultured bacterial cells were inoculated in 0.5 ml aliquots into a similar bottle containing 10 ml of the medium with toluene for subculture. The subculture was performed every three days under the same conditions. After three subcultures, by which time the effect of glycerol in the stock medium was eliminated completely, the bacterial cells (OD_{660}=0.40–0.45) were inoculated into the bioreactor for further experiments. The low final cell concentration was considered to be due to the use of toluene as the sole carbon source for the bacterial subculture.

The culture system consisting of a 0.5-*l* glass bottle (Kinoshita-type for gas washing, Tokyo Glass Instruments Co., Tokyo) and a 1-*l* flask used as the air stripping tank and bioreactor, respectively, is shown in Fig. 1 (abbreviated as ASTB system). The effective volumes of the air stripping tank and the bioreactor were 0.4 and 0.8 *l*, respectively. The aqueous solution of toluene and the basal medium were placed in the air stripping tank and the bioreactor, respectively. The air stripping was carried out using a compressor through a sparger installed at the bottom of the tank. To maintain the bioreactor under fully aerobic conditions, an extra sparger for aeration using an air pump was also used in addition to the sparger for dispersing the stripped toluene gas into the medium. The air flow rate (Q_a) in the stripping tank was varied from 1.8 to 7.2 *l*/h (0.075 to 0.3 vvm), and the aeration rate in the bioreactor was controlled to 3.6 *l*/h (0.075 vvm). For a comparison, cultivation in a single glass bottle (ST system shown in Fig. 1a) of the same type as that used in the ASTB system containing 0.4 *l* medium with the aqueous solution of toluene and the basal medium at volume ratio of 1 : 1 was also performed at an air flow rate of 1.8 *l*/h (0.075 vvm). All the cultivations were carried out by inoculating the subcultured bacterial cells (OD_{660}=0.40–0.45) at an inoculum size of 1/20 (v/v), a stirring speed of 150 rpm and at 28°C in a temperature-controlled water bath. The use of lower bacterial cell concentration of the inoculum was to examine the growth kinetics of the culture utilizing toluene as the sole carbon source in the ASTB system. After the start of the cultivation, changes in the cell density, toluene concentrations in the gas and liquid phases and pH with culture time were measured.

To obtain the bacterial growth yield, cultivation in a closed vial of 100 ml was also carried out. Nine vials each containing 5 ml of the basal medium and 5 ml of the aqueous solution of toluene were inoculated with 0.5 ml aliquots of the subcultured bacterial cells, then sealed and cultured under the same conditions as the subculture. After a predetermined cultivation period, one of the bottles was withdrawn for measurement of cell density and toluene concentrations. Toluene amounts in the gas and liquid phases were calculated from the respective concentrations and volumes, and the total toluene amount divided by the volume of the medium was considered as the total toluene concentration for further quantitative analysis.

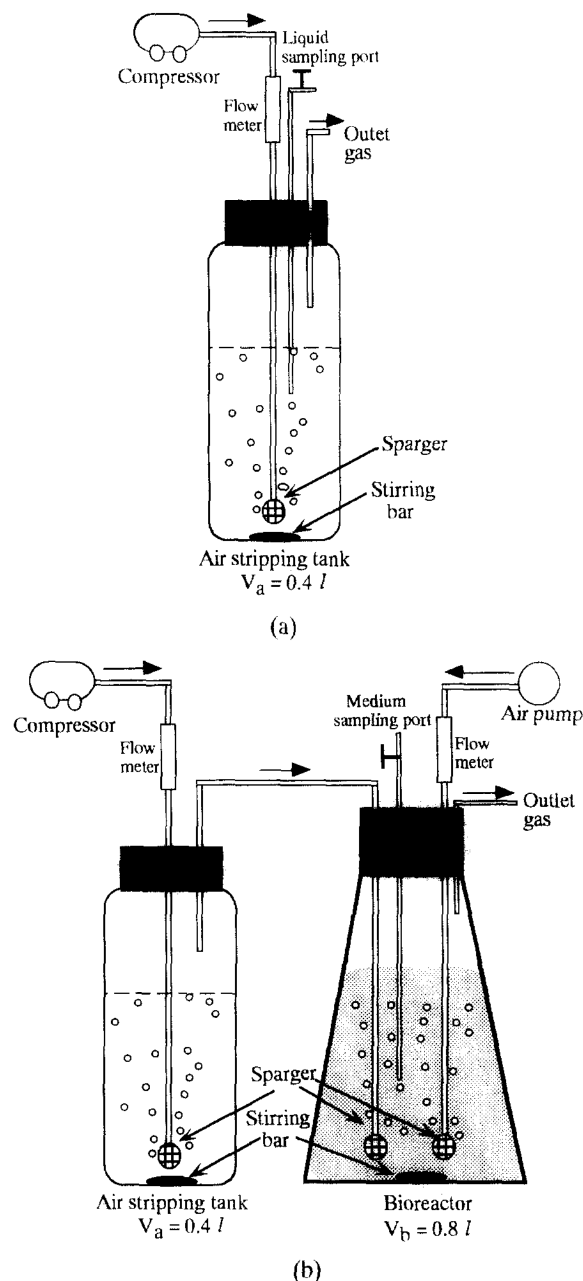

FIG. 1. Experimental apparatus used for air stripping of toluene and bacterial cultivation. (a) Apparatus for air stripping experiment or single-tank culture (ST system); (b) apparatus of air stripping tank linked with a bioreactor (ASTB system).

In order to evaluate the air stripping process, the time

course of changes in toluene concentrations in the gas phase at the outlet and the liquid phase in the air stripping tank shown in Fig. 1a was measured, and the data were analyzed by the Truong and Blackburn's method (3).

Analysis The cell density was measured by the optical density at a wavelength of 660 nm (OD_{660}). Toluene concentration was measured with a gas chromatograph (GC) equipped with a flame ionization detector (GC-14A, Shimadsu, Kyoto). A glass column, Unisole F-200 30/60 (2 m×3ϕ in I.D., GL Science Inc., Tokyo) was used. The temperatures of both the injector and detector were maintained at 150°C, and the column temperature was controlled at 100°C. The flow rate of nitrogen carrier gas was 40 ml/min. The concentration of toluene in the culture broth was measured by the GC method after centrifugation at 4°C and 24.1×g for 5 min. The pH of the culture broth was measured with a pH meter (F-13, Horiba Co., Tokyo).

Chemicals All the chemicals, of commercial grade, were purchased from Wako Pure Chemicals Co. (Osaka).

RESULTS AND DISCUSSION

Estimation of air stripping rate constant As reported by Truong and Blackburn (3), air stripping of toluene dissolved in water can be expressed as a first-order kinetic process as represented in Eq. 1.

$$R_a = -dC_a/dt = K_a{}^s a C_a \quad (1)$$

where, R_a is the rate of air stripping of toluene from the aqueous phase into the gas phase, $K_a{}^s a$ is the air stripping rate constant based on disappearance of the aqueous phase, and C_a is the toluene concentration in the aqueous phase.

Integrating the results from Eq. 1 to Eq. 2,

$$\ln(C_{a0}/C_{at}) = K_a{}^s a t \quad (2)$$

where C_{a0} and C_{at} are the organic concentrations at times 0 and t, respectively. $K_a{}^s a$ can thus be obtained from the plot of $\ln C_{a0}/C_{at}$ vs. t.

Figure 2 shows the relationship of C_{a0}/C_{at} in the exponential scale vs. t based on the experimental data concerning toluene concentration in the aqueous phase at different air flow rates in the air stripping tank shown in Fig. 1a. In all of the cases, $\log(C_{a0}/C_{at})$ vs. t was nearly linear. The air stripping rate constants were obtained from the slopes.

Through research on air stripping of volatile chemicals in biological wastewater treatment under air flow rates ranging from about 0.07 to 0.31 vvm, Truong and Blackburn concluded that the $K_a{}^s a$ is nearly equal to $(Q_a/V_a)(H_C)^{0.9}$ (H_C is a dimensionless Henry's law constant, which is 0.15 for toluene at 20°C) (3). Figure 3 shows a double logarithmic plot of $K_a{}^s a$ vs. (Q_a/V_a) obtained from the above air stripping experiment. From the result shown in Fig. 3, $K_a{}^s a$ was correlated to (Q_a/V_a) as $K_a{}^s a = 0.17(Q_a/V_a)$, which was similar to Truong and Blackburn's results.

A mass balance for toluene in both the liquid and gas phases in the air stripping tank can be expressed by Eq. 3.

$$Q_a(C_g{}^{ao} - C_g{}^{ai}) = V_a R_a = V_a K_a{}^s a C_{at} \quad (3)$$

where $C_g{}^{ai}$ and $C_g{}^{ao}$ are toluene concentrations in the gas phase at the inlet and outlet of the air stripping tank,

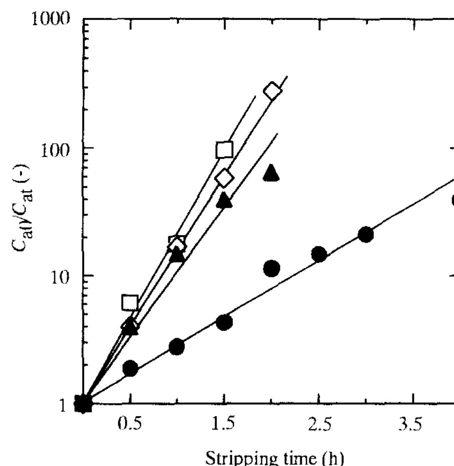

FIG. 2. Plot of C_{a0}/C_{at} vs. stripping time under different air flow rates (Q_a) in the air stripping tank with lines of best fit. Symbols: ●, Q_a=1.8 l/h; ▲, Q_a=3.6 l/h; ◇, Q_a=5.4 l/h; □, Q_a=7.2 l/h.

respectively, Q_a is the air flow rate and V_a is the volume of the tank. As $C_g{}^{ai}=0$ and $V_a=0.4\,l$, from Eq. 3 and the above correlation between $K_a{}^s a$ and Q_a, the relationship between $C_g{}^{ao}$ and C_{at} is given by Eq. 4.

$$C_g{}^{ao}/C_{at} = (V_a/Q_a)K_a{}^s a = \alpha = 0.17 \quad (4)$$

Figure 4 shows a comparison of the results of calculation using Eq. 4 and the experimental data for $C_g{}^{ao}$ and C_{at} which were obtained from the same air stripping experiment as that shown in Fig. 2. In all cases, the results of calculation using Eq. 4 agreed satisfactorily with the experimental results, which supports the validity of Eq. 4.

Analysis of bacterial growth and toluene degradation under different air stripping rates Bacterial cells grew by degrading toluene and the growth curves in the ASTB system under different air stripping rates and the control culture system of ST are shown in Fig. 5. After the start of the cultivations, the OD_{660} increased and gradually reached an almost constant level (X_m) which indicated the cessation of bacterial growth. The X_m decreased with an increase in Q_a in the ASTB system, while that in the ST system was the lowest. The X_m at Q_a=1.8 l/h was almost twice that at Q_a=7.2 l/h and in the ST system.

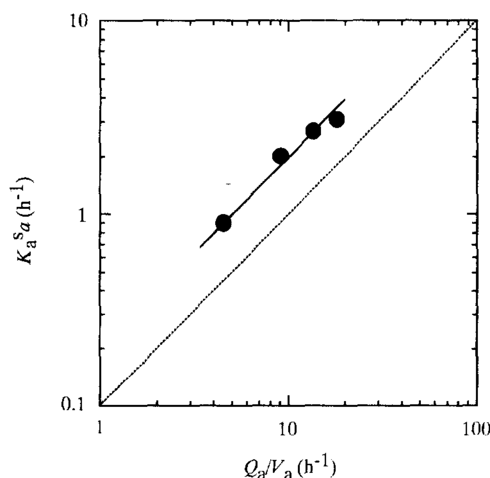

FIG. 3. Relationship between $K_a{}^s a$ and Q_a/V_a in the air stripping tank.

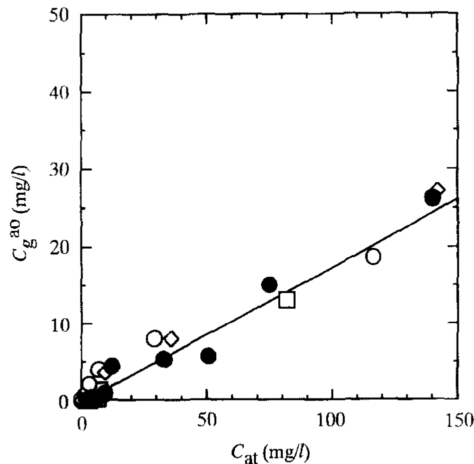

FIG. 4. Comparison of calculation using Eq. 4 (line) and experimental results of toluene concentrations in the gas phase and liquid phase in the air stripping tank (symbols). Symbols: ●, Q_a =1.8 l/h; ◇, Q_a=3.6 l/h; ○, Q_a=5.4 l/h; □, Q_a=7.2 l/h.

Because bacterial growth was dependent on the degradation of toluene which was used as the sole carbon source, the results shown in Fig. 5 indicate that controlling the air stripping rate in the ASTB system was more effective for the removal of toluene than that in the single-tank culture system with aeration. Air stripping of toluene by aeration in the ST system reduced the bacterial degradation rate of toluene.

In order to analyze the performance of the ASTB system, the following assumptions were first made: (i) the bioreactor in the ASTB system is completely mixed; (ii) the bacterial growth is described by the Monod equation, where toluene is the growth limiting substrate; (iii) the gas and liquid phases at the interface are in equilibrium (ratio of the toluene concentration in the gas phase to that in the liquid phase at the interface is constant (H_C), *i.e.*, Henry's law is applicable).

Under the above assumptions, the bacterial growth and toluene utilization rates are expressed by Eqs. 5 and 6.

$$R_X = dX/dt = \mu_m X C_b/(K_s + C_b) \quad (5)$$

$$R_C = -(dC_b/dt)_g = R_X/Y_C \quad (6)$$

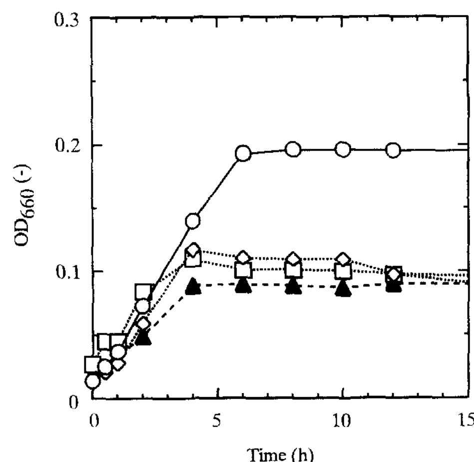

FIG. 5. Growth curves in the ASTB system under different Q_a and in the ST system as the control. Symbols: ○, Q_a=1.8 l/h; ◇, Q_a =3.6 l/h; □, Q_a=7.2 l/h, ▲, control (ST).

where, R_X and R_C are the cell growth rate and substrate consumption rate, respectively, Y_C is the observed cell yield on toluene, μ_m is the bacterial maximal growth rate constant and K_s is the Monod constant.

In the bioreactor of the ASTB system, the change in toluene concentration (C_b) in the liquid phase is dependent on the transfer rate of toluene from the outlet gas phase of the air stripping tank into the medium of the bioreactor, the bacterial utilization rate and the air stripping by aeration. The transfer process of toluene from the air-stripped gas into the medium of the bioreactor can be considered to be similar to that of oxygen (9). The change of C_b can thus be expressed by Eq. 7.

$$R_b = dC_b/dt = K_La(C_b^* - C_b) - R_C - K_b^s a C_b \quad (7)$$

where K_La is the overall mass transfer coefficient of toluene from the gas phase into the liquid phase, $K_b^s a$ is the air stripping rate constant of toluene by aeration in the bioreactor, and C_b^* is the liquid-side toluene concentration at the gas-liquid interface. At a constant temperature, C_b^* will correspond to the toluene partial pressure, *i.e.*, the toluene concentration in the inlet gas phase of the bioreactor (C_g^{bi}), and can be expressed by the following equation under the above assumption that the gas and liquid phases at the interface are in equilibrium.

$$C_b^* = C_g^{bi}/H_C \quad (8)$$

Because the effect of the distance between the air stripping tank and the bioreactor could be neglected, C_g^{bi} is approximated by the toluene concentration in the outlet gas phase of the air stripping tank (C_g^{ao}). From Eqs. 2 and 4, C_g^{bi} can be given by the following equation:

$$C_g^{bi} = C_g^{ao} = (V_a/Q_a)K_a^s a C_a = \alpha C_{a0} \exp(-K_a^s a\, t) \quad (9)$$

By substituting Eq. 9 into Eq. 7, Eq. 10 is obtained.

$$dC_b/dt = K_La[\alpha/H_C C_{a0} \exp(-K_a^s a\, t) - C_b] - R_C - K_a^{bs} a\, C_b \quad (10)$$

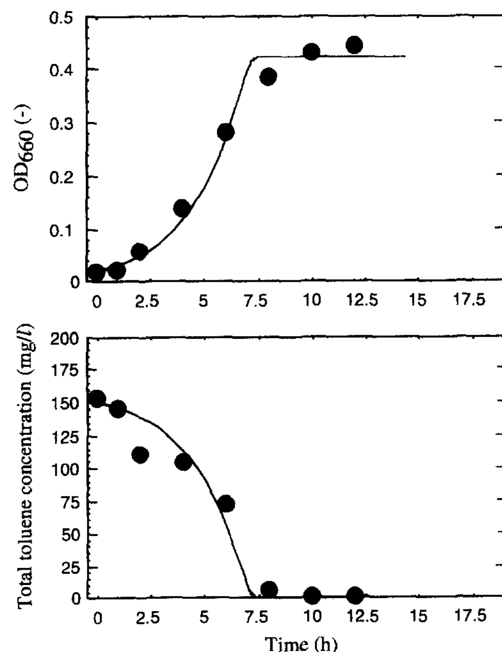

FIG. 6. Growth curve (A) and change in total toluene concentration (B) during cultivation in closed vials. Circles, experimental data; lines, calculation by the Monod equation.

TABLE 1. Parameters and constants used for the simulation

Constants			Parameters		
μ_m	0.45	h^{-1}	C_{a0}	500	mg/l
Y_C	2.67×10^{-3}	OD_{660}/mg/l	C_{b0}	0	mg/l
K_s	6.00	mg/l	C_{T0}	161	mg/l
H_C	0.15*	—	C_g^{ai}	0	mg/l
$K_b{}^s a$	0.86	h^{-1}	X_0	0.02	—

* From ref. (3)

Toluene concentration in the outlet gas phase of the bioreactor (C_g^{bo}) is expressed by Eq. 11.

$$(Q_a+Q_b)C_g^{bo}-Q_aC_g^{bi}=V_bR_b \qquad (11)$$

Therefore, Eqs. 5, 6 and 9–11 can be used to calculate the cell growth rate and changes in toluene concentrations in the liquid and gas phases of the bioreactor, when the values of Y_C, μ_m, K_s and K_La are known. Because the volume of the bioreactor was different from that of the air stripping tank, the air stripping rate constant $K_b{}^s a$ at the aeration rate of 3.6 l/h could be estimated by Eq. 4.

The parameters of Y_C, μ_m and K_s were obtained from the experimental data in the culture in closed vials as described in Materials and Methods. Figure 6 shows the growth curve and change in the total toluene concentration. The total toluene concentration (C_T) calculated from the measured toluene concentrations in the liquid and gas phases by taking the respective phase volumes into consideration, decreased gradually with bacterial growth. After the toluene was entirely exhausted, the bacterial growth stopped. Figure 6 shows the results of fitting Eqs. 5 and 6 to the respective experimental data under the initial condition of C_{T0}=161 mg/l (Table 1). By curve-fitting, the above parameters could be determined and are also summarized in Table 1. From Fig. 6, it is clear that analysis of the growth kinetics considering toluene as the growth-limiting substrate mentioned above is appropriate.

TABLE 2. Value of K_La obtained by curve fitting (Fig. 7) and ratio of K_La to $K_b{}^s a$

Q_a (l/h)	K_La (h^{-1})	$K_La/K_b{}^s a$ (−)
1.8	0.55	0.61
3.6	1.20	0.60
7.2	2.00	0.65

A simulation of bacterial growth and changes in toluene concentrations during the cultivations based on Eqs. 5, 6 and 10–11 at a given K_La was carried out with the software *Mathematica* for Macintosh (Version 2.2) using the parameters and initial conditions of $K_b{}^s a$, C_{a0}, C_{b0} and X_0 shown in Table 1, and the values of $K_a{}^s a$ shown in Fig. 3. A comparison of the simulation with the experimental data in the ASTB system under different values of Q_a is shown in Fig. 7. The mathematical simulation agreed well with the experimental results obtained in all of the cases. The values of K_La obtained by the simulation are listed in Table 2. It was clear that K_La

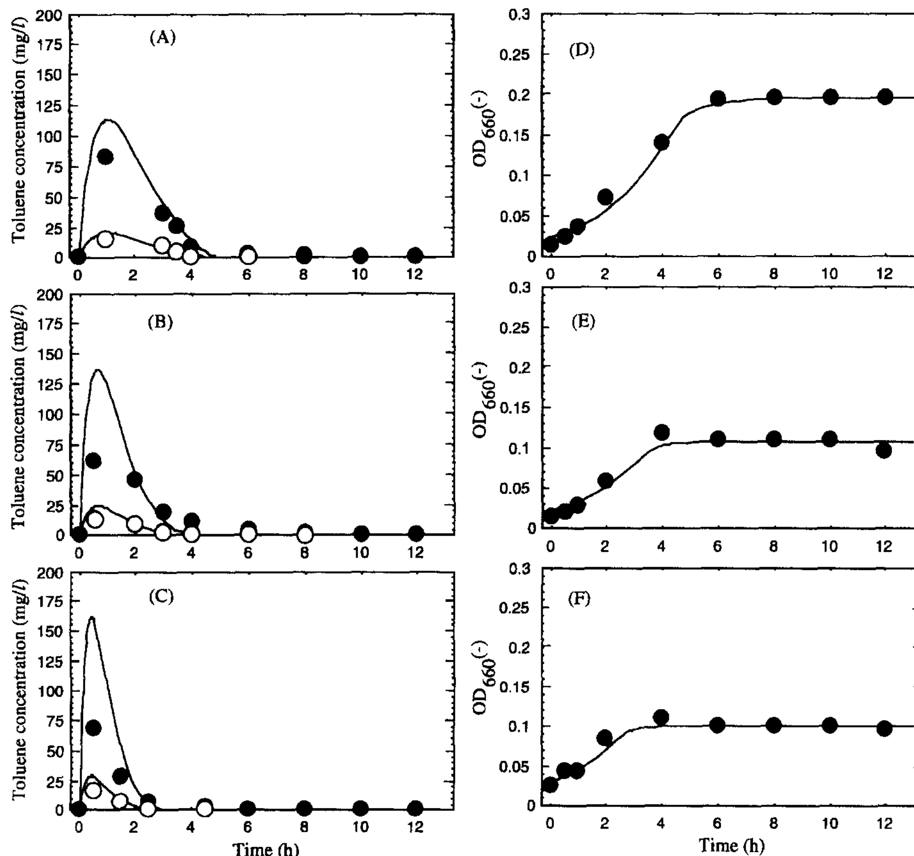

FIG. 7. Comparison of results of simulation (lines) and experimental data (circles) for various toluene concentrations (A, B, C) and OD_{660} (D, E, F). The experimental data were the same as those shown in Fig. 5. Open circles, Toluene concentration in the gas phase; closed circles, toluene concentration in the liquid phase and OD_{660}. Q_a=1.8 l/h (A, D); 3.6 l/h (B, E); 7.2 l/h (C, F).

increased with an increase in Q_a, *i.e.* the air stripping rate, and $K_La/K_a{}^sa$ remained almost constant. This result was considered reasonable because the aeration rate in the bioreactor of the ASTB system was kept constant, and Q_a would therefore affect both $K_a{}^sa$ and K_La identically. The reason that X_m decreased with an increase in Q_a, even though K_La was raised, was presumably due to the fact that the exponential item in Eq. 10 decreased more quickly than the increase in K_La.

In summary, the proposed culture system consisting of a gas stripping tank followed by a bioreactor was effective for the degradation of volatile organic chemicals in wastewater, and system performance was analyzed satisfactorily by taking the growth kinetics and mass transfer of the chemicals into consideration.

From the values of X_m obtained in all of the experiments (Fig. 7) and Y_C, removal efficiency of toluene by the bacterium could be calculated. Approximately 30% of the initial total amount of toluene placed in the air stripping tank was removed after 6-h cultivation at $Q_a = 1.8$ *l*/h. The relatively poor rate of toluene removal was considered to be due to the use of a completely mixed bioreactor and the relatively lower inoculum size. Based on the findings obtained here, further research for improving the removal efficiency of the air-stripped toluene is being carried out using a plug-flow type bioreactor with a high cell concentration.

NOMENCLATURE

C_a : toluene concentration in the aqueous phase of the air stripping tank, mg/*l*

C_{a0} : initial toluene concentration in the aqueous phase of the air stripping tank, mg/*l*

C_{at} : toluene concentration in the aqueous phase of the air stripping tank at time *t*, mg/*l*

$C_g{}^{ao}$: toluene concentration in the outlet gas phase of the air stripping tank, mg/*l*

$C_g{}^{ai}$: toluene concentration in the inlet gas phase of the air stripping tank, mg/*l*

C_b : toluene concentration in the liquid phase of the bioreactor, mg/*l*

$C_g{}^{bo}$: toluene concentration in the outlet gas phase of the bioreactor, mg/*l*

$C_g{}^{bi}$: toluene concentration in the inlet gas phase of the bioreactor, mg/*l*

$C_b{}^*$: liquid-side toluene concentration at the interface between gas and liquid in the bioreactor corresponding to $C_g{}^{bo}$, mg/*l*

C_{b0} : initial toluene concentration in the liquid phase of the bioreactor, mg/*l*

C_{T0} : initial total toluene concentration calculated from the measured toluene concentrations in the gas and liquid phases in a closed culture system, mg/*l*

H_C : dimensionless Henry's law constant, —

$K_a{}^sa$: air stripping rate constant of toluene in the aqueous phase of the air stripping tank, h^{-1}

$K_b{}^sa$: air stripping rate constant of toluene in the liquid phase by aeration in the bioreactor, h^{-1}

K_La : overall mass transfer coefficient of toluene from the gas phase into the liquid phase, h^{-1}

K_s : Monod constant, mg/*l*

Q_a : air flow rate in the air stripping tank, *l*/h

Q_b : aeration rate in the bioreactor, *l*/h

R_a : rate of air stripping of toluene from the aqueous phase into the gas phase in the air stripping tank, mg/*l*/h

R_b : rate of change of toluene concentration in the liquid phase in the bioreactor, mg/*l*/h

R_C : substrate consumption rate, mg/*l*/h

R_X : cell growth rate, OD_{660}/h

t : time, h

V_a : air stripping tank volume, ml

V_b : bioreactor volume, ml

X : cell concentration (OD_{660}), —

X_m : maximum cell concentration achieved at the stationary phase (OD_{660}), —

Y_C : cell yield on toluene, OD_{660}/mg/*l*

μ_m : bacterial maximal growth rate constant, h^{-1}

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