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Development and mathematical modeling of a two-stage reactor system for trichloroethylene degradation using *Methylosinus trichosporium* OB3b

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Abstract

A two-stage reactor system was developed for the continuous degradation of gas-phase trichloroethylene (TCE). *Methylosinus trichosporium* OB3b was immobilized on activated carbon in a TCE degradation reactor, trickling biofilter (TBF). The TBF was coupled with a continuous stirred tank reactor (CSTR) to allow recirculation of microbial cells from/to the TBF for the reactivation of inactivated cells during TCE degradation. The mass transfer aspect of the TBF was analyzed, and mass transfer coefficient of 3.9 h^{-1} was estimated. The loss of soluble methane monooxygenase (sMMO) activity was modeled based on a material balance on the CSTR and TBF, and transformation capacity (T_c) was determined to be 20.2 μmol mg^{-1}. Maximum TCE degradation rate of 525 mg l^{-1} d^{-1} was obtained and reactor has been stably operated for more than 270 days.

Nomenclature: a – activity of sMMO (TCE per cell and time, nmol mg^{-1} min^{-1}); A – cross-sectional area of TBF (cm^2); a_{max} – maximum activity of sMMO (nmol min^{-1} mg^{-1}); B – bleed rate (ml min^{-1}); C_g – outlet TCE concentration in gas phase of TBF (mol l^{-1}); C_{g0} – inlet TCE concentration in gas phase of TBF (mol l^{-1}); C_l – TCE concentration in liquid phase of TBF (mol l^{-1}); C^*_l – TCE concentration in interface of liquid phase of TBF (mol l^{-1}); C_s – TCE concentration in activated carbon support of TBF (mol l^{-1}); C_b – TCE concentration in biofilm of TBF (mol l^{-1}); C_x – TCE concentration in liquid phase of TBF (mol l^{-1}); $\Delta C_g - C_{g0} - C_g$ (mol l^{-1}); F_g – gas flow rate of TBF (ml min^{-1}); F_l – liquid flow rate to TBF (ml min^{-1}); $F'_l - F_l - B$ (ml min^{-1}); h – height of TBF (cm); H – Henry's constant (atm 1 mg^{-1}); k_la – gas/liquid mass-transfer coefficient (h^{-1}); R – ideal gas constant (atm 1 mol^{-1} K^{-1}); P_g – partial pressure of gas (atm); r – rate of TCE degradation (mg l^{-1} d^{-1}); S – growth substrate concentration in CSTR (mol l^{-1}); S_0 – growth substrate concentration of inflow to CSTR (mol l^{-1}); S' – growth substrate concentration of outflow from TBF (mol l^{-1}); $\Delta S - S_0 - S$ (mol l^{-1}); t – time (h); T – kelvin temperature (K); T_c – transformation capacity (μmol mg^{-1}); V_1 – CSTR working volume (l); X_d – dead cell concentration in CSTR (mg ml^{-1}); X'_d – dead cell concentration of outflow from TBF (mg ml^{-1}); X_{max} – maximum cell concentration (mg ml^{-1}); X_v – viable cell concentration in CSTR (mg ml^{-1}); X'_v – viable cell concentration of outflow from TBF (mg ml^{-1}); X_t – total cell concentration (mg ml^{-1}); Y – yield coefficient (cell per substrate, mg mg^{-1}); *Greek symbols*: $\alpha_1 - F'_l/F_l$ (dimensionless); $\alpha_2 - B/F_l$ (dimensionless); $\alpha_3 - V_1/F_l$ (min); $\alpha_4 - F_g/F_l$ (dimensionless); ε_b – fraction of biofilm (dimensionless); ε_g – fraction of gas phase (dimensionless); ε_s – fraction of activated carbon support (dimensionless); ε_x – fraction of liquid phase (dimensionless); μ – specific grow rate in CSTR (h^{-1}); μ_{max} – maximum specific grow rate in CSTR (h^{-1}); φ – viable cell fraction in CSTR (dimensionless).

Introduction

Trichloroethylene (TCE) is one of the most widespread contaminants in underground water, soil and air due to its extensive industrial use as an industrial solvent and degreasing agent (Ensley 1991; Lee 2001; Westrick et al. 1984). Since TCE is a suspected carcinogen and constitutes public health concern for drinking water, many efforts have been devoted to treat this hazardous waste (Alvarez-Cohen & McCarty 1991; Sun & Wood 1997).

Conventional counter-current air stripping and granular activated carbon (GAC) adsorption processes are expensive and simply transfer TCE to a new phase without destroying it, which requires subsequent treatment (Love & Eilers 1982). Microbial degradation is a promising alternative for the remediation of TCE, where TCE can be completely mineralized into harmless end products by enzymes. Since TCE is not a growth substrate, it can be treated through co-metabolism in which an oxygenase fortuitously degrades TCE (Chang & Alvarez-Cohen 1995; Kang et al. 2001). Among microorganisms employed in biodegradation process, *Methylosinus trichosporium* OB3b has the highest TCE degradation rate ($V_{max} = 278$ nmol mg^{-1} min^{-1}). *M. trichosporium* possesses a TCE-degrading enzyme, soluble methane monooxygenase (sMMO), and sMMO is effectively expressed when cells are grown in a copper-deficient medium (Tsien et al. 1989).

Numerous biofilter systems have been investigated to treat TCE in a continuous mode. A single-stage trickling biofilter (TBF) has received much attention for the treatment of TCE because of its cost-effectiveness and complete degradation of TCE into harmless end products (Fennel et al. 1993; Fitch et al. 1996; Lee et al. 2003b; Livingston 1991; Tschantz et al. 1995). However, TCE is not easily treated in a continuous mode by a simple TBF system. This is related to the toxicity of TCE to microbial cells. TCE and its degradation intermediates inactivate enzymes and decrease the degradation activity of cells. A higher removal efficiency of TCE in a simple TBF has been maintained only for short operational periods, and the microbial activities of TCE degradation rapidly decrease with time (Oldenhuis et al. 1991; Strandberg et al. 1989; van Hylckama Vlieg et al. 1997). The addition of growth substrate is needed to recover the microbial activity for a continuous degradation. When methane is provided to TBF containing *M. trichosporium*, a severe competitive inhibition between methane and TCE occurs, which hamper an efficient treatment of TCE. Therefore, the development of a novel biofilter system is required to solve these problems. A two-stage treatment system consisting of growth and treatment reactors is an attractive system when competitive inhibition between a growth substrate and TCE is involved and degradation products are toxic to the microbial cells (Chang & Alvarez-Cohen 1997; Lee 2003; Sipkema et al. 1999).

The objective of this work was to develop and model the treatment of gas-phase TCE in a continuous mode using a two-stage reactor system. The two-stage system consists of one TBF unit and one continuous stirred tank reactor (CSTR) unit in a serial mode. *M. trichosporium* OB3b was immobilized on activated carbon in the TCE degradation reactor, TBF. The CSTR with cell recycle from/to the TBF provided the reactivation of inactivated microbial cells during TCE degradation in the TBF. The effects of operation conditions on TCE removal and degradation rate were studied, and reactor performance was evaluated for the continuous treatment of gas-phase TCE. The analysis of mass-transfer limitation in the TBF and estimation of the transformation capacity (T_c) of the two-stage reactor using mathematical modeling were carried out to determine parameters for reactor design and scale-up.

Materials and methods

Microorganism, culture medium and chemicals

The microorganism employed in this study was *M. trichosporium* OB3b provided by Dr. R.T. Taylor at the Biomedical Sciences Division of the Lawrence Livermore National Laboratory (USA). A copper deficient modified Higgins nitrate medium was used as the culture medium to avoid copper suppression of sMMO expression (Taylor & Hanna 1995). Seed culture of *M. trichosporium* OB3b was performed in a 300 ml side-arm flask in a shaking incubator (KMC-8480sf, Vision Scientific Co., Korea). Flow rates of methane and air were controlled by a gas proportioner (Cole-Parmer II

60648, USA), and the gases were injected through the sampling septum. Analytical grade TCE was obtained from Aldrich Chemical Co. (USA).

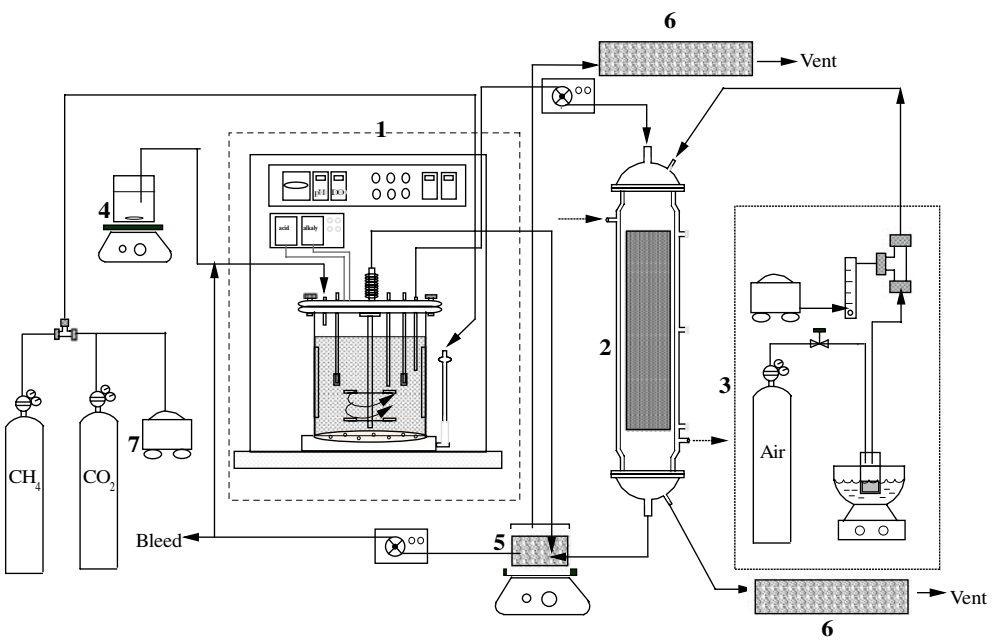
Analytical methods

The concentrations of TCE were measured by analysis of 5 μl of inlet and outlet gas-phase samples on a gas chromatograph (Hewlett Packard 5890, USA) equipped with a flame ionization detector and Graphac C column (Alltech Inc., USA). Nitrogen of 30 ml min^{-1} was used as a carrier gas, and the temperatures of oven, detector and injector were 150, 190 and 170 °C, respectively. For the determination of liquid TCE concentrations, liquid samples were taken into Teflon-cap vials and then the vials were incubated at 30 °C for 30 min with shaking. After equilibrium between gas and liquid phases had been reached, 5 μl of a gas-phase sample was analyzed by GC and the concentration was determined by TCE-in-air calibration curve prepared by the values of partition coefficient and the volumes of two phases in the vials (Lee et al. 2003a). Methane concentration in a gas phase was determined from 300 μl of gas sample by GC (Young-In GC, Korea) with a thermal conductivity detector, helium (as carrier gas; 30 ml min^{-1}), and a packed column (6 ft stainless steel with Porapak Q, Alltech, USA; oven temperature, 40 °C).

The activity of sMMO was analyzed by measuring the epoxidation rate of propene as described in the reference (Lee et al. 2003a). Cell concentration was determined by measuring the absorbance at 660 nm (UV1240, Shimazhu, Japan) with the use of calibration curve.

Development and operation of the two-stage reactor system

We employed a TBF containing biofilms to treat a high concentration of TCE since a biofilm is more resistant to opportunistic shock loading of a high inlet TCE concentration. A schematic diagram of the two-stage reactor system is shown in Figure 1. *M. trichosporium* OB3b was cultured in CSTR at



1. CSTR 2. Trickling filter 3. TCE Feeding Unit 4. Nutrient 5. Stripping Unit 6. Activated Carbon Trap 7. Air Pump

Figure 1. Schematic diagram of the two-stage reactor system. The TBF consists of a 1.9 l glass cylinder diameter: 7 cm, height: 50 cm). Activated carbon as supporter matrix was packed to a depth of 20 cm. *M. trichosporium* OB3b was cultured in the CSTR at 500 rpm, 30 °C and a dilution rate of 0.017 h^{-1}. The liquid volume of the CSTR was 3.5 l.

500 rpm, 30 °C and a dilution rate of 0.017 h^{-1}. Here, the dilution rate means to be a hydraulic retention time in the CSTR. The liquid volume of the CSTR was 3.5 l. The operation condition of CSTR was previously examined and optimized to achieve maximal levels of sMMO activity (Shah et al. 1992). Methane and oxygen were supplied at rates of 0.2 l min^{-1} and 0.4 l min^{-1}, respectively. To increase the cell concentration in the CSTR, the concentration of methane together with the concentrations of medium components of the mineral salts medium were increased.

The TBF consists of a 1.9 l glass cylinder (diameter: 7 cm, height: 50 cm). Activated carbon as supporter matrix was packed to a depth of 20 cm. All fittings and connectors were gas-tight and the temperature of the TBF was controlled using a water circulator. Prior to biofilm development, the activated carbon was soaked in a medium solution for 18 h. The biofilm on the activated carbon in the TBF was developed several days after seeding by circulation of the culture broth from/to the CSTR. After biofilms had been developed, contaminated gas containing TCE was introduced to the top of the TBF by a syringe pump at various inlet air flow rates, ranging from 50 to 600 ml min^{-1}. The culture broth containing *M. trichosporium* OB3b was pumped concurrently from the CSTR to the TBF at various flow rates to secure an efficient degradation of TCE at the TBF, and then the broth was recirculated back to the CSTR.

Results and discussion

Mathematical modeling on mass transfer limitation in the TBF

In the following, a mathematical model is presented for describing the biodegradation of gas-phase TCE in the two-stage reactor. In biofilm reactors like TBF, modeling considers two basic processes: mass transfer of TCE from gas phase to the microbial cells and intrinsic kinetics of TCE degradation by the cells. It can be expected that there is mass transfer limitation in the TBF because of transfer resistance of TCE from gas to liquid phase and poor solubility of TCE.

For better understanding of mass transfer-limited characteristics of the TBF, a mathematical model is developed. The TBF is considered to be a plug flow reactor and material balance equations for TCE over dh are as follows (Figure 2):

$$\text{TCE Accumulation} = \text{input} - \text{output} + \text{generation}$$
$$\text{(i)} \qquad \text{(ii)} \qquad \text{(iii)} \qquad \text{(iv)} \qquad (1)$$

(i) at steady state

$$\frac{dC_s}{dt}(A \cdot dh \cdot \varepsilon_s) + \frac{dC_b}{dt}(A \cdot dh \cdot \varepsilon_b) + \frac{dC_\chi}{dt}(A \cdot dh \cdot \varepsilon_x) + \frac{dC_g}{dt}(A \cdot dh \cdot \varepsilon_g) = 0$$

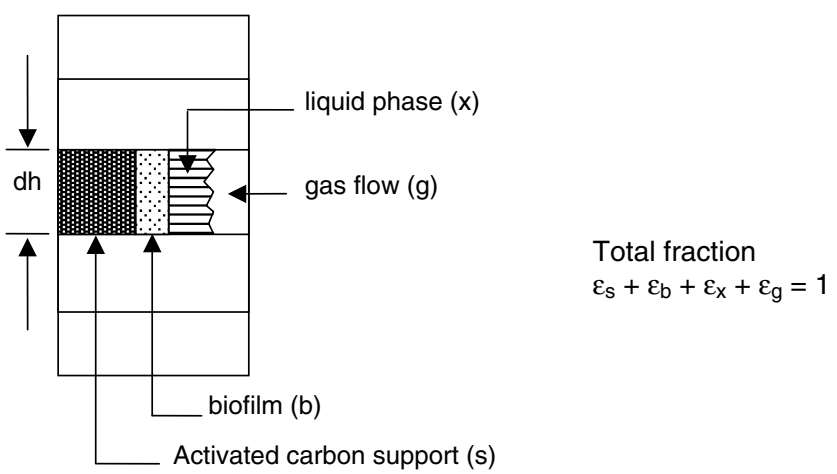


Figure 2. Schematic diagram on a slab in the TBF.

(ii) and (iii)

$$\left(F_l \cdot C_l|_h + F_g \cdot C_g|_h\right) - \left(F_l \cdot C_l|_{h+dh} + F_g \cdot C_g|_{h+dh}\right)$$

(iv) $-r \cdot A \cdot dh$

When $F_g \cdot C_g$ is higher than $F_l \cdot C_l$ and F_g is constant, Equation (1) can be written as:

$$F_g dC_g = -rAdh \tag{2}$$

If TCE removal rate at steady state where volumetric degradation rate is equal to TCE mass transfer from gas to liquid phase is mass transfer-limited ($C^*_l \gg C_l$ or $C_l \approx 0$):

$$r = k_l a(C^*_l - C_l) \cong k_l a \cdot C^*_l \tag{3}$$

Here, we consider Henrys' law:

$$P_g = HC^*_l \text{ or } C^*_l = \frac{P_g}{H} \tag{4}$$

Substitution of Equation (4) into (3) and ideal gas assumption yields:

$$r = k_l a \frac{P_g}{H} = \frac{k_l a \cdot RT}{H} C_g \tag{5}$$

Equation (5) can be combined with Equation (2) to yield:

$$F_g dC_g = -\frac{k_l aRTA}{H} C_g dh \tag{6}$$

Integration of Equation (6) yields a mass transfer-limitation model equation:

$$\ln \frac{C_{g0}}{C_g} = \frac{k_l a \cdot RTA}{H \cdot F_g} h \tag{7}$$

From Equation (7), mass transfer coefficient can be obtained from the slope if the left-hand side is plotted as a function of the inverse of gas flow rate. The Equation (7) can also be used as a design equation to determine the TBF height for a desired level of TCE removal.

Mathematical modeling on the microbial growth rate in the CSTR

A sMMO activity and transformation capacity (T_c) can be enhanced in the two-stage reactor system because the CSTR can promote the recovery of damaged proteins during TCE degradation and supply reducing powers like NADH as the addition of formate does. However, additional cost for the CSTR operation can hamper commercial application of the two-stage reactor system. Therefore, it is important to determine the relative size of the CSTR to the TBF and optimal operation conditions to secure the maintenance of appropriate level of sMMO for an efficient TCE treatment and minimize operation cost. The quantitative analysis of the extent of sMMO inactivation and estimation of transformation capacity in the two-stage TBF system is prerequisite to meet above objectives.

For a mathematical modeling of the specific growth rate in the CSTR and the effect of TCE removal on sMMO inactivation, the following mathematical descriptions are derived based on a cell-death model. This model assumes that the cells can be divided into viable cells (X_v) possessing the active sMMO and dead cell (X_d).

Cell-death model assumption:

$$X_t = X_v + X_d \tag{8}$$

$$\varphi = \frac{a}{a_{max}} \cong \frac{X_v}{X_t} = \text{ viable cell fraction} \tag{9}$$

The material balance on the cells of the two-stage reactor shown in Figure 3 is as follows at steady state:

Active cells balance in CSTR:

$$\frac{d(X_v \cdot V_l)}{dt} = 0 = F'_l X'_v - F_l X_v + \mu X_v V_l$$

(accumulation) (input)(output)(growth) (10)

Dead cells balance in CSTR:

$$\frac{d(X_d \cdot V_l)}{dt} = 0 = F'_l X'_d - F_l X_d \tag{11}$$

Substrate balances in CSTR:

$$\frac{d(SV_l)}{dt} = 0 = BS_0 + F'_l S' - F_l S - \frac{\mu}{Y} X_v V_l \tag{12}$$

Since $F_l = F'_l + B$ and $(S_0 - S)$ is larger than $(S' - S)$, Equation (12) can be rewritten to yield a equation for the specific growth rate as follows:

$$\mu = \frac{YB(S_0 - S)}{X_v V_l} \tag{13}$$

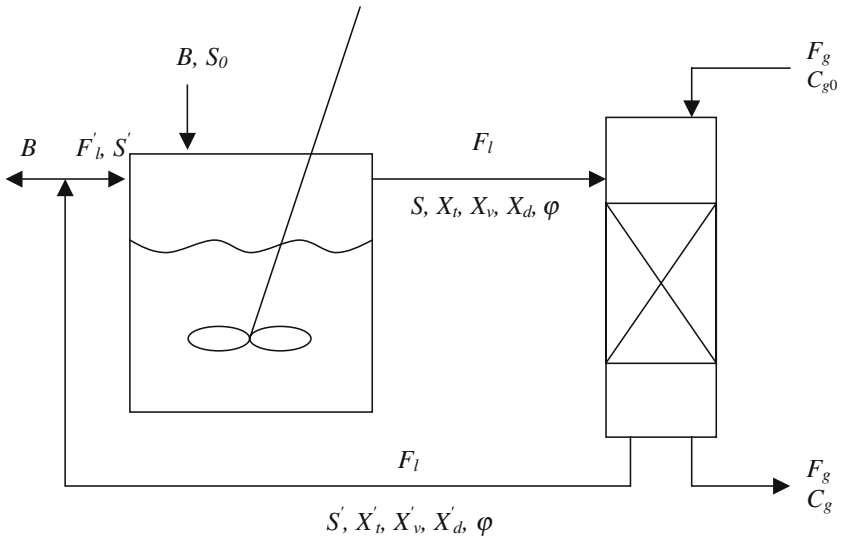


Figure 3. Schematic diagram for a mathematical modeling of the two-stage reactor system.

Equation (10) also gives μ:

$$\mu = \frac{F_l X_v - F'_l X'_v}{X_v V_l} \tag{14}$$

We like to measure μ in the CSTR from Equation (13) or (14), but it is not acceptable since S' or X'_v is not readily available. In order to relate $X'_v(X'_d)$ to $X_v(X_d)$, we need to consider the TBF.

The cell death rate and TCE degradation rate in the TBF can be expressed as:

$$\text{Cell death rate} = F_l X_v - F_l X'_v = F_l(X_v - X'_v)$$
$$\text{(amount of cell death/time) (input)(output)} \tag{15}$$

$$\text{TCE degradation rate} = F_g(C_{g0} - C_g)$$
$$\text{(amount of TCE degradation/time)} \tag{16}$$

Transformation capacity (T_c) which relates Equation (15) and (16) is expressed as:

$$T_c = \frac{\text{TCE degradation rate}}{\text{cell death rate}} = \frac{F_g(C_{g0} - C_g)}{F_l(X_v - X'_v)} \tag{17}$$

Let $F'_l/F_l = \alpha_1, B/F_l = \alpha_2, V_l/F_l = \alpha_3, F_g/F_l = \alpha_4$, and $C_{g0} - C_g = \Delta C_g$, then viable cell concentration can be expressed as a function of TCE removal by combination of Equations (14) and (17):

$$X_v = \frac{\alpha_1 \alpha_4 \Delta C_g}{(\alpha_3 \mu - \alpha_2) T_c} \tag{18}$$

For dead cell and total cell concentration, similar procedures lead to the following equations:

$$X_d = \frac{\alpha_1 \alpha_4 \Delta C_g}{\alpha_2 T_c} \tag{19}$$

$$X_t = \frac{\alpha_1 \alpha_3 \alpha_4 \mu \Delta C_g}{\alpha_2(\alpha_3 \mu - \alpha_2) T_c} \tag{20}$$

Combination of Equations (18) and (20) yield the following relationship between the activity loss of sMMO and specific growth rate in the CSTR:

$$\varphi = \frac{a}{a_{max}} = \frac{\alpha_2}{\alpha_3}\frac{l}{\mu} = \frac{B}{V_l}\frac{l}{\mu} \tag{21}$$

There are several important points related with Equation (21): (1) If φ is measured by experiment, μ can be determined using the above equation. (2) If the activity loss increased, μ of the CSTR should be increased (Figure 4). (3) Since the maximum value of φ is 1.0, dilution rate $(D = (B + F'_l)/V_l)$ equals to the minimum specific growth rate in the CSTR, $\mu_{min} = B/V_l$. (4) Since μ_{max} was determined to be 0.080 h^{-1} from the batch experiments, the minimal value of φ can be determined to be 0.187 from Equation (21) assuming B/V_l is constant. This implies that washout will be happen if the value of φ is smaller than the minimal value of 0.187.

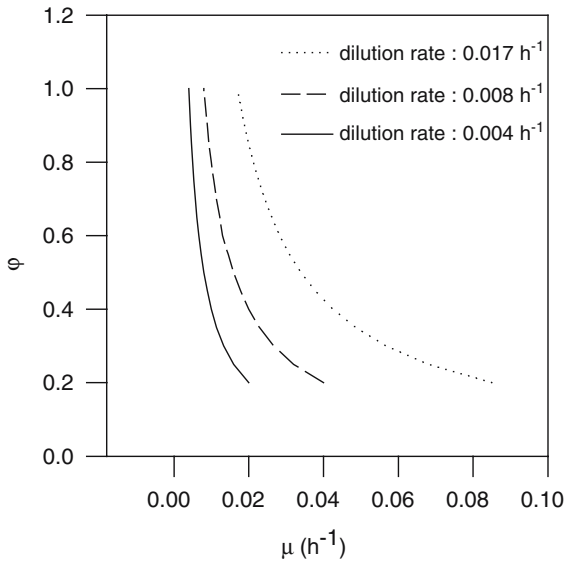


Figure 4. Relationship between sMMO activity loss and specific growth rate of the CSTR at various dilution rates.

Mathematical modeling on the effect of TCE removal on sMMO activity loss

For reactor design, it is important to quantitatively describe the effect of TCE removal on sMMO activity loss. First, we need to express μ as a function of ΔC_g, and the relationship between specific growth rate and ΔC_g can be obtained from Equations (13) and (18):

$$\mu = \frac{\alpha_2 T_c B Y \Delta S}{\alpha_3 T_c B Y \Delta S - \alpha_1 \alpha_4 V_l \Delta C_g} \qquad (22)$$

By combination of Equations (21) and (22), the sMMO activity loss can be expressed as a function of TCE removal, ΔC_g:

$$\varphi = 1 - \frac{\alpha_1 \alpha_4 \Delta C_g}{\alpha_2 T_c Y \Delta S} = \frac{\alpha_1 \alpha_4 \Delta C_g}{\alpha_2 T_c X_t} \qquad (23)$$

The effect of TCE removal on sMMO activity loss can be quantitatively analyzed using Equation (23), and transformation capacity (T_c) can be estimated from the slope by the linear regression of the model Equation (23).

Bioreactor operation

It is necessary to achieve maximum expression of sMMO of the cells in the CSTR, and operation conditions of the CSTR were previously optimized

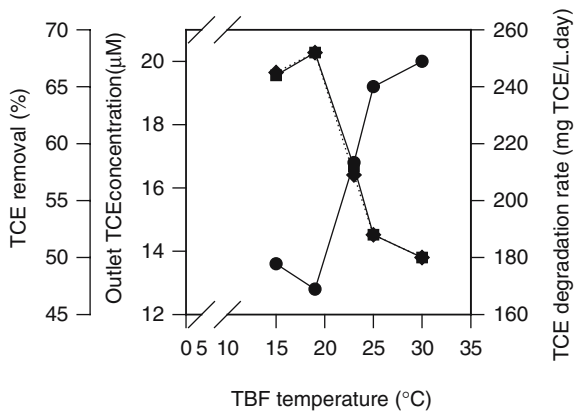


Figure 5. The effect of temperature of the TBF on TCE removal and degradation rate. (-●- : outlet TCE concentration; -◆- : TCE degradation rate; -■- : conversion)

using batch culture results. Cell concentrations of 2.1–2.2 mg ml^{-1} were maintained in the CSTR with the dilution of rate of 0.017 h^{-1}, methane flow rate of 0.2 l min^{-1} and oxygen flow rate of 0.4 l^{-1} min. During steady-state conditions, the TCE concentration difference between inlet and outlet of the TBF was due to biodegradation by *M. trichosporium* OB3b. Although bioreactor operation was not maintained aseptically, relative population of contaminants was not exceeded more than 5% of total populations determined by plate counting and contamination did not seem to affect significantly on TCE degradation.

Effect of temperature

Figure 5 shows the effect of temperatures of the TBF on TCE removal and degradation rate with the TBF operation condition of 40 μmol l^{-1} inlet TCE concentration, 100 ml min^{-1} liquid flow rate to the TBF, 100 ml min^{-1} gas flow rate and dilution rate of 0.017 h^{-1}. The TCE removal efficiency increased to 68% at 20 °C, whereas 50% at 30 °C. This enhancement of TCE removal was due to the increase in solubility of gas-phase TCE at lower temperature (i.e., decrease in partition coefficient). From the experimental result that the increased TCE mass transfer at lower temperature resulted in the enhancement of TCE removal, the analysis of mass transfer limitation should be considered for the TBF.

If experiments of the effect of temperature on TCE removal were done in the region of

mass-transfer limitation, Equation (7) can be applied for the analysis of the temperature effect on TCE removal. The Equation (7) can be rewritten as:

$$\ln \frac{C_{g0}}{C_g} = \frac{T}{H} \frac{(k_l a) \cdot (RAh)}{F_g} \qquad (24)$$

where $k_l a \cdot RAh/F_g$ can be considered to be constant if F_g is constant. If we compare the experimental results of $C_{g0} = 40\ \mu M$ and $C_g = 12\ \mu M$ at 20 °C and $C_{g0} = 40\ \mu M$ and $C_g = 20\ \mu M$ at 30 °C with Equation (24), the value of Henry's constant at 30 °C was determined to be 1.68 times higher than that of H at 20 °C and this calculation agrees well with the reported result that H changes about 1.6 times for every 10 °C increment in case of TCE (Chang & Alvarez-Cohen 1995).

Mass transfer aspects of TCE removal in the TBF

The experiments for the effects of gas flow rates on TCE removal at various cell concentrations were performed to investigate the mass transfer aspects of TCE removal in the TBF with the operation conditions of 40 μmol l^{-1} of inlet TCE concentration, 50 ml min^{-1} of liquid flow rate to the TBF and dilution rate of 0.017 h^{-1}. Cell concentrations of 2.2 and 3.0 mg ml^{-1} were obtained by changing nutrient concentrations. The specific sMMO activities were similar level for each case, which means that total sMMO activity is directly proportional to total cell concentrations. If the kinetic limitation dominates the TBF performance, TCE removal efficiency should be affected by the change in the cell concentrations of the CSTR because there are large amounts of active cells in the circulating broth in addition to the cells of the biofilm in the TBF. However, as shown in Figure 6, the TCE removal of about 51% at 100 ml min^{-1} of gas flow rate was not changed with the increment in cell concentrations of the CSTR. It can be concluded that TCE removal in the two-stage reactor system was in the mass transfer-limited region based on the experimental result that TCE removal was not affected by the cell concentration. TCE was not detected in the outflow liquid of TBF, also supported that TCE degradation reaction in the two-stage reactor system was mass transfer-limited.

The left-hand side of Equation (7) is plotted as a function of the inverse of the gas flow rates as shown in Figure 7. The mass transfer coefficient was determined to be 3.9 h^{-1} by linear regression. TCE removal at various gas flow rates can be predicted using Equation (7) with the estimated value of $k_l a$ and other constants. It is seen from Figure 6 that fairly good agreement was obtained between experimental data and predicted values, which showed TBF was operated in mass transfer-limited conditions.

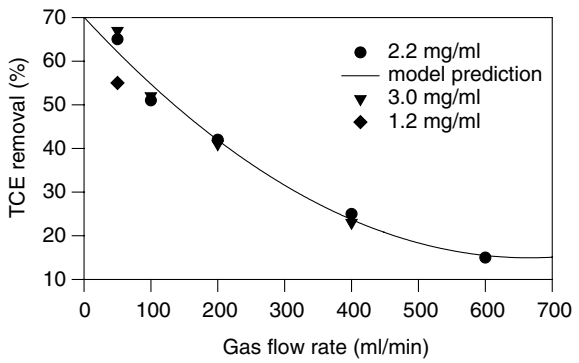


Figure 6. The effect of gas flow rates on TCE removal at various cell concentrations in the CSTR.

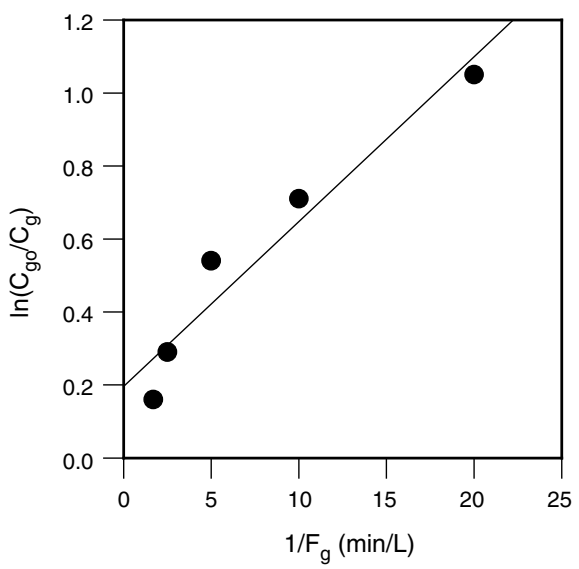


Figure 7. Determination of mass transfer coefficient ($k_l a$) by plotting of $\ln(C_{g0}/C_g)$ against $1/F_g$.

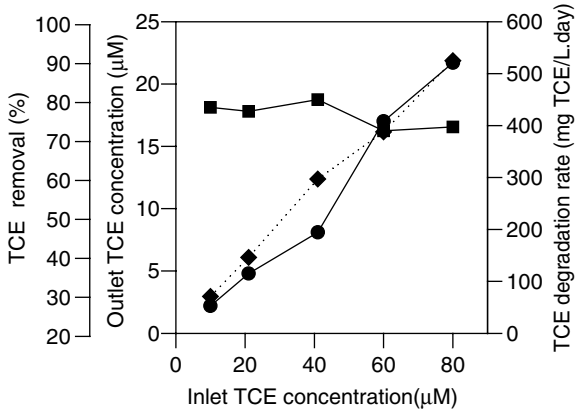


Figure 8. The effect of inlet TCE concentrations on TCE removal and degradation rate of the two-stage reactor system. (-●- : outlet TCE concentration; -◆- : TCE degradation rate; -■- : conversion)

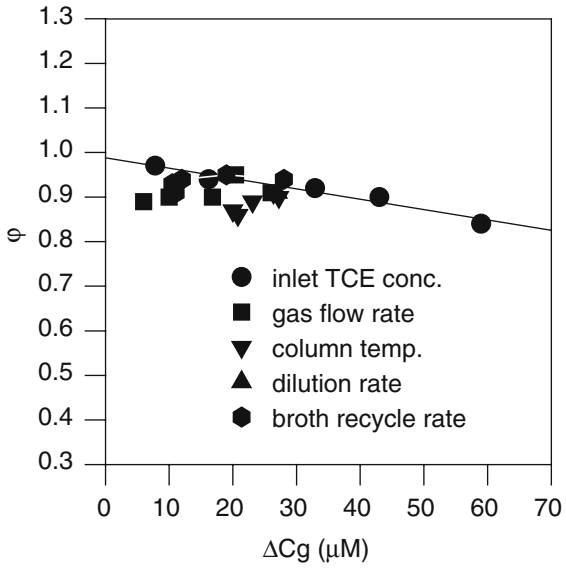


Figure 9. The effect of TCE removal on sMMO activity loss at various operation conditions and determination of transformation capacity of the two-stage reactor system.

Effect of TCE removal on sMMO activity loss

The effects of inlet TCE concentrations on TCE removal and degradation rate were investigated at TCE concentrations ranging from 10 to 80 μmol l^{-1}. As shown in Figure 8, TCE removal and degradation rate increased with increase in inlet TCE concentration up to 80 μmol l^{-1} and further increase in inlet TCE concentration resulted in sudden decrease in cell concentration in the CSTR due to the toxic effects of TCE degradation products and TCE itself. More than 75% of TCE was degraded for inlet TCE concentrations ranging from 10 to 80 μmol l^{-1}, and almost 100% removal was achieved when TCE was introduced at concentration of less than 10 μmol l^{-1}. An eight-fold increase in inlet TCE concentration resulted in seven-fold increase in degradation rate with the maximum volumetric degradation rate of up to 525 mg l^{-1} d^{-1}. The increase in TCE removal coincided with decline in sMMO activity as shown in Figure 9, which obviously shows the toxic effects of TCE degradation. When 80 μmol l^{-1} of TCE was fed, a reduction of 20% in sMMO activity for 75% of TCE removal was observed.

Transformation capacity (T_c) was determined to be 20.2 μmol mg^{-1} by the linear regression of the model Equation (23). The model describing sMMO activity loss as a function of TCE removal represented the experimental results relatively well, as shown in Figure 9. The T_c value is higher than those of other literatures (Alvarez-Cohen & McCarty 1991; Taylor & Hanna 1995). It can be inferred that the efficient recovery of sMMO activity and the plentiful supply of reducing power like NADH in the CSTR can increase T_c value. The transformation capacity was proposed to quantify the cytotoxicity of TCE and its degradation products on cells. The active cell concentration is normally decreased with the increase of TCE degradation and cells are expected to have a finite transformation capacity (Alvarez-Cohen & McCarty 1991). However, as indicated in our results, the original concept of transformation capacity is inadequate for the describing the TCE transformation capacity in the two-stage reactor system. It is more reasonable and useful to consider the transformation capacity as a changeable process parameter that quantitatively describes the relationship between the TCE removal and sMMO activity loss at various reactor operation conditions.

Mathematical model equation has been shown to correlate the effect of TCE removal on sMMO activity loss. Mathematical model equation can be used to predict the amount of sMMO activity loss for a desired TCE removal, which can be served as a basic equation for the determination of the relative volumes of the CSTR and the TBF or for the determination of optimal operation condition.

Reactor performance comparison

Although we didn't analyze the portion of TCE degraded by the cells in the biofilm or the liquid phase of cell suspension from the CSTR, TCE was definitely degraded by both the cells in biofilm and the liquid phase. The CSTR plays a key role in the reactivation of the cells which were inactivated during TCE degradation in the TBF. Therefore, it was effective to separate the degradation in the TBF in the absence of primary substrate and the reactivation in the CSTR in the presence of primary substrate.

Due to the difficulty of the determination of total cell concentration in the TBF, a volumetric TCE degradation rates was calculated. The maximum volumetric degradation rate of our system was determined to be 525 mg l^{-1} d^{-1}, significantly higher TCE degradation rate than those from literatures (Sun & Wood 1997). The reactor has been stably operated more than 270 days and this system also appeared to be stable in response to the fluctuations in operation conditions such as inlet TCE concentration. This result represented that the two-stage reactor system is a better approach than a simple one-stage biofilm reactor for the continuous treatment of gas-phase TCE. Overall, these experimental and modeling results demonstrated the advantages and commercial feasibility of the two-stage reactor system, but several weak points remain to be investigated. For example, a CSTR size and liquid flow rate to the TBF should be minimized for lower operating cost but this may result in decrease in efficiency of sMMO reactivation, which indicates that there is an optimal condition for economic operation.

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