J Ind Microbiol Biotechnol (2006) 33: 37–44
DOI 10.1007/s10295-005-0049-0

ORIGINAL PAPER

Chi-Yuan Lee · Ching-Hsing Lin

Bacterial growth and substrate degradation by BTX-oxidizing culture in response to salt stress

Received: 11 March 2005 / Accepted: 29 September 2005 / Published online: 12 November 2005

Abstract Interactions between microbial growth and substrate degradation are important in determining the performance of trickle-bed bioreactors (TBB), especially when salt is added to reduce biomass formation in order to alleviate media clogging. This study was aimed at quantifying salinity effects on bacterial growth and substrate degradation, and at acquiring kinetic information in order to improve the design and operation of TBB. Experiment works began by cultivating a mixed culture in a chemostat reactor receiving artificial influent containing a mixture of benzene, toluene, and xylene (BTX), followed by using the enrichment culture to degrade the individual BTX substrates under a particular salinity, which ranged 0–50 g l^{-1} in batch mode. Then, the measured concentrations of biomass and residual substrate versus time were analyzed with the microbial kinetics; moreover, the obtained microbial kinetic constants under various salinities were modeled using noncompetitive inhibition kinetics. For the three substrates the observed bacterial yields appeared to be decreased from 0.51–0.74 to 0.20–0.22 mg mg^{-1} and the maximum specific rate of substrate utilization, $\hat{q}$, declined from 0.25–0.42 to 0.07–0.11 h^{-1}, as the salinity increased from 0 to 50 NaCl g l^{-1}. The NaCl acted as noncompetitive inhibitor, where the modeling inhibitions of the coefficients, $K_{T(S)}$, were 22.7–29.7 g l^{-1} for substrate degradation and $K_{T(\mu)}$, 13.0–19.0 g l^{-1}, for biomass formation. The calculated ratios for the bacterial maintenance rate, m_S, to $\hat{q}$ further indicated that the percentage energy spent on maintenance increased from 19–24 to 86–91% as salinity level increased from 0 to 50 g l^{-1}. These results revealed that the bacterial growth was more inhibited than substrate degradation by the BTX oxidizers under the tested salinity levels. The findings from this study demonstrate the potential of applying NaCl salt to control excessive biomass formation in biotrickling filters.

Keywords Benzene · Toluene · Xylene · Salinity · Trickle-bed bioreactor · Kinetics

C.-Y. Lee (✉) · C.-H. Lin
Water Resources and Environmental Engineering Program, Department of Harbor and River Engineering, National Taiwan Ocean University, Keelung, 20224 Taiwan, ROC
E-mail: cylee@mail.ntou.edu.tw
Tel.: +886-2-24622192
Fax: +886-2-24624770

Introduction

Aromatic chemicals, such as benzene, toluene, and xylene (BTX) are of special significance because they are toxic volatile organic compounds (VOC_S), having been implicated in human health [6]. For example, benzene is suspected to be carcinogen for all exposures and is a risk factor for leukemia and lymphomas [1, 19, 20]; inhalation of toluene or xylene may damage kidney and liver [2, 3]. The BTX compounds are major hydrocarbons in gasoline and are among the most commonly used organic solvents. They can widely be found in automobile exhaust, industrial waste gases, and wastewater treatment plants, which make them to be the prevalent air pollutants.

Recent developments in environmental biotechnology have made it possible for the BTX gases to be effectively degraded by trickle-bed bioreactors (TBB). The TBB consists of bacteria immobilized on a packing medium and circulating liquid that contains inorganic minerals and nutrients for the needs of cell growth [4, 8, 11, 14]. When this treatment option is implemented, however, there appears to be a significant problem due to clogging or pressure drop [4, 8, 11]. Thus, the addition of inert salt NaCl to the aqueous phase of TBBs to limit biomass formation has been proposed and tested [7, 17]. This practice, nevertheless, might result in a substantial reduction in substrate degradation concomitant with the inhibition of bacterial growth.

Though numerous investigations have been carried out to unravel the impact of salinity on wastewater treatment by biological processes [9, 10], the interrelationship between bacterial growth and substrate degra-

dation during VOC biodegradation affected by salinity has been rarely studied [7, 17]. Among the limited studies available, Dikes [7] investigated dichloromethane degradation by *Hyphomicrobium* GJ21 and by an enriched culture isolated using a trickling filter and showed that biomass growth was more inhibited than substrate degradation. In an examination of ethyl acetate and toluene degradations by a TBB, Schönduve et al. [17] reported that the fractional inhibition of biomass formation was stronger than the fractional inhibition of degradation under 0.4 M NaCl in the liquid phase. Lee et al. [12] also indicated that salinity caused both reductions in bacterial growth and trichloroethylene degradation by microorganisms under phenol enrichment. With regard to BTX degradations, the effect of salinity on the interactions between biomass formation and biodegradation is still unclear.

The purpose of this study was to combine experimental and kinetic modeling designed to acquire kinetic information that will help to improve the design and operation of TBBs. After cultivating a mixed bacterial consortia enriched on a mixture containing BTX, a batch study was carried out on the degradation of individual BTX substrates by the enriched bacteria in response to the change of salt stress. The focus of modeling is placed on solving the kinetic constants by a nonlinear regression technique in order to clarify the interactions between bacterial growth and substrate biodegradation across NaCl salinities from 0 to 50 g l^{-1}.

Methods

Bacterial cultures

The bacteria used for the BTX biodegradation tests were cultivated using a chemostat reactor operated at an ambient temperature of 25°C. Seeding bacterial sludge was obtained as sediment from Keelung harbor (located at E:121°45′ 38.82″, N:25°9′ 16.09″) which was contaminated with oil. Culture enrichment was carried out by the fill-and-draw technique, in a 4.8 l glass reactor. The feeding solution was prepared by dissolving the BTX mixture and mineral salts in distilled water. The composition of mineral salts (in g l^{-1}) was: $K_2HPO_4 \cdot 3H_2O$, 4.25; $NaH_2PO_4 \cdot H_2O$, 1.00; NH_4Cl, 2.00; $MgSO_4 \cdot 7H_2O$, 0.20; $FeSO_4 \cdot 7H_2O$, 0.012; $MnSO_4 \cdot H_2O$, 0.003; $ZnSO_4 \cdot 7H_2O$, 0.003; $CoSO_4 \cdot 7H_2O$, 0.001. The BTX mixture contained benzene (purity > 99% v/v, Ishizu Seiyaku Ltd., Japan), toluene (purity > 99% v/v, Ajax Chemicals, Australia), and *p*-xylene (purity 98% v/v, Nacalai Tesque, Inc., Japan), with each compound at a concentration of 30 mg l^{-1}. The cultivation of the BTX bacteria involved 48 h of aeration and this was followed by 1 h settling. The total period of culture enrichment was 20 days. After this, the fill-and-draw operation was changed to continuous flow with a 7 days hydraulic retention time. The influent, with a flow rate of 0.69 l day^{-1}, was transported by a peristaltic pump into the reactor, which was aerated through a diffuser at the upper level of mixed liquid.

During the period of cultivation, the performance of chemostat reactor was monitored frequently. The concentrations of influent BTX averaged 82.3 mg l^{-1} and the concentrations of effluent BTX averaged 1.24 mg l^{-1}, and the amount of biomass was 52.5 mg l^{-1}. About 20 µl of off-gas was sampled from the gas line before the activated carbon filter using a syringe, and analyzed by gas chromatography (see Analytical methods). There were 84.2 mg m^{-3} of BTX found in the off-gas. Based on a mass-balance calculation, the BTX reduction efficiency performed by biological activity was about 70%. The dissolved oxygen concentration inside the reactor was monitored regularly to ensure aerobic conditions, which were maintained in a range 4–6 mg l^{-1} oxygen throughout the period.

Batch biodegradation tests

Biodegradation tests of individual BTX compounds were conducted in 12.5 ml serum bottles containing 5 ml of liquid phase. The headspace was 7.5 ml to ensure sufficient oxygen supply. A small magnetic stirring bar was placed inside the bottle and maintained at a rotating speed of 200 rpm to assure equilibrium between gas and liquid phase. Prior to the biodegradation testing, 0.5 ml of fresh bacterial suspension taken from the chemostat reactor, mixed with appropriate volume of growth medium was placed in serum bottles, followed by the sealing of the serum bottle with a Teflon-lined rubber septum and aluminum crimp-top cap. Then a predetermined volume of each individual substrate was taken from saturated stock solution (1,780 mg l^{-1} for benzene, 515 mg l^{-1} for toluene and 175 mg l^{-1} for xylene) and injected into the serum bottle to yield a final liquid phase substrate concentration of 30 mg l^{-1}. In each test run, the starting biomass concentration was in the range of 3.3–4.8 mg volatile suspended solids (VSS) l^{-1}, giving ratios of initial concentrations of bacteria to substrate between 0.11 and 0.16 (mg VSS mg^{-1} substrate). Each test was carried out in duplicate and during the course of the study the vials were kept at 25°C in an incubator. The abiotic loss of individual BTX compounds during batch biodegradation assays was less than 1%, indicating that the non-biological activity could be negligible.

Determinations of kinetic coefficients

Two data sets of kinetic coefficients were determined to quantify the effect of salinity on bacterial growth and substrate degradation.

Data set 1: microbial kinetic coefficients

The microbial growth model employed in this study is comprised of three important mechanisms: the cellular

maintenance demands from exogenous substrate utilization, the endogenous respiration from storage materials, and the Monod relationship between bacterial growth rate and substrate concentration [13, 15]. For the substrate consumption rate under a particular salinity, it is expressed as the combination of cell synthesis from the true growth yield, Y_G [defined as $Y_G = \Delta X/_{\text{growth}}$, mg VSS mg^{-1} substrate] and maintenance-substrate utilization, m_S (h^{-1}), as indicated in Eq. 1a. The biomass production rate is equal to the difference between the bacterial growth from substrate consumption minus endogenous decay due to self respiration, shown in Eq. 1b.

$$-\frac{dS}{dt} = \left(\frac{1}{Y_G}\right)\mu X + m_S X, \tag{1a}$$

$$\frac{dX}{dt} = \mu X - bX, \tag{1b}$$

where μ the specific growth rate (h^{-1}), X biomass concentration (mg l^{-1}), and b bacterial decay coefficient (h^{-1}).

To solve Eqs. 1a, and 1b, substitution of appropriate kinetic constants in these two equations should be made. The procedure is illustrated below. First, the specific growth rate is replaced with Monod expression, i.e., $\mu = \hat{\mu}[S/(K+S)]$. Then, a parameter of observed bacterial yield, Y [defined as $Y = /\left(\Delta S_{\text{growth}} + \Delta S_{\text{maintenance}}\right)$, mg VSS mg^{-1} substrate] is substituted for Y_G to eliminate the term of m_S (see Eq. 3 for the relationship of Y to Y_G). And finally, define a parameter $\hat{q}$, the maximum specific substrate utilization rate (h^{-1}), as $\hat{q} = \hat{\mu}/Y$ and substitute $Y\hat{q}$ for $\hat{\mu}$.

$$-\frac{dS}{dt} = \frac{1}{Y}\frac{\hat{\mu}XS}{(K+S)}, \tag{2a}$$

$$\frac{dX}{dt} = Y\frac{\hat{q}XS}{K+S} - bX, \tag{2b}$$

where $\hat{\mu}$ maximum specific growth rate (h^{-1}), S substrate concentration (mg l^{-1}), and K half-velocity concentration (mg l^{-1}).

The growth yields of Y_G to Y are related through maintenance coefficient and specific growth rate as indicated in Eq. 3 [7, 15] by assuming $S \gg K$ (30≫2.6–5.1 mg l^{-1}, during growth phase) and neglecting the constant b, for b (0.003–0.006 h^{-1}, Table 1) is very small compared with $\hat{\mu}$ (0.01–0.31 h^{-1}, obtained from Table 1).

$$\frac{1}{Y} = \frac{1}{Y_G} + \frac{m_S}{\mu}. \tag{3}$$

In serum bottles, the volatile substrate was present in both the liquid and gas phases. Though substrate utilization rate is related to the substrate in liquid phase, it must account for the substrate transported from the gas to liquid phase during the course of incubation [16]. Therefore, the expression of the substrate utilization of Eq. 2a becomes:

$$-\frac{dS}{dt} = \frac{1}{\left(1 + H_c\frac{V_g}{V_l}\right)} \cdot \frac{\hat{q}XS}{K+S}, \tag{4}$$

where V_g volume of gas phase, V_l volume of liquid phase, H_c Henry's constant. When NaCl concentrations were 0, 10, 20, 30, 40 and 50 g l^{-1}, the dimensionless Henry's constants for benzene were 0.2288, 0.2468, 0.2647, 0.2827, 0.3007 and 0.3186; for toluene were 0.2746, 0.2982, 0.3218, 0.3454, 0.3690 and 0.3926; and for xylene were 0.2038, 0.2318, 0.2597, 0.2877, 0.3156 and 0.3436, respectively.

Using the measured data of residual substrate and biomass formation with respect to the elapsed time of incubation under a particular salinity, the procedure to determine the microbial kinetic coefficients was as follows. The decay coefficient, b, was computed first from the following equation:

$$b = -\frac{1}{t}\ln\left(\frac{X}{X_0}\right), \tag{5}$$

where b decay coefficients (h^{-1}), t time (h), X biomass (mg l^{-1}) at time t, X_0 biomass (mg l^{-1}) in the beginning of endogenous phase.

Given the b value, the Microbial kinetic constants of Y, $\hat{q}$, K are then solved from Eqs. 2b and 4 using nonlinear regression. The objective function of the numerical searching was defined as the minimization of the residual square errors between measurements and predictions, which is

$$\text{Minimize}\sum_i^m \left\{ \left[S_{\text{obs}} - S_{\text{pre}}\right]^2 + \left[X_{\text{obs}} - X_{\text{pre}}\right]^2 \right\}, \tag{6}$$

where m data set of measurements; S_{obs}, X_{obs} are measured substrate and biomass concentrations, respectively, and S_{pre}, X_{pre} represent predicted substrate and biomass concentrations, respectively.

Data set 2: salt inhibition kinetic coefficients

After the parameters of $\hat{q}$ and K were determined at a particular salinity, the effects of salinity changes on substrate degradation and biomass production were modeled by following noncompetitive inhibition kinetics [18]. The choice of noncompetitive inhibition kinetics was based on the fact that the obtained parameters $\hat{q}$ and $\hat{\mu}$ become lower when salinity is increased, but K remains unchanged. Thus, at a given salinity T (mg l^{-1}), the relationships of $\hat{q}$ to T and $\hat{\mu}$ to T are [7, 21]:

$$\hat{q} = \hat{q}_f \cdot \frac{1}{1 + \left(\frac{T}{K_{T(S)}}\right)^n} \tag{7a}$$

and

Table 1 Estimated biokinetic coefficients of Y, $\hat{q}$ and K for the degradation of benzene, toluene and xylene by mixed BTX cultures at different NaCl concentrations

Compounds	NaCl (g l^{-1})	Input parameter	Obtained biokinetic coefficient[b]		
		b[a] (h^{-1})	Y (mg VSS mg^{-1} substrate)	$\hat{q}$(h^{-1})	rss[c]
Benzene	0	0.005	0.70	0.36	0.2082
	10	0.004	0.53	0.28	0.3992
	20	0.004	0.41	0.23	0.2885
	30	0.004	0.37	0.11	0.1630
	40	0.003	0.27	0.09	0.9751
	50	0.003	0.20	0.08	1.6277
Toluene	0	0.006	0.74	0.42	0.9298
	10	0.004	0.62	0.39	0.5522
	20	0.004	0.55	0.31	0.6591
	30	0.004	0.32	0.16	1.3417
	40	0.004	0.27	0.13	1.3404
	50	0.003	0.22	0.11	1.0337
Xylene	0	0.005	0.51	0.25	0.7093
	10	0.004	0.42	0.23	0.6216
	20	0.004	0.37	0.19	0.2974
	30	0.004	0.26	0.10	0.3720
	40	0.003	0.23	0.09	0.4100
	50	0.003	0.20	0.07	0.3726

[a] b the bacterial decay coefficient
[b] Y the observed bacterial yield, and $\hat{q}$ the maximum specific substrate utilization rate. The obtained values for K (half-velocity concentration) across the tested salinities were 2.6 for benzene, 5.1 for toluene, and 3.4 mg l^{-1} for xylene
[c] rss, the minimization of the residual square errors between measurements and predictions

$$\hat{\mu} = \hat{\mu}_f \cdot \frac{1}{1 + \left(\frac{T}{K_{T(\mu)}}\right)^n}, \tag{7b}$$

where $\hat{q}_f$ maximum specific substrate utilization rate at salt-free solution (h^{-1}), n a constant, $K_{T(S)}$ inhibition coefficients on substrate degradation (mg l^{-1}), $\hat{\mu}_f$ maximum specific growth rate at salt-free solution (h^{-1}), and $K_{T(\mu)}$ inhibition coefficient on growth rate (mg l^{-1}).

Numerical iterations were performed to search for the constants of $K_{T(S)}$, n and $K_{T(\mu)}$, n, using the following objective functions:

$$\text{Minimize} \sum_i^m \left(\hat{q}_{\text{obs}} - \hat{q}_{\text{pre}}\right) \tag{8a}$$

and

$$\text{Minimize} \sum_i^m \left(\hat{\mu}_{\text{obs}} - \hat{\mu}_{\text{pre}}\right), \tag{8b}$$

where m data set of measurement; $\hat{q}_{\text{obs}}$ observed rate of maximum specific substrate utilization (h^{-1}), $\hat{q}_{\text{pre}}$ predicted rate of maximum specific substrate utilization (h^{-1}), $\hat{\mu}_{\text{obs}}$ observed rate of maximum specific growth (h^{-1}); and $\hat{\mu}_{\text{pre}}$ predicted rate of maximum specific growth (h^{-1}).

Analytical methods

Concentrations of dissolved oxygen and salinity were measured using an YSI 52 m (Yellow Spring Instrument, USA). Biomass was determined by a spectrophotometer (Spectronic 20 D+, Spectronic Instrument) at a wavelength of 600 nm. The obtained optical density, denoted as OD_{600}, was calibrated against dry cell mass, which was measured gravimetrically from the difference in cell mass at 105°C overnight and after combustion at 550°C for 1 h (APHA) [5]. Since the serum bottle was selected to fit the cell port of the spectrophotometer, optical density could be measured directly by placing the serum bottle in the port, which avoided removing liquid sample during routine measurement of biomass. Substrate depletion was determined by the headspace technique, where a volatile gas sample of 20 µl was withdrawn from vial using a gas-tight syringe (Hamilton 1702RN syringe with side port needles 22Sga.) and then was injected into a gas chromatograph (Hewlett Packard 6890) equipped with flame ionized detector (FID) and a DB-5 column (30 m L, 0.32 mm ID, and 0.25 µm film thickness). The temperature was maintained 65°C for the oven, 250°C for the injection port, and 275°C for the FID.

Results and discussion

Salinity effects on BTX degradations and bacterial growth

The study was initiated by placing 30 mg l^{-1} benzene as a single substrate in a serum bottle containing an initial biomass of 3.3–4.8 mg VSS l^{-1} of BTX-grown cultures. The profiles of substrate disappearance and biomass production over the course of the incubation in response to NaCl salinities from 0 to 50 g l^{-1} are depicted in Fig. 1. The increasing salinity caused reductions both in

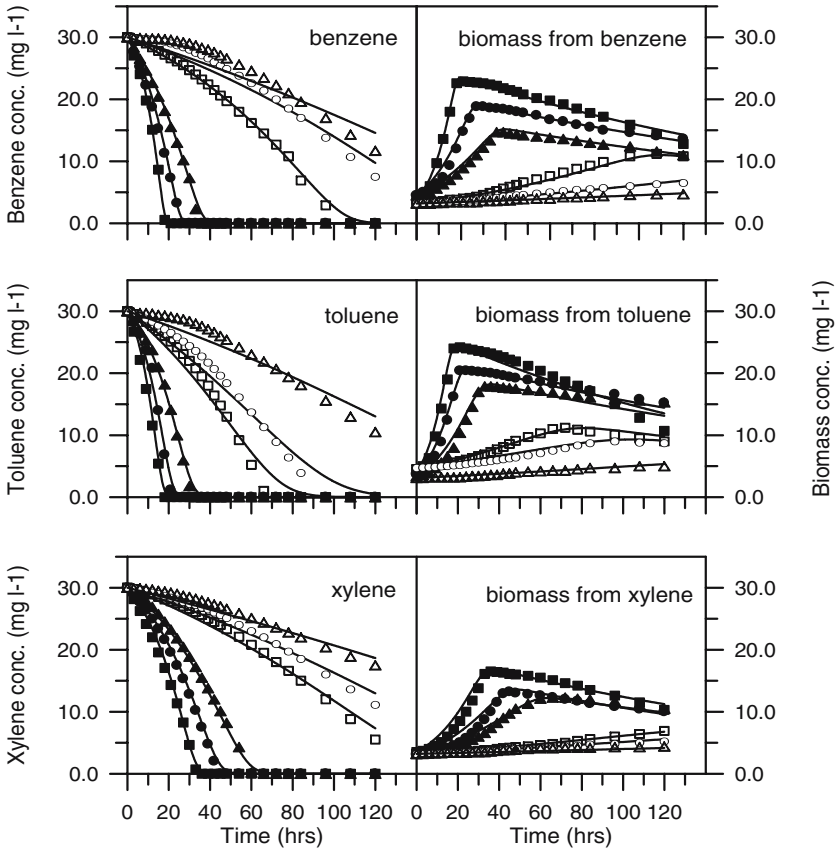

Fig. 1 Experimental data (*filled square*, 0 g l^{-1}; *filled circle*, 10 g l^{-1}; *filled triangle*, 20 g l^{-1}; *open square*, 30 g l^{-1}; *open circle*, 40 g l^{-1}; *open triangle*, 50 g l^{-1} NaCl) and model predictions (*lines*) are plotted to illustrate substrate degradation and growth of BTX oxidizers in different NaCl concentrations. Model predictions are generated using Eqs. 2b and 4 through inputs of kinetic coefficients, Y, b, $\hat{q}$, and K, listed in Table 1. Each point represents the averaged value from duplicate experiments

substrate degradation and bacterial growth. For example, in the salt free system, benzene was degraded completely within 21 h of incubation and in this period the biomass production reached 22.9 mg VSS l^{-1}. However, in 50 g l^{-1} NaCl saline solution even after 120 h of incubation, residual benzene was still present at 13.7 mg l^{-1} and biomass production remained below 4.3 mg VSS l^{-1}. The profiles for degradation of toluene and xylene in response to changes in salt stress also indicated that salinity caused reductions in both substrate utilization and biomass formation. However, it is difficult to compare the degradation behavior across the three compounds by themselves using the profiles in Fig. 1. The degradation characteristics of each compound needs to be unraveled through kinetic analyses, as illustrated below.

Kinetic coefficients of degradations of BTX

Therefore, we present the results of a kinetic analysis of substrate disappearance and biomass production as affected by salt stress. Quantification of the salinity effect on the behaviors of BTX-grown culture in degrading individual BTX compounds is based on four kinetic parameters, Y, b, $\hat{q}$, and K. For the case of benzene degradation, it appeared that Y decreased from 0.70 to 0.20 mg VSS mg^{-1} benzene as salt concentrations increased from 0 to 50 g l^{-1} (Table 1). The decay coefficients, b, remained in a rather limited range of 0.003–0.005 h^{-1}. The obtained optimal values of $\hat{q}$ decreased from 0.36 to 0.08 h^{-1}, and the K value for each salinity remained at 2.6 mg l^{-1} as the tested NaCl salinities increased from 0 to 50 g l^{-1}.

The changes in the kinetics parameters for toluene and xylene as salinity increased exhibited a similar pattern to benzene (Table 1). In the case of toluene, the coefficient Y decreased from 0.74 to 0.22 mg VSS mg^{-1} toluene, the $\hat{q}$ value dropped from 0.42 to 0.11 h^{-1}, and K values remained at 5.1 mg l^{-1}. For xylene, throughout the tested salinities, there were rather low values of Y (0.51–0.20 mg VSS mg^{-1} xylene), and of $\hat{q}$ (0.25–0.07 h^{-1}), as compared to benzene and toluene. The K value for xylene remained at 3.4 mg l^{-1}.

Inhibition of salinity

The impact of salinity changes on the substrate degradation is further generalized by solving the parameters for the inhibition coefficients, $K_{I(S)}$ and n from Eq. 7a. As indicated in Fig. 2, the estimated parameters were $K_{I(S)} = 22.7$ g l^{-1}, $n = 1.8$ for benzene; $K_{I(S)} = 28.2$ g l^{-1}, $n = 2.3$ for toluene; and $K_{I(S)} = 29.7$ g l^{-1}, $n = 2.2$ for xylene. This implies that among the three compounds, although the constants are in the same order of

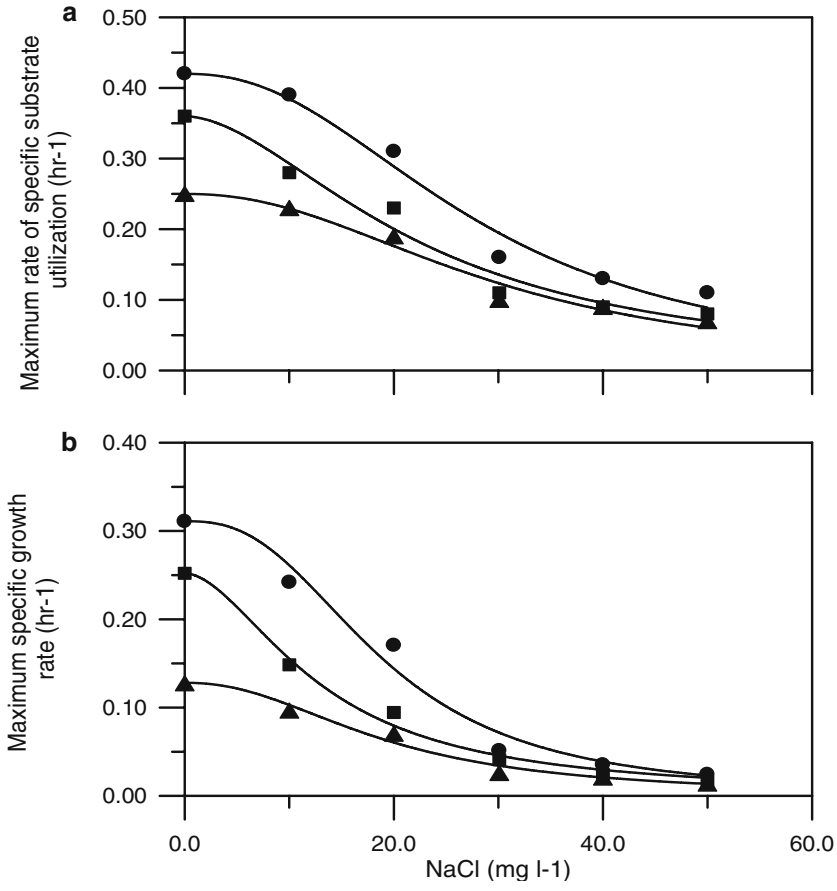

Fig. 2 The effects of NaCl inhibition on the maximum rate of specific substrate utilization $\hat{q}$ for degrading benzene, toluene, and xylene by BTX oxidizers (**a**) and on the maximum specific growth rate $\hat{\mu}$ of BTX oxidizers during utilizing benzene, toluene, and xylene (**b**). The values of $\hat{q}$ are obtained from Table 1 and $\hat{\mu}$ are from $\hat{q}$ multiplied by Y (*filled square*, benzene; *filled circle*, toluene; *filled triangle*, xylene). The predictions (*lines*) are generated from Eqs. 7a and 7b

magnitude, there is still a difference in substrate degradation that is affected by salinity stress. The degradation of benzene is most easily inhibited by salinity, and the degradation of xylene is least (Fig. 2), which supports the analysis done earlier in this study and shown in Table 1. Diks et al. [7] reported a $K_{T(S)}$ value of 16.8 g l^{-1}, using Eq. 7a with $n = 2.2$, for the inhibition of the dichloromethane in a biological trickling filter system. Kargi et al. [9, 10] modeled the removal rate of diluted molasses in synthetic wastewater with Eq. 7a under circumstance of $n = 1$, indicating $K_{T(S)}$ values of 950 g l^{-1} for activated sludge and of 70 g l^{-1} for rotating biological contacters. Compare with the $K_{T(S)}$ values of these reports, BTX and dichloromethane seem most sensitive to salinity during substrate degradation.

In a similar way, the effect of salinity on bacterial growth could be generalized through solving $K_{T(\mu)}$ and n from Eq. 7b. The $K_{T(\mu)}$ values for BTX were only 13.0, 18.9 and 19.0 g l^{-1} and n values were 1.8, 2.6, 2.2, respectively (Fig. 2). Such relatively low values of $K_{T(\mu)}$, compared with $K_{T(S)}$, implies that the inhibition of bacterial growth caused by salinity pressure is stronger than the reduction of substrate degradation. Additionally, when benzene was used as growth substrate, the BTX-oxidizing culture was more susceptible to salinity stress than with toluene and xylene.

Implications for biological treatment of BTX vapors

Treatment of volatile organic compounds by TBB has received much attention because of its low operating cost. However, the excess biomass in a biotrickling filter leads to a reduction in run time and increased energy use to overcome the pressure drop. The addition of NaCl to the liquid phase of TBB represents a novel approach that should limit biomass formation [7, 17]. Thus, quantification of the interactions between the inhibitions of biomass production and substrate degradation under the changes of salinity load are vital to the successful design and operation of TBB.

This study has shown that the effect of changes in salinity on the bacterial growth and substrate degradation by BTX oxidizers can be quantified using the parameters Y, b, $\hat{q}$, and K. As indicated previously, the observed bacterial yield Y decreased with increasing salinity stress, implying that more maintenance energy was needed in higher salinity solutions. To clarify the interactions between bacterial growth and substrate degradation, the parameters of maintenance energy, m_S, and true bacterial yield, Y_G, need to be calculated first.

In Eq. 3, as the parameter μ is replaced with $\hat{\mu}$ by assuming $S \gg K$, and thus a linear equation to solve m_S and Y_G can be obtained [7, 15]:

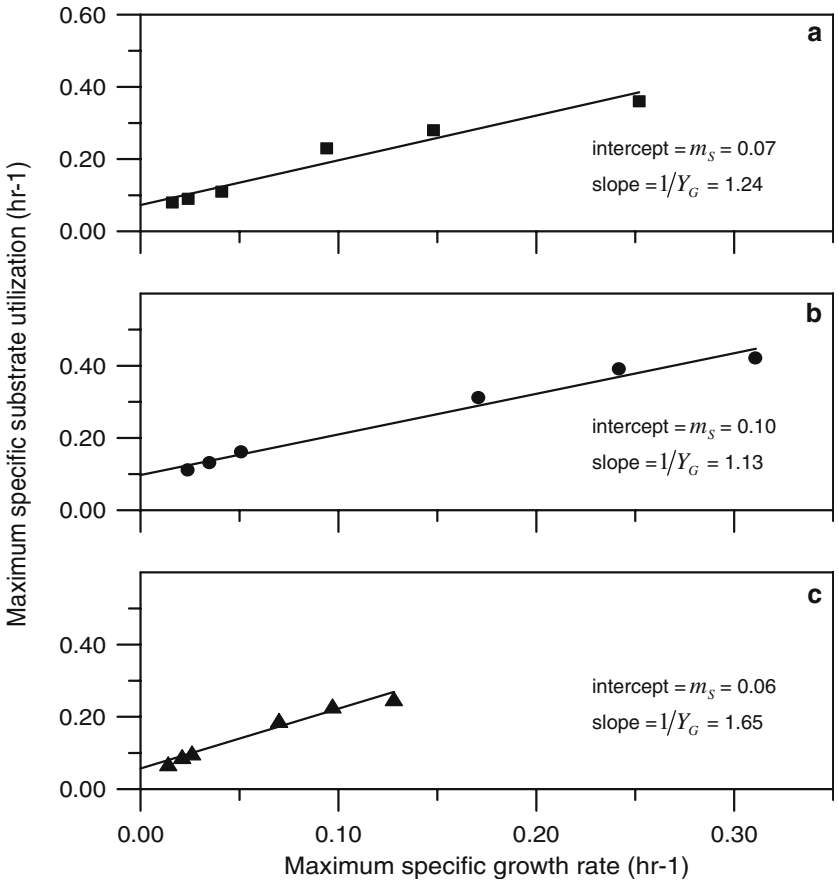

Fig. 3 The maximum rate of specific substrate utilization (Fig. 2a) is plotted against the maximum growth rate (Fig. 2b) for the degradation of benzene (**a**), toluene (**b**), and xylene (**c**) to solve the constants of maintenance energy m_S and true growth yield Y_G according to Eq. 9

$$\hat{q} = \frac{\hat{\mu}}{Y_G} + m_S. \quad (9)$$

Based on Eq. 9, the plot of $\hat{q}$ versus $\hat{\mu}$ will be a straight line with the intercept = m_S and slope = $1/Y_G$. As shown in Fig. 3, the obtained values of m_S are 0.07 for benzene, 0.10 for toluene, and 0.06 g (g h)$^{-1}$ for xylene. Additionally, the obtained values of Y_G are 0.81 for benzene, 0.88 for toluene, and 0.61 mg VSS mg^{-1} substrate for xylene. If m_S is further compared with $\hat{q}$, it appears that about 20% of substrate consumption results is needed for maintenance in a salt-free system (Table 2), a value similar to the dichloromethane degradation by *Hyphomicrobium* [7]. However, when the salinity concentration is increased to 50 g l^{-1}, the maintenance energy increases to 88% $\hat{q}$ for degrading benzene, 91% $\hat{q}$ for degrading toluene, and 86% for degrading xylene.

Table 2 The ratios of m_S to $\hat{q}$ for the degradation of benzene, toluene, and xylene at different NaCl concentrations

NaCl (g l^{-1})	m_S/q[a]		
	Benzene	Toluene	Xylene
0	0.19	0.24	0.24
10	0.25	0.26	0.26
20	0.30	0.32	0.32
30	0.64	0.63	0.60
40	0.78	0.77	0.67
50	0.88	0.91	0.86

[a] m_S the maintenance coefficient (h^{-1}), $\hat{q}$ the maximum specific substrate utilization rate (h^{-1})

The relative effects of salinity on bacterial growth and substrate degradation can be also illustrated by a parameter R [17]. The R value is defined as the ratio of the fraction inhibition of biomass formation to the fraction decrease in degradation, i.e., $R = \left[\left(\frac{\hat{\mu}_f - \hat{\mu}}{\hat{\mu}}\right) \Big/ \left(\frac{\hat{q}_f - \hat{q}}{\hat{q}}\right)\right]$. Accordingly, a value of R greater than 1 is favorable, when NaCl is added into TBB to limit biomass formation. In case of R values higher than 1, this implies that under salinity stress, the BTX oxidizers exhibited active maintenance, leading to the consumption of more substrate than is normally required for growth. In our study, under salinity 10 g l^{-1} the values of R were 1.85 for benzene, 3.11 for toluene, and 3.03 for xylene. However, the R values declined as the salinity increases. For example, they decreased to 1.20 for benzene, 1.25 for toluene, and 1.24 for xylene at 50 g l^{-1}. For the three substrates over the salinity range 10–50 g l^{-1} the R values were larger than 1.20, indicating that the fractional inhibition of biomass is greater than fractional reduction in degradation. This result agrees with a study of the treatment of toluene vapor by

a TBB [17], where the R value was 1.32 when the NaCl concentration in the liquid phase was 0.4 M (23.4 g l^{-1}).

In summary, both the indicators, $m_S/\overline{q}$ and R, have revealed that it is advantageous to apply NaCl as salt as a means of controlling excessive biomass, during the treatment of BTX waste gases by TBB. However, since these indicators were obtained from batch experiment with a short-term salinity load, the application of these findings to engineering in practice should be done with care. Future research should seek to elucidate the effect of salt addition on long-term reactor performance in TBB systems in order to identify the optimal dosage of NaCl for TBB.

Acknowledgements The authors thank Wen-Der Liu for assistance with kinetic modeling. This research was supported by National Science Council (NSC 92-2211-E-019-004), Taiwan, the Republic of China.

References

1. Agency for Toxic Substances and Disease Registry (ATSDR) (1997) Toxicological profile for benzene. U.S. Public Health Service, U.S. Department of Health and Human Services, Atlanta, GA
2. Agency for Toxic Substances and Disease Registry (ATSDR) (1994) Toxicological profile for toluene. U.S. Public Health Service, U.S. Department of Health and Human Services, Atlanta, GA
3. Agency for Toxic Substances and Disease Registry (ATSDR) (1995) Toxicological profile for xylenes. Public Health Service, U.S. Department of Health and Human Services, Altanta, GA
4. Alonso C, Suidan MT, Sorial GA, Smith FL, Biswas P, Smith PJ, Brenner RC (1997) Gas treatment in trickle-bed biofilters: biomass, how much is enough? Biotechnol Bioeng 54:583–594
5. APHA (1998) Standard methods for the examination of water and wastewater, 20th edn. American Public Health Association, Washington DC, p.2-54-2-60 (ISBN 0-87553235-7)
6. Dean BJ (1985) Recent findings on the genetic toxicology of benzene, toluene, xylenes and phenols. Mutat Res 154:153–181
7. Diks RMM, Ottengraf SPP, van den Oever AHC (1994) The influence of NaCl on the degradation rate of dichloromethane by *Hyphomicrobium* sp. Biodegradation 5:129–141
8. Hemakt D, Linn A, Stephan M, Vortmeyer D (1997) Biodegradation dynamics of aromatic compounds from waste air in a trickle-bed reactor. Appl Microbiol Biotechnol 48:129–134
9. Kargi F, Dincer AR (1996) Enhancement of biological treatment performance of saline wastewater by halophilic bacteria. Bioproc Eng 15:51–58
10. Kargi F, Dincer AR (1999) Salt inhibition effects in biological treatment of saline wastewater in RBC. J Environ Eng 125:966–971
11. Laurenzis A, Heirs H, Wübker SM, Heinze U, Friedrich C (1998) Continuous biological waste gas treatment in stirred trickled-bed reactor with discontinuous removal of biomass. Biotechnol Bioeng 57:497–503
12. Lee CY, Chan YC, Lin CL (2005) The effect of salinity on trichloroethylene co-metabolism by mixed cultures enriched on phenol. World J Microbiol Biotechnol 21:359–365. DOI:10.1007/s11274-004-2611-8
13. Monod J (1949) The growth of bacteria cultures. Ann Rev Microbiol 3:371–394
14. Oh YS, Bartha R (1997) Removal of nitrobenzene vapors by a trickling air biofilter. J Ind Microbiol Biotechnol 18:293–296
15. Pirt SJ (1965) The maintenance energy of bacteria in growing cultures. Proc R Soc London 163B:224–231
16. Reardon KF, Mosteller DC, Bull Rogers JD (2000) Biodegradation kinetics of benzene, toluene, and phenol as single and mixed substrates for *Pseudomonas putida* F1. Biotechnol Bioeng 69:385–400
17. Schönduve P, Sàra M, Friedl A (1996) Influence of physiologically relevant parameters on biomass formation in a trickle-bed bioreactor used for waste gas cleaning. Appl Microbiol Biotechnol 45:286–292
18. Tinoco I Jr, Sauer K, Wang JC (1985) Physical chemistry: principles and applications in biological sciences, 2nd edn. Prentice-Hill, New Jersey, pp 383–385 (ISBN 0-13-666280-3)
19. U.S. Environmental Protection Agency (2002) Integrate risk information system (IRIS) on benzene. National Center for Environmental Assessment, Office of Research and Development, Washington DC
20. U.S. Environmental Protection Agency (2000) The integrated urban strategy, report to congress. EPA-453/R-99-007; U.S. Environmental Protection Agency, office of Air Quality Planning and Standards Research Triangle Park, North Carolina
21. Yano T, Koga S (1969) Dynamic behavior of the chemostat subject to substrate inhibition. Biotechnol Bioeng 11:139–153