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The effect of salinity conditions on kinetics of trichloroethylene biodegradation by toluene-oxidizing cultures

Chi-Yuan Lee *, Wen-Der Liu

Water Resources and Environmental Engineering Program, Department of Harbor and River Engineering, National Taiwan Ocean University, Keelung 20224, Taiwan, ROC

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Abstract

This study investigates the effect of salt (NaCl) conditions on the biodegradations of trichloroethylene (TCE) by mixed cultures enriched on toluene. Two cultures were separately cultivated in this investigation, involving culture LHTO4, cultivated with freshwater and culture HHTO4, cultivated with 3.5% (w/v) NaCl solution. Batch tests were conducted to elucidate the degradations of toluene, TCE and a mixture of toluene and TCE by cultures LHTO4 at salinities of 0, 2 and 3.5% and by HHTO4 at salinity of 3.5%. The measurements were analyzed with microbial kinetics. The results show that for culture LHTO4 in the resting cells, when the transient salinities increased from 0 to 3.5%, the maximum specific rate of TCE degradation, k_{TCE}, declined from 2.28 to 1.45 d^{-1}, and the observed TCE transformation capacity, $T_{c,obs}$, decreased from 0.060 to 0.036 mg TCE/mg VSS. In the presence of toluene, TCE degradation was more inhibited by toluene (inhibition coefficients, $K_{I,TOL}$ were 0.8, 2.2, and 0.96 mg/L for salinity 0, 2, and 3.5%, respectively) than toluene degradation was by TCE ($K_{I,TCE}$ were 14, 5.8, and 1000 mg/L for salinity 0, 2, and 3.5%, respectively). Under long-term salinity stress, the culture HHTO4 maintained its capacity to utilize toluene but lost its effectiveness in the cometabolic transformation of TCE: k_{TCE} fell to 0.25 d^{-1} and $T_{c,obs}$ dropped to 0.024 mg TCE/mg VSS. This work reveals that the degradation of TCE by toluene-oxidizing cultures under saline conditions can be best described by the chosen kinetic equations and experimentally estimated constants, which can thus be used to lay a foundation for the rational design of biological processes to remove TCE from saline solutions.

Keywords: Cometabolic transformation; Competitive inhibition; Kinetics; Noncompetitive inhibition; Toluene

1. Introduction

Trichloroethylene (TCE), a chlorinated chemical, is prevalent in industrial waste streams and contaminated sites. It constitutes a serious healthy risk because it is carcinogenic [1]. Except for fresh water, this compound can be found in saline environments. Examples are the TCE contaminated groundwater in coastal aquifers having seawater intrusion and the leachate from landfills [2,3]. In addition, when TCE vapor is treated using a biotrickling filter, where NaCl accumulates in the liquid phase, a phenomenon occurs that is similar to that observed during the treatment of dichloromethane vapor [4]. And in the biotrickling treatment of the waste gas, sodium chloride may be added to prevent the excessive accumulation of biomass [5]. Kinetic analysis of TCE biodegradation in saline solutions is very complicated for two reasons. Firstly, TCE cannot support bacterial growth, so its aerobic removal should employ cometabolic biodegradation, in which a growth substrate, such as phenol, toluene or methane, is used to cultivate bacteria and thus induce the required nonspecific enzyme to catalyze the oxidation of TCE [6]. Second, the presence of salt may cause an osmotic pressure to the bacteria during the cometabolic transformation of TCE, rendering the quantification of degradation behaviors difficult. While extensive studies on the degradation of TCE by toluene-oxidizing cultures in freshwater have been undertaken [7–10] and in soils [11], the need to improve our understanding of the effects of salinity on TCE degradation is increasing.

Research on TCE degradation in salt solutions is relatively limited, despite the fact that numerous studies have been conducted on the metabolism of growth substrates that have been affected by salinity [12–15]. Lee et al. [16] indicated that salinity substantially influences TCE degradation by phenol-grown

* Corresponding author. Tel.: +886 2 2462 2192x6147; fax: +886 2 24624770.
E-mail address: cylee@mail.ntou.edu.tw (C.-Y. Lee).

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Nomenclature

B_f	effectiveness factor for volatile substrate utilization in a serum bottle with headspace
H_c	Henry's law constant
HHTO4	holotolerant mixed culture grown on toluene in saline solution with 3.5% NaCl
k	maximum specific substrate utilization rate (d^{-1})
K_I	inhibition coefficient (mg/L)
K_S	half-velocity concentration (mg/L)
LHTO4	mixed culture grown on toluene in fresh water
m	number of observations
m_s	maintenance coefficient of bacteria (d^{-1})
n	number of observations
RSS	residual sum of squares
S	substrate concentration in liquid phase (mg/L)
t	time (d)
$T_{c,obs}$	observed TCE transformation capacity (mg TCE/mg VSS)
TCE	trichloroethylene
TOL	toluene
V_a	volume of the gas phase in a serum bottle (mL)
V_w	volume of the liquid phase in a serum bottle (mL)
VSS	volatile suspend solids
X	biomass concentration (mg/L)
Y_G	true bacterial yield (mg VSS/mg TOL)
Y_{obs}	observed bacterial yield (mg VSS/mg TOL)

Greek letter

μ	specific growth rate (d^{-1})
μ_{max}	maximum specific growth rate (d^{-1})

Subscripts

obs	observed
pred	predicted
TCE	trichloroethylene
TOL	toluene

cultures produced from chemostat reactors, and the degree of the effect depends on the growth conditions, the operating hydraulic retention time of the reactor and the salinity of the growth medium. Nevertheless, kinetic information on the biodegradation interactions between growth substrate and TCE in saline water is still lacking.

This work examines the degradation of TCE by the toluene-grown cultures in response to salt stress. Specifically, TCE degradations during substrate interactions between TCE and toluene at salinities from 0 to 3.5%, are analyzed with microbial kinetics.

2. Model development

2.1. Toluene degradations

The overall rate of toluene utilization in solution at a particular salinity is described by coupling the rate of substrate utilization for cell synthesis, $\mu_{max}S_{TOL}X/Y_G(K_{S,TOL}+S_{TOL})$ and that required for the maintenance of cells due to osmotic pressure, m_sX [4,17]. Accordingly, the substrate balance equation can be expressed as

$$-\frac{dS_{TOL}}{dt} = \frac{\mu_{max}S_{TOL}X}{Y_G(K_{S,TOL}+S_{TOL})} + m_sX \quad (1)$$

where $-dS_{TOL}/dt$ is the toluene utilization rate (mg/L d), μ_{max} the maximum specific growth rate (d^{-1}), S_{TOL} the toluene concentration (mg/L), X the biomass concentration (mg/L), $K_{S,TOL}$ the toluene half-velocity concentration (mg/L), m_s the maintenance coefficient (d^{-1}) and Y_G is the true bacterial yield (mg volatile suspended solids (VSS)/mg toluene). The rate of decay is negligible, so the net rate of bacterial growth is simplified to

$$\frac{dX}{dt} = \frac{\mu_{max}S_{TOL}X}{K_{S,TOL}+S_{TOL}} \quad (2)$$

The observed bacterial yield, Y_{obs}, is defined as the ratio of bacterial production to overall substrate utilization, $-dX/dS_{TOL}$. Eqs. (1) and (2) yield the following relationship between Y_{obs} and Y_G,

$$\frac{1}{Y_{obs}} = \frac{1}{Y_G} + \frac{m_s}{\mu} \quad (3)$$

where $\mu = \mu_{max}S_{TOL}/(K_{S,TOL}+S_{TOL})$. The maximum specific substrate utilization rate, k_{TOL} (d^{-1}), is defined as the maximum specific growth rate divided by the observed bacterial yield, μ_{max}/Y_{obs}. The following substitutions are made into Eq. (1):

$$m_s = \mu\left[\frac{1}{Y_{obs}} - \frac{1}{Y_G}\right]; \qquad k_{TOL} = \frac{\mu_{max}}{Y_{obs}}$$

Eq. (1) becomes

$$-\frac{dS_{TOL}}{dt} = \frac{k_{TOL}S_{TOL}X}{K_{S,TOL}+S_{TOL}} \quad (4)$$

Similarly, μ_{max} in Eq. (2) can be replaced by $Y_{obs}k_{TOL}$, yielding another expression of bacterial growth:

$$\frac{dX}{dt} = Y_{obs}\frac{k_{TOL}S_{TOL}X}{K_{S,TOL}+S_{TOL}} = -Y_{obs}\frac{dS_{TOL}}{dt} \quad (5)$$

When the test is conducted in a serum bottle with headspace, toluene is present in both the liquid and the gas phases. While bacteria are consuming toluene, the substrate depletion rate depends on the concentration of the liquid, but the amount transferred from the gas phase to the liquid phase must be considered. Therefore, an effectiveness factor of B_f [18] is used to account for this effect, which is expressed as

$$B_f = \frac{1}{1+H_c(V_a/V_w)} \quad (6)$$

where H_c is the Henry's constant (dimensionless), V_a the volume of the gas phase (mL), and V_w the volume of the liquid phase (mL). Thus, the toluene utilization rate in the serum bottle will be

$$-\frac{dS_{TOL}}{dt} = \frac{B_fk_{TOL}S_{TOL}X}{K_{S,TOL}+S_{TOL}} \quad (7)$$

2.2. Degradations of TCE

During TCE degradation by toluene degraders, the bacteria will be inactivated because of the toxicity of intermediate compounds [6]. Therefore, at a certain salinity, the measurement of TCE mass degraded by a unit mass of bacteria before deactivation is important to quantify the bacterial activity, for which the parameter of transformation capacity developed by Alvarez-Cohen and McCarty [19] can be employed. Incorporating the concepts of transformation capacity and Monod kinetics, the equations of TCE degradations and net growth rate in resting cells can be expressed as [19–22]:

$$-\frac{dS_{TCE}}{dt} = \frac{B_f k_{TCE} S_{TCE} X}{K_{S,TCE} + S_{TCE}} \quad (8)$$

$$\frac{dX}{dt} = \frac{1}{T_{c,obs}} \frac{dS_{TCE}}{dt} \quad (9)$$

where $T_{c,obs}$ is the observed transformation capacity (mg TCE/mg VSS). B_f is determined from Eq. (6), which is used to account for the effect of TCE transferred from the gas phase to the liquid phase. In the presence of toluene and TCE, competitive inhibition of TCE degradation probably occurs, so the kinetics of toluene and TCE depletions, as well as of bacterial growth are modified as follows [22]:

$$-\frac{dS_{TOL}}{dt} = \frac{B_{f,TOL} k_{TOL} S_{TOL} X}{K_{S,TOL}(1 + S_{TCE}/K_{I,TCE}) + S_{TOL}} \quad (10)$$

$$-\frac{dS_{TCE}}{dt} = \frac{B_{f,TCE} k_{TCE} S_{TCE} X}{K_{S,TCE}(1 + S_{TOL}/K_{I,TOL}) + S_{TCE}} \quad (11)$$

$$\frac{dX}{dt} = -Y_{obs} \frac{dS_{TOL}}{dt} + \frac{1}{T_{c,obs}} \frac{dS_{TCE}}{dt} \quad (12)$$

where $K_{I,TCE}$ is the coefficient that express the inhibition by TCE to toluene utilization (mg/L) and $K_{I,TOL}$ is the coefficient that expresses the inhibition by toluene to TCE degradation (mg/L). A small coefficient reveals a high toxicity that the compound can exert.

3. Materials and methods

3.1. Chemicals

TCE (GR grade) was purchased from Merck Co. (Merck Taiwan Ltd.). TCE-saturated water solution was prepared by injecting 10 mL of pure TCE into a 125 mL vial that had been filled with 80 mL of distilled water and capped with a Teflon-lined rubber septum. The contents of the vial were vigorously mixed for 1 min and allowed to settle for 2 h before they were used, resulting in a saturated TCE concentration of 1100 mg/L. Toluene with a purity of 99.7% was obtained from Alps Chem. Co., Ltd. (Taiwan). Similarly, the toluene-saturated stock solution was prepared by injecting 5 mL of pure toluene into a 125 mL serum bottle that had been filled with 80 mL of distilled water, finally yielding a saturated toluene concentration of 515 mg/L. Sodium chloride (NaCl purity of 100.2%), used to prepare saline solutions, was obtained from Baker Analyzed (USA).

3.2. Mixed cultures cultivated with fresh solution

The mixed culture LHTO4, a low halotolerant toluene oxidizer, was cultivated with freshwater to examine the effect of salinity on the activity of the bacteria in degrading toluene and TCE. The culture was initially seeded with sediment obtained from Keelung Harbor, Taiwan. About 500 mL of culture was cultivated in a 1 L flask using the fill-and-draw method, in which the toluene was the only source of carbon and the mineral medium contained (in g/L) K_2HPO_4 (0.68), KH_2PO_4 (0.52), $(NH_4)_2SO_4$ (0.71), $MgSO_4 \cdot 7H_2O$ (0.88), $MnCl_2 \cdot 4H_2O$ (0.001), $CaCl_2 \cdot 2H_2O$ (0.026), $FeSO_4 \cdot 7H_2O$ (0.0012) and Na_2MoO_4 (0.002) [23]. It was cultivated in 1-week cycles, in each of which approximately 400 mL of mixed liquor was decanted and replaced with liquid medium, and then toluene was added, yielding an initial toluene concentration of 65 mg/L in the liquid phase.

3.3. Mixed cultures cultivated with saline solutions

The mixed culture HHTO4, a halotolerant toluene oxidizer, was cultivated in parallel with saline solution to explore the effect of long-term salinity stress on the toluene-grown culture in degrading toluene and TCE. Cultivations of the saline culture began with placing culture LHTO4 in 3.5% salt (w/v) solution. The medium composition of the saline solutions, the experimental setups, and operational procedures were those used to cultivate LHTO4.

During the cultivation of cultures LHTO4 and HHTO4, toluene concentration, volatile suspended solid content, and salinity were periodically measured. Both cultures had been cultivated for 16 months before degradation tests were performed.

3.4. Batch experiments

Three test sets were carried out to evaluate the ability of culture LHTO4 to degrade TCE under transient salt stress. In the first set (set LH-I), the influence of transient salinity on toluene degradation was investigated. The batch test began by taking about 50 mL fresh bacterial suspension from the flask, to which toluene had been added 1 d before. Prior to the experiment, the bacterial suspension had been aerated for 2 h to expel residual toluene, and then placed in a 125 mL serum bottle. Each bottle contained the required amount of NaCl salt to yield desired salinity (covered 0, 2, and 3.5%). The serum bottle was sealed with a 3 mm Teflon-lined rubber septum and crimp-top caps before the appropriate mass of substrate was injected. In set LH-I, with a cell mass of 36 mg/L, only toluene-saturated solutions (3.89 mL for test at 0% NaCl, 4.04 mL at 2% NaCl, and 4.18 mL at 3.5% NaCl) were added, yielding 30 mg/L of toluene in the liquid phase. In set LH-II, the initial biomass concentration was 51 mg/L and only TCE-saturated solutions (208 μL for test at 0% NaCl, 220 μL at 2% NaCl, and 227 μL at 3.5% NaCl) were injected to yield a liquid TCE concentration of 3 mg/L.

In set LH-III, the initial biomass concentration was maintained at 38.5 mg/L and the solutions contained mixtures of toluene (30 mg/L) and TCE (3 mg/L). After the substrates had been added, the serum bottles were mixed vigorously by hands for 1 min and then were shaking at 165 rpm to ensure that equilibrium is reached between the liquid and the headspace. During incubation, these serum bottles were kept at 25 °C chamber. All batch experiments were conducted in triplicate.

The degradation capability of culture HHTO4 was examined at 3.5% NaCl concentration. Three test sets were used: set HH-I to investigate the degradation of toluene, set HH-II to examine TCE degradation, and set HH-III to explore the degradation of mixtures of toluene and TCE. The experimental setups and procedures were the same as those used for the culture LHTO4.

3.5. Analytical methods

The mass of the volatile suspended solids (VSS) was measured gravimetrically from the difference between the mass of cells stored at 103 °C overnight and the mass of the cells after combustion at 550 °C for 1 h. The toluene and TCE concentrations were determined from the headspace, from which a gas sample of 20 μL was withdrawn using a gas-tight syringe and injected into a gas chromatograph (GC, Hewlett Packard 6890). The GC was equipped with a flame-ionized detector (FID) and a column of DB-5 (30 m, 0.32 mm, and 0.25 μm). The temperature of the oven was maintained at 65 °C, that of the injection port was maintained at 250 °C and that of the detector was maintained at 275 °C. The dimensionless Henry's constant, H_c, at 25 °C and at various salinities were 0.224 (for an NaCl salinity of 0%), 0.259 (2%) and 0.290 (3.5%) for toluene and 0.351 (for an NaCl salinity of 0%), 0.407 (2%) and 0.442 (3.5%) for TCE [24]. H_c was used to calculate the substrate concentration in the liquid phase from the concentration of the headspace and to determine B_f from Eq. (6).

3.6. Determinations of kinetic constants

3.6.1. Determinations of Y_{obs}, k_{TOL} and $K_{S,TOL}$ using test sets LH-I and HH-I

Test sets LH-I and HH-I were employed to determine the coefficients of Y_{obs}, k_{TOL}, and $K_{S,TOL}$ and thus elucidate toluene degradation. The observed bacterial yield, Y_{obs}, is presented as the ratio of the increment of biomass to the depletion of toluene during the growth phase [10]. The parameters k_{TOL} and $K_{S,TOL}$ were evaluated simultaneously from Eqs. (5) and (7), by inputting the initial biomass and the measured toluene concentrations during incubation. The optimal values of k_{TOL} and $K_{S,TOL}$ were found by nonlinear regression, as presented below. The objective function for the optimal coefficients of k_{TOL} and $K_{S,TOL}$ is

$$\min \mathrm{RSS} = \min \sum_{i=1}^{n} \frac{1}{n} (S_{i,\mathrm{pred}} - S_{i,\mathrm{obs}})^2 \tag{13}$$

where $S_{i,obs}$ and $S_{i,pred}$ are the toluene concentrations measured and predicted at time i, respectively, and n is the number of observations.

3.6.2. Determinations of $T_{c,obs}$, k_{TCE} and $K_{S,TCE}$ by test sets LH-II and HH-II

The TCE mass that transformed must be determined to yield $T_{c,obs}$. The amount of TCE degraded in the resting cells is the difference between the TCE mass initially added to the serum bottle and that at the conclusion of test. In the absence of toluene, $T_{c,obs}$ equals the removed TCE mass divided by the amount of biomass initially added; in the presence of toluene, the biomass must be adjusted, such that the adjusted biomass consists of that initially added and that synthesized by the consumption of toluene (Y_{obs} times the mass of toluene is consumed).

k_{TCE} and $K_{S,TCE}$ were determined by inputting the initial biomass, the values of $T_{c,obs}$, and the TCE concentrations after various elapsed times of incubation into Eqs. (8) and (9). The optimal values of k_{TCE} and $K_{S,TCE}$ were found by nonlinear regression. The objective function is Eq. (13), where $S_{i,obs}$ and $S_{i,pred}$ are the TCE concentrations measured and predicted at time i, respectively, and n is the number of observations.

3.6.3. Determinations of $K_{I,TOL}$, $K_{I,TCE}$ by test sets LH-III and HH-III

In the presence of both toluene and TCE, coefficients $K_{I,TOL}$, $K_{I,TCE}$ specify the extent of competitive inhibition. For each run with test sets LH-III and HH-III, the measured concentrations of TCE and toluene, and initial biomass and parameters of Y_{obs}, k_{TOL}, $K_{S,TOL}$, k_{TCE}, $K_{S,TCE}$ and $T_{c,obs}$ were substituted into Eqs. (10)–(12) to yield the optimal parameters $K_{I,TOL}$ and $K_{I,TCE}$. The optimal values of $K_{I,TOL}$ and $K_{I,TCE}$ were determined by nonlinear regression based on the objective function,

$$\min \mathrm{RSS} = \min \left[\sum_{i=1}^{n} \frac{1}{n} \frac{(S_{i,\mathrm{TOL,pred}} - S_{i,\mathrm{TOL,obs}})^2}{S_{o,\mathrm{TOL}}^2} + \sum_{j=1}^{m} \frac{1}{m} \frac{(S_{j,\mathrm{TCE,pred}} - S_{j,\mathrm{TCE,obs}})^2}{S_{o,\mathrm{TCE}}^2} \right] \tag{14}$$

where $S_{i,TOL,pred}$ and $S_{i,TOL,obs}$ are the predicted and observed toluene concentrations, and $S_{j,TCE,pred}$ and $S_{j,TCE,obs}$ are the predicted and observed TCE concentrations, respectively. $S_{o,TOL}$ and $S_{o,TCE}$ are the initial concentrations of toluene and TCE, respectively. n and m represent the numbers of observations.

3.6.4. Numerical method for evaluating optimal parameters

Nonlinear regression was applied to search for the optimal values of the kinetic parameters. Fourth-order Runge–Kutta algorithms were coded to solve the differential equations. A Matlab (Matlab 6.5, The Math Works, Inc.) computer program was run on a personal computer to perform an iterative search to find a least-square fit to the data.

4. Results

4.1. Toluene degradations

The application of transient salinity to freshwater culture LHTO4 suppressed toluene degradation. Given an initial con-

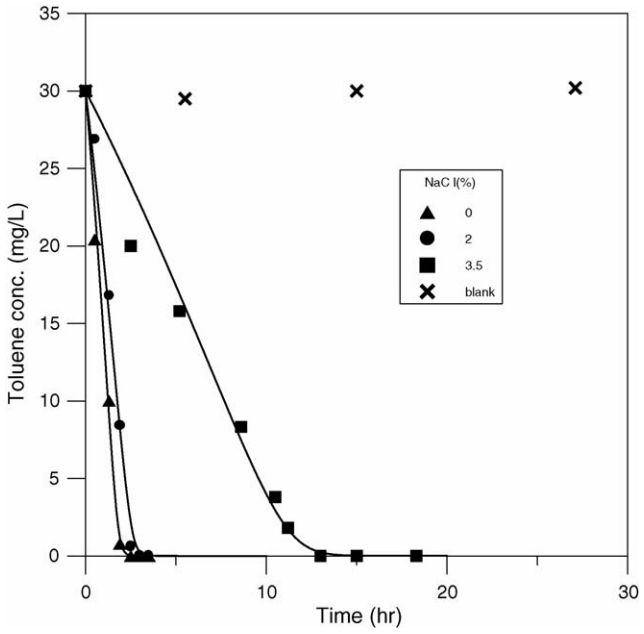

Fig. 1. Toluene biodegradation by culture LHTO4 under different NaCl concentrations. Symbols are observations of batch test set LH-I; curves are generated using Eqs. (5) and (7) by inputs of parameters listed in Table 1.

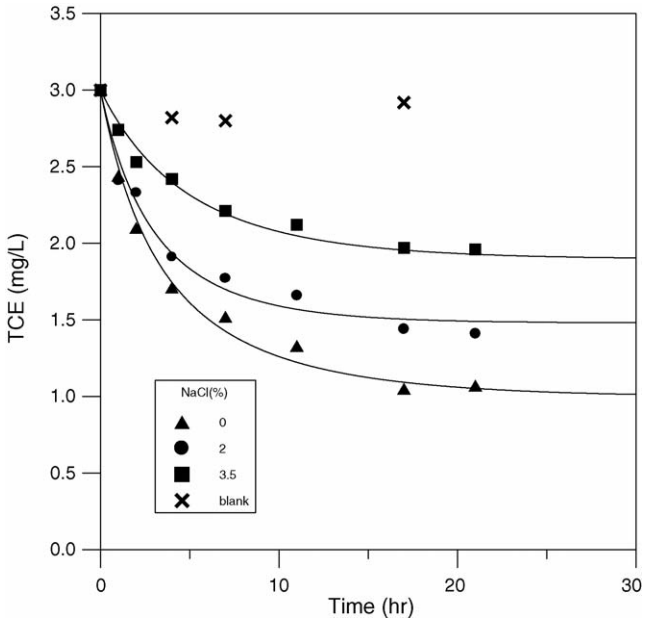

Fig. 2. TCE biodegradation by culture LHTO4 under different NaCl concentrations. Symbols are observations of batch experiments; curves are generated using Eqs. (8) and (9) by inputs of parameters listed in Table 1.

centration of toluene of 30 mg/L and an initial concentration of biomass of 36 mg/L in salt-free solutions, 2 h are required to degrade the compound completely. Complete degradation took 13 h when the salt concentration was increased to 3.5% (Fig. 1). This culture also exhibited a trend of declining observed cell yield, Y_{obs} from 0.61 to 0.54 mg VSS/mg TOL as the salt concentration is raised from 0 to 3.5% (Table 1).

Table 1 presents the best-fit kinetic coefficients of toluene degradation by culture LHTO4 in response to salt stress. The k_{TOL} values fell from 20.9 to 3.6 d^{-1} as the NaCl concentration increased from 0 to 3.5%, while $K_{S,TOL}$ was maintained at 2.9 mg/L at all tested salinities. The decline of k_{TOL} at 2% NaCl was 25%, and that at 3.5% was 89%. The response of culture LHTO4 to the salt stress was similar to those in the trickle-bed bioreactor, reported by Schönduve et al. [5], who found that the toluene reaction rate decreased by 25% when the NaCl concentration reached 2.3%. Fig. 1 reveals a good agreement between the predictions and the observations, suggesting that the degradation of toluene under shock salinity follows noncompetitive inhibition kinetics.

4.2. Degradation of TCE by resting cells

Fig. 2 displays the variations of TCE degradation by resting cells at salinity from 0 to 3.5%. Even after 30 h of incubation, TCE cannot be completely degraded, and the residual TCE concentration increased with salinity. These data demonstrate that the transient salinity sharply reduced the capability of microorganisms to cometabolically degrade TCE. Based on the removed TCE mass, the values of $T_{c,obs}$ were 0.060, 0.048 and 0.036 mg TCE/mg VSS at 0, 2 and 3.5% salinities, respectively.

Table 1
Biodegradation kinetic parameters for culture LHTO4 under different NaCl concentrations

Test set	Substrate	Parameters	NaCl (%)		
			0	2	3.5
LH-I	Toluene	Maximum specific toluene degradation rate, k_{TOL} (d^{-1})	20.9 ± 0.37[a]	15.7 ± 0.37	3.55 ± 0.042
		Half-velocity concentration for toluene, $K_{S,TOL}$ (mg/L)	2.9	2.9	2.9
		Observed bacterial yield, Y_{obs} (mg VSS/mg TOL)	0.61	0.57	0.54
LH-II	TCE	Maximum specific TCE degradation rate, k_{TCE} (d^{-1})	2.28 ± 0.10	1.83 ± 0.39	1.45 ± 0.39
		Half-velocity concentration for TCE, $K_{S,TCE}$ (mg/L)	7.9	7.9	7.9
		Observed TCE transformation capacity, $T_{c,obs}$ (mg TCE/mg VSS)	0.060	0.048	0.036
LH-III[b]	Mixture of toluene and TCE	Observed TCE transformation capacity, $T_{c,obs}$ (mg TCE/mg VSS)	0.041	0.039	0.039
		Competitive inhibition constant of toluene on TCE, $K_{I,TOL}$ (mg/L)	0.80 ± 0.45	2.2 ± 1.2	0.96 ± 0.50
		Competitive inhibition constant of TCE on toluene, $K_{I,TCE}$ (mg/L)	14 ± 4.5	5.8 ± 0.67	1000

[a] Average ± standard deviation.
[b] For modeling degradation data in test set LH-III, the kinetic constants of k_{TOL}, $K_{S,TOL}$, and Y_{obs} are obtained from the determinations by LH-I, and the kinetic constants of k_{TCE}, and $K_{S,TCE}$ are obtained from the determinations by LH-II.

The best-fit kinetic degradation parameters of $K_{S,TCE}$ were maintained at 7.9 mg/L, but k_{TCE} decreased from 2.28 to 1.45 d^{-1} as the salinity was increased from 0 to 3.5% (Table 1). Again, Fig. 2 shows that the predications are highly consistent with the observations, indicating that NaCl is a noncompetitive inhibitor during TCE biodegradation by culture LHTO4.

4.3. Competitive inhibition of TCE degradation

In the presence of toluene, the degradations of TCE by culture LHTO4 in response to saline pressure became very complicated, because competitive inhibition occurs between TCE and toluene. The inhibition of TCE degradation by toluene was observed in the beginning period of incubation as the TCE degradation began only after the toluene had almost completely degraded (Fig. 3). However, toluene degradation was less inhibited by TCE, because the utilization rate remained almost the same as that when TCE was absent; this result is similar to that in other reports [9,25–27].

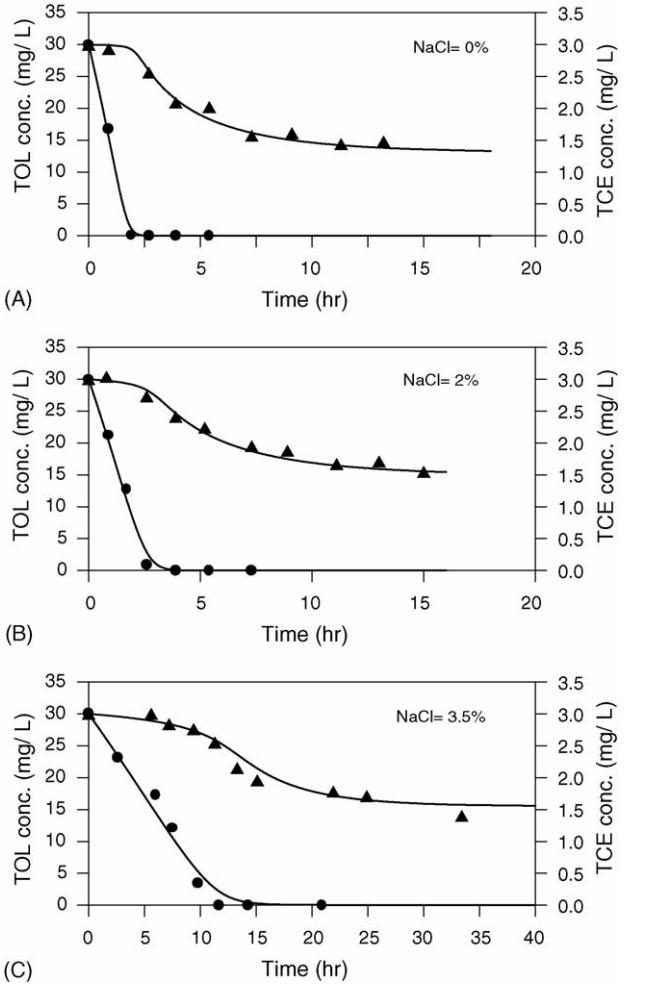

Fig. 3. Biodegradation of toluene (TOL) and TCE mixture by culture LHTO4 under different saline conditions: NaCl = 0% (A); NaCl = 2% (B); NaCl = 3.5% (C). Symbols (dots: toluene, triangles: TCE) are observations of batch experiments; curves are generated using Eqs. (10)–(12) by inputs of parameters listed in Table 1.

The relative toxicity between TCE and toluene at 0–3.5% salinity was determined by simultaneously solving Eqs. (10)–(12) for parameters $K_{I,TOL}$ and $K_{I,TCE}$. Table 1 summarizes the values of $K_{I,TOL}$ (0.80, 2.2 and 0.96 mg/L) and $K_{I,TCE}$ (14, 5.8 and 1000 mg/L). This kinetic analysis supports two inferences. First, a comparison of $K_{I,TOL}$ with $K_{I,TCE}$ confirms that the TCE degradation is more inhibited by toluene than the degradation of toluene is inhibited by TCE, because $K_{I,TOL}$ was less than $K_{I,TCE}$. Second, the inhibition by toluene on TCE degradation was generally unrelated to salinity but the inhibition by TCE was largely affected by salinity, e.g., at salinity of 3.5% the toxicity of TCE was considerably reduced, as $K_{I,TCE}$ rose to 1000 mg/L.

4.4. Degradation of TCE by saline culture HHTO4

Fig. 4 depicts the degradation patterns of toluene, TCE in resting cells, and mixtures of TCE and toluene by halotolerant culture HHTO4. These halotolerant cultures rapidly

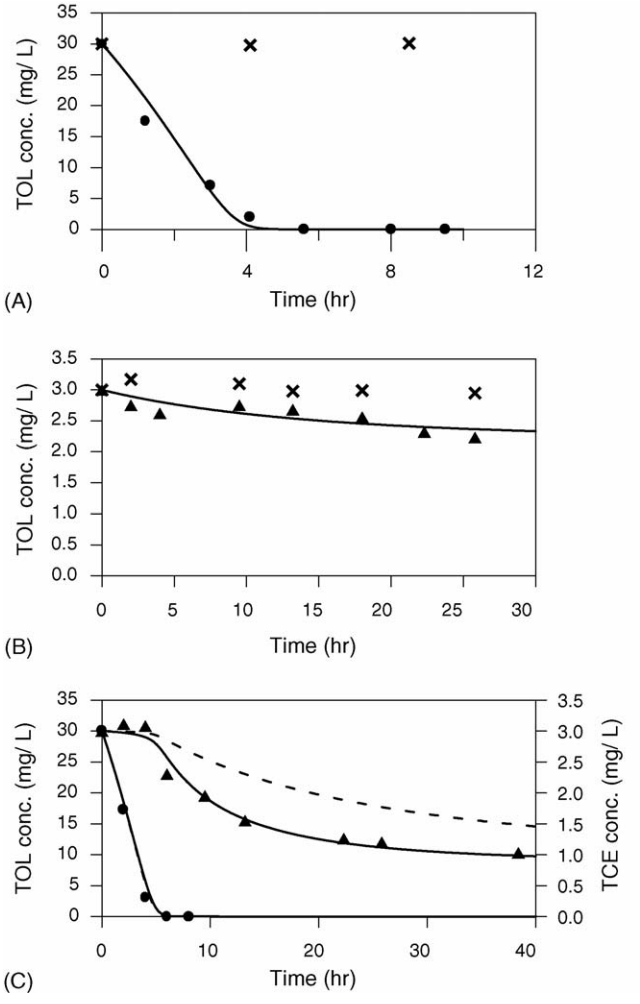

Fig. 4. Biodegradations of toluene (TOL) (A), TCE (B), and toluene and TCE mixture (C) by HHTO4 in NaCl = 3.5% solutions. Symbols (dots: toluene, triangles: TCE, crosses: control) are observations of batch experiments; curves are generated using Eqs. (5)–(12). In panel (C) dash line is generated by using $k_{TCE} = 0.25\ d^{-1}$ (the same as that estimated in resting cells) where the solid line is generated by using the best-fit value of $k_{TCE} = 1.02\ d^{-1}$.

Table 2
Biodegradation kinetic parameters for culture HHTO4 under NaCl concentration at 3.5%

Test set	Substrate	Parameters	NaCl (%) 3.5
HH-I	Toluene	Maximum specific toluene degradation rate, k_{TOL} (d^{-1})	5.52 ± 1.25[a]
		Half-velocity concentration for toluene, $K_{S,TOL}$ (mg/L)	3.1
		Observed bacterial yield, Y_{obs} (mg VSS/mg TOL)	1.0
HH-II	TCE	Maximum specific TCE Degradation rate, k_{TCE} (d^{-1})	0.25 ± 0.022
		Half-velocity concentration for TCE, $K_{S,TCE}$ (mg/L)	7.8
		Observed TCE transformation capacity, $T_{c,obs}$ (mg TCE/mg VSS)	0.024
HH-III[b]	Mixture of toluene and TCE	Maximum specific TCE Degradation rate, k_{TCE} (d^{-1})	1.02 ± 0.19
		Half-velocity concentration for TCE, $K_{S,TCE}$ (mg/L)	7.8
		Observed TCE transformation capacity, $T_{c,obs}$ (mg TCE/mg VSS)	0.036
		Competitive inhibition constant of toluene on TCE, $K_{I,TOL}$ (mg/L)	2.0 ± 0.8
		Competitive inhibition constant of TCE on toluene, $K_{I,TCE}$ (mg/L)	1000

[a] Average ± standard deviation.
[b] For modeling degradation data in test set HH-III, the kinetic constants of k_{TOL}, $K_{S,TOL}$, and Y_{obs} are obtained from the determinations by HH-I.

degraded toluene in 4 h when an initial liquid concentration of 30 mg/L toluene was incubated, yielding $k_{TOL} = 5.52\ d^{-1}$, a value greater than that obtained using culture LHTO4 at 3.5% salinity (Table 2). However, the HHTO4 culture in resting cells had a very small capability to catalyze the cometabolic transformation of TCE. For instance, the corresponding value of $T_{c,obs}$ was 0.024 mg TCE/mg VSS and that of k_{TCE} was only $0.25\ d^{-1}$; both values were much less than those for freshwater culture LHTO4 at 3.5% salinity. However, in the presence of 30 mg/L of toluene, TCE degradation by HHTO4 culture was markedly improved. Specifically, the $T_{c,obs}$ rose to 0.036 mg TCE/mg VSS, and k_{TCE} increased to $1.02\ d^{-1}$.

The inhibition of TCE degradation also occurred in the beginning 5 h of incubation as toluene is present (Fig. 4). By comparing inhibition coefficients, the relative toxicity between TCE and toluene was easily distinguished, where the $K_{I,TCE}$ value (1000 mg/L) is two orders of magnitude larger than $K_{I,TOL}$ (2.0 mg/L), indicating that the inhibition of toluene by the presence of TCE is weaker than that of TCE degradation by toluene.

5. Discussion

5.1. Kinetic equations and associated constants

Kinetic analysis of TCE cometabolic transformation in saline water is very difficult because NaCl hinders the degradation of substrate. In this work, the behaviors of toluene-oxidizing cultures in degrading TCE at 0–3.5% salinity were accurately predicted using the kinetic equations and the best-fitted parameters. It is the incorporation of important concepts, involving the competitive inhibition kinetics for substrate interactions between toluene and TCE ($K_{I,TCE}$ and $K_{I,TOL}$), the finite capacity for TCE transformation ($T_{c,obs}$), and modified Monod kinetics for bacterial growth caused by osmotic pressure (Y_{obs}) that makes TCE degradation modeling successful.

Some investigators [10,20,22,26] hold that for salt-free solutions, the competitive inhibition coefficients (such as $K_{I,TOL}$) can be simply replaced with half-velocity coefficients (such as $K_{S,TOL}$). This replacement can only be made for TCE degradation because $K_{I,TOL}$ (0.8, 2.19, and 0.96 mg/L for salinity 0, 2, 3.5%, respectively) are very close to $K_{S,TOL}$ (2.9 mg/L). This method cannot be used to describe toluene degradation at high salinity, since the values of $K_{I,TCE}$ (1000 mg/L at 3.5% salinity) differ greatly from $K_{S,TCE}$ (7.9 mg/L).

5.2. Influence of salinity on toluene-grown cultures

Although numerous studies reported the degradation of TCE by cultures enriched on toluene [7–10,20,26,28,29], relatively few have addressed the effect of salinity on the biodegradation kinetics. This study first quantified the effect of the salinity conditions on the ability of toluene-grown cultures to degrade toluene and TCE through kinetic study. Based on the quantification results, toluene-oxidizing cultures exhibit three distinctive features in response to osmotic stress. First, applying a transient salinity stress to freshwater cultures consistently reduced the degradation rates of toluene and TCE, as well as the biomass yields. However, long-term salinity caused only the culture to lose its capability to degrade TCE but not toluene. Second, the presence of toluene greatly improved the degradation of TCE by halotolerant culture. The k_{TCE} value in the presence of toluene is four times higher than that in the absence of toluene. This phenomenon differs from that for freshwater culture under shock salinity stress, for which the k_{TCE} is the same in both the presence and absence of toluene. Finally, applying a short-term salt stress to freshwater culture reduced bacterial yield, but the observed bacterial yield, Y_{obs}, for halotolerant culture HHTO4 was increased to 1.0 mg VSS/mg TOL, a value even greater than obtained that in freshwater. The above results imply that the toluene-oxidizing culture copes with salt stress by two different mechanisms. Under shock salinity loads, the culture might produce compatible solutes from the substrate to maintain a turgor pressure, but under long-term salinity stress, the culture underwent evolutionary changes in their intracellular composition as a mechanism of osmotic adaptation, resulting in totally different physiologies of the culture [30].

The findings from this study lay a foundation in the rational design of biological process systems to remove TCE from saline water, e.g., biotrickling filters for TCE vapor treatment.

Since the NaCl salt appears to be a significant impact on bacterial activity, it is necessary that the level and duration of salt loads to the reactors be properly controlled. Subsequent investigations should be conducted toward revealing process kinetics of treatment systems under various salinity conditions, as well as to optimize operational parameters.

6. Conclusions

The following conclusions can be drawn from this study.

1. Applying transient saline stress to the culture cultivated in freshwater (LHTO4 culture) reduces the toluene utilization rate, the TCE degradation rate and the cell yields. NaCl is a noncompetitive inhibitor during the degradation of toluene and TCE.
2. In long-term 3.5% salinity, the culture underwent physiological changes into a halotolerant culture (HHTO4). This microorganism maintained most of its capability to consume toluene but its power to catalyze the cometabolic transformation of TCE declined substantially.
3. The inhibition by toluene on TCE degradation exceeds that by TCE on toluene degradation for cultures LHTO4 across 0–3.5% salinity and HHTO4 at 3.5% salinity. For culture LHTO4 under 0–3.5% salt stress, the inhibition of toluene is unrelated to salt content but the inhibition of TCE falls considerably as the NaCl concentration is raised to 3.5%.
4. Through incorporating important concepts of modified Monod kinetics, a finite capacity for TCE transformation, and competitive inhibition kinetics into the kinetic equations, this study successfully predicts the degradations of toluene, TCE and mixtures of toluene and TCE by cultures LHTO4 at 0–3.5% salinities and HHTO4 at 3.5% salinity. The findings from this work demonstrated the applicability of the employed microbial kinetics to the rational design of biological processes for removing TCE from saline solutions.

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