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Biodegradation of tetrachloroethylene by a newly isolated aerobic *Sphingopyxis ummariensis VR13*

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Abstract−Chlorinated aliphatic solvents are major sources of groundwater and soil contamination. In this study, an aerobic bacterial strain, *Sphingopyxis ummariensis VR13,* which has been newly isolated from petrochemical wastewater sludge, was used for the dechlorination of PCE in relatively high concentrations. The addition of a co-substrate as glucose and yeast extract enhanced the dechlorination of PCE. An adaptation of the bacterial cells to PCE resulted in a significant increase in the PCE degradation yield (62.9-39.4%) at relatively high initial PCE concentrations (0.4-5 mM). The adapted cells achieved the highest biodegradation yield (64.8%) in 1.2 mM. However, the maximum dechlorination percentage (41.6%) was measured in lower PCE concentration. The kinetic studies showed that PCE degradation was associated with the biomass growth because a higher removal of PCE (64.8%) occurred in a higher cell density. The degradation kinetics of PCE was properly fitted by Monod-like equation with the specific degradation rate of 7.2 mmol PCE (g biomass)$^{-1}$d^{-1}, which was even faster than the reported anaerobic bacteria at this concentration. This strain can be used in the aerobic degradation of PCE.

Keywords: Biodegradation, Tetrachloroethylene (PCE), *Sphingopyxis ummariensis VR13*, Kinetics, Dechlorination

INTRODUCTION

Chlorinated aliphatic solvents are recalcitrant compounds [1-5] that are commonly present in groundwater, and soil as pollutants [6]. Among them, tetrachloroethylene (PCE) is an artificial waste which is degraded slowly in the environment and is of great concern due to its carcinogenic properties. Therefore, PCE is listed as a priority pollutant by the United States Environmental Protection Agency (US-EPA) [7]. The maximum contaminant level (MCL) of PCE in drinking water is 5 mg/L [3].

The methods of removing PCE in water and groundwater include adsorption [8], aeration stripping [9,10], photocatalytic degradation [11], advanced oxidation [12], and bioremediation [8,13]. Engineered bioremediation processes are efficient methods for the PCE remediation of polluted sites; biotechnological methods are less expensive and the least complex when compared to other approaches. PCE is one the most difficult pollutants to biologically degrade, due to its artificial nature [4].

Many researchers have reviewed the metabolism of anaerobic bio-reductive degradation of PCE, in which it transforms to trichloroethylene (TCE), *cis*-1,2-dichloroethylene (*cis*-DCE), vinyl chloride (VC) and ethane [6,14]. Until almost twenty years ago, it was thought that PCE was non-biodegradable in aerobic conditions. In recent years, the possibility of aerobic dechlorination of chloroethylene compounds has been considered because of some critical obstacles of anaerobic biodegradation. The slow anaerobic processes compared to the aerobic biodegradation [14,15], limiting steps in the bio-reductive dechlorination [15], and the strain limitation for completing the bio-reductive dechlorination of PCE to ethane [4,6,16] are some of the anaerobic dechlorination disadvantages. To solve these problems, anaerobic/aerobic biological processes are used for the treatment of complex chlorinated compounds such as PCE and TCE. The lower chlorinated compounds resulting from anaerobic degradation of the higher chlorinated compound are more easily degraded under aerobic conditions [4]. Microbial anaerobic/aerobic degradation is a complex process that needs to be carefully controlled. Therefore, it would be beneficial to find new microbial species capable of aerobic degradation of chlorinated hydrocarbons. An efficient aerobic alternative may result in performance improvements and the reduction of PCE removal costs.

There are limited reports available for aerobic bacterial degradation of PCE [4,17-19].

Ryoo et al. initially investigated the degradation of PCE by aerobic *Pseudomonas stutzeri* OX1 and found that its degradation was the result of toluene-o-xylene monooxygenase enzyme (ToMO) expression, while toluene was used as the carbon source. Then, the enzyme ToMO was successfully cloned and expressed in *Escherichia coli*, which was capable of degrading PCE and generating stoichiometric amounts of products (chloride) at low concentrations using glucose as the energy source [17]. Subsequently, the aerobic biodegradation of PCE by *P. stutzeri OX1* and recombinant *E. coli* in the binary mixture solution of PCE, TCE, *cis*-DCE, *trans*-DCE, and VC

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was studied at a higher concentration of PCE [18].

In addition, an activated sludge process was successfully used to remove PCE from polluted air. The dominant strains in the activated sludge were gram-negative bacteria including *Pseudomonas aeruginosa*, *Ralstonia picketii*, *Pseudomonas putida*, and *Ochrobactrum anthropii* [20].

Researchers believe that chloroethylenes, such as PCE, degrade in a co-metabolism way under aerobic conditions. In this regard, the degrading enzymes are produced for degradation of a primary substrate for cell growth, while the chloroethylenes co-metabolically degrade as a non-growth substrate. Note that a few reports are published about aerobic PCE co-metabolism [6,14]. The present study investigates the ability of a newly isolated strain namely, *Sphingopyxis ummariensis VR13*, to aerobically degrade PCE in relatively high concentrations (up to 1.2 mM) after cell adaptation. The use of adapted strains to metabolize calcitrant compounds is an attractive method to reduce the lag phase of cell growth and increase degradation in industrial wastewater treatment [21,22].

The applied bacterium was a gram-negative, aerobic, light-yellow pigmented, and non-fermentative bacterium. It was previously isolated from a petrochemical wastewater sludge and used to degrade some polyaromatic hydrocarbons [23]. A co-substrate was used to increase cell mass and achieve higher biodegradation capacities [24]. The PCE degradation kinetics is of great concern due to its importance in industrial applications.

MATERIALS AND METHODS

1. Materials and Microorganism

The PCE, TCE, and toluene (>99.9%), nutrient broth, nutrient agar, glucose and yeast extract were purchased from Merck Co. (Darmstadt, Germany). The growth medium was composed of a mineral salts solution (MSS), trace element solution, glucose, and yeast extract. The mineral salts solution (MSS) contained the following salts (g) per liter of distilled water: KH_2PO_4 (1.0), $(NH_4)_2SO_4$ (1.8), $MgSO_4{\cdot}7H_2O$ (0.2), $CaCl_2{\cdot}2H_2O$ (0.03), and $Na_2HPO_4{\cdot}12H_2O$ (2.5) [25]. The trace elements solution was composed of (in mg/L) $Ca(NO_3)_2{\cdot}4H_2O$ (880), $FeSO_4{\cdot}7H_2O$ (200), $ZnSO_4{\cdot}7H_2O$ (10), H_3BO_3 (10), $CoSO_4{\cdot}7H_2O$ (10), $CuSO_4{\cdot}5H_2O$ (10), $MnSO_4{\cdot}H_2O$ (4), $Ni(NO_3)_2{\cdot}6H_2O$ (4), $Na_2MoO_4{\cdot}2H_2O$ (3), $Na_2WO_4{\cdot}2H_2O$ (2), and H_2SO_4 (0.05 N) [26]. In all the experiments, the same weight ratio of glucose and yeast extract was added to the MSS solution containing 0.5% (v/v) of the trace element solution to prepare the culture medium. The pH of the medium was adjusted to 7-7.5.

The *S. ummariensis VR13* strain, which has been newly isolated from petrochemical wastewater sludge, was obtained from Persian Type Culture Collection (PTCC 1895).

2. Adaptation of *S. ummariensis* to PCE

The PCE-utilizing strain of *S. ummariensis* was grown in a nutrient broth solution at 30 °C on a rotary shaker (150 rpm) for 48 h to reach the exponential growth phase. The cells were harvested by centrifugation at 7,000 rpm for 15 min and subsequently washed twice with physiological saline solution. The harvested cells were then used for adaptation experiments in a packed-bed aerobic bioreactor with gas recycle. The cells were grown in a culture medium containing glucose/yeast extract as co-substrate. The adaptation process was carried out by a gradual increase in PCE concentration from 0 to 1.0 mM in the culture medium within a month. Then, the adapted cells were harvested and washed twice with physiological saline solution. Prior to incubation to the serum bottles, the purity of the bacteria was checked under the microscope and their colonies were checked on nutrient agar plates. PCE degradation experiments were done by the harvested cells before and after adaptation.

3. Experimental Growth Conditions

Due to the high volatilization of PCE, serum bottles were used for the growth of *S. ummariensis VR13* cells to prevent PCE evaporation. The cells were diluted by the culture medium to an optical density (OD_{600}) of 1.0 and used as preculture. The culture media containing predefined PCE and glucose/yeast extract concentrations was added to 150 mL serum bottles with a 9 : 1 airspace/liquid ratio and inoculated by 100 µL of preculture [25]. The serum bottles were immediately sealed with silicone rubber septa, parafilm, and aluminum crimp caps; they were then incubated at 30 °C on a rotary shaker (150 rpm). Oxygen was available for the bacteria from the headspace of ambient air in the bottles. Dissolved oxygen (DO) measurement in the beginning and at the end of bacterial cultivation time was between 6 and 2 mg/L, which ensured the aerobic conditions [24,27]. TCE, PCE, chloride ion and biomass concentrations were measured during bacterial growth.

The effect of different initial concentrations of co-substrate was investigated on the PCE degradation yield (Y) and PCE dechlorination (G) to find the optimum concentrations. Therefore, different culture media were prepared with glucose/yeast extract concentrations in the range 0.125-1 g/L and *S. ummariensis VR13* was cultivated in the media. Y was also measured at different initial concentrations. Each experiment included control bottles (without bacteria) that were also incubated under the same conditions to monitor possible changes in the PCE by volatilization. The experiments were carried out in triplicate and expressed as Mean±SD.

4. Analytical Methods

The TCE and PCE concentrations were analyzed by gas chromatography. To prepare the samples, the culture medium were extracted by toluene according to Fathepure and Boyd [28]. The recovery efficiencies for PCE and TCE were determined using standard solutions, which were measured as 95-97%. The concentrations of PCE and TCE were measured by a gas chromatograph (YL Instrument Model 6500) equipped with a flame ionization detector (FID) and a capillary column (TRB5: 30 m×0.53 mm×1.5 µm). The temperatures of the injector and detector were kept at 280 °C. The temperature for the column was programmed and maintained at 50-160 °C for 12 min and incrementally increased (10 °C/min). The carrier gas was nitrogen at a flow rate of 4 cm^3/min. The biodegradation for PCE and TCE was determined using standard solutions.

The initial biomass concentration was determined by measuring the optical density (OD) of the medium at 600 nm utilizing a UV spectrometer (Philips Pye Unicam PU 8620 UV/VIS/NIR, UK).

Dissolved oxygen (DO) of the culture medium was measured using a DO meter (Hach HQ30d). The concentration of the chloride ions of the medium, generated by the dissociation of PCE, were determined by a Jenway 3045 Ion Analyser using selective ion electrodes (ISE).

The PCE degradation yield (Y) was measured by the following equations:

$$Y = \frac{C_T - C_C}{C_T} \times 100 \quad (1)$$

where, C_T is initial concentration of PCE (mM), and C_C is concentration of PCE (mM).

The PCE dechlorination (G) was defined as the percentage of stoichiometric chloride that was released to the solution by PCE biodegradation and calculated as:

$$G = \frac{S_C - S_{Ctrl}}{S_T - S_{Ctrl}} \times 100 \quad (2)$$

where, S_{Ctrl} is control chloride solution concentration without bacterial cells (mg/L), S_C is released chloride (mg/L), and S_T is initial concentration of chloride in PCE solution (mg/L).

5. Kinetic Studies of PCE Biodegradation

The kinetics of PCE biodegradation by adapted *S. ummariensis VR13* was studied in a batch suspended cell mode for 50 h at 30 °C. The culture media contained glucose/yeast extract (0.5 g/L) with different concentrations of PCE. The kinetics of TCE degradation with the initial concentration of 0.47 mM was also studied by adapted *S. ummariensis* to find the TCE degradation and chloride ion production in the presence of 0.5 g/L glucose/yeast extract.

The degradation kinetics was formulated using three kinetic models: Monod-like, first-order, and second-order equations. The Monod-like equation which has been previously applied to describe PCE degradation kinetics in an anaerobic enrichment culture [29], is described as Eq. (3).

$$\frac{dC}{dt} = \frac{\mu_m C}{K_S + C} X \quad (3)$$

where, C (mM) is the concentration of PCE in the culture medium, t (h) is degradation time, K_S (mM) is the maximum specific biodegradation yield and the half-saturation constant, μ_m (mmol PCE/(g biomass)·h) is the maximum growth rate, and X (g biomass) is the amount of biomass [29].

For the batch cell growth, Eqs. (4) and (5) describe the first-order kinetics, while Eqs. (6) and (7) characterize the second-order reaction:

$$\frac{dC}{dt} = r_C = A \cdot C \quad (4)$$

$$\ln(C/C_0) = Bt \quad (5)$$

$$\frac{dC}{dt} = r_C = A \cdot C^2 \quad (6)$$

$$\frac{1}{C} - \frac{1}{C_0} = B't \quad (7)$$

where, C (mM) is the concentration of PCE in the culture medium, and A and B are constants of the equation. The coefficient of determination (R^2) was used to assess the error of fitting.

6. Statistical Analysis

The results of adaptation were compared using student's t-test method. The significant effect of different factors on the responses was evaluated via one-way ANOVA (analysis of variance). All statistical analysis was carried out with a 95% confidence interval.

RESULTS

The *S. ummariensis VR13* bacterium was used to study the aerobic biodegradation of PCE. The release of dissociated chloride ions into the medium was the primary indication of PCE degradation. Therefore, the chloride concentrations were measured during the experiments. The operating conditions that influence microbial growth and PCE degradation, including growth co-substrates and PCE concentration were investigated in this study. The primary experiments focused on the biodegradation ability of non-adapted cells on PCE, followed by the PCE biodegradation study using the adapted cells.

1. Dechlorination of PCE by *S. ummariensis*

The experiments revealed the ability of *S. ummariensis VR13* cells in the dechlorination of PCE and TCE by aerobic metabolic pathway. Initially, PCE was used as the sole carbon source and the use of the bacterium resulted in 10% dechlorination for PCE. Phenol, glucose, glucose/yeast extract, nutrient broth (NB), and toluene were then tested as growth co-substrates. The mixture of glucose and yeast extract with an equal weight ratio as co-substrate, a pH of 7.0 and a temperature of 30 °C showed the best results in aerobic PCE dechlorination (data not shown). The optimized conditions were used in the following experiments.

Fig. 1 shows the effect of the initial PCE concentration on degradation efficiency and dechlorination percentage by *S. ummariensis VR13* (non-adapted) in the presence of the glucose/yeast extract co-substrate. Both the PCE degradation (from 24.8 to 9.6%) and dechlorination yield (from 17.3 to almost 4.9%) decreased by increasing the initial concentration of PCE from 0.5 to 5 mM.

2. Effect of Adaptation on PCE Degradation Efficiency

Fig. 2 represents the results of PCE degradation at different initial concentrations in the presence of glucose/yeast extract (0.2 g/L) before and after adaptation. The adaptation of the bacterial cells to PCE at 0.4 mM was performed and resulted in an increase in the Y from 27.3 to 62.9%. By increasing the initial PCE concentration, the biodegradation of PCE slightly increased to 64.7% at 1.2 mM, then decreased to 51.7, and 39.4% for 2.5 and 5 mM, respectively. In addition, the measurement of the G of PCE at 1.2 mM

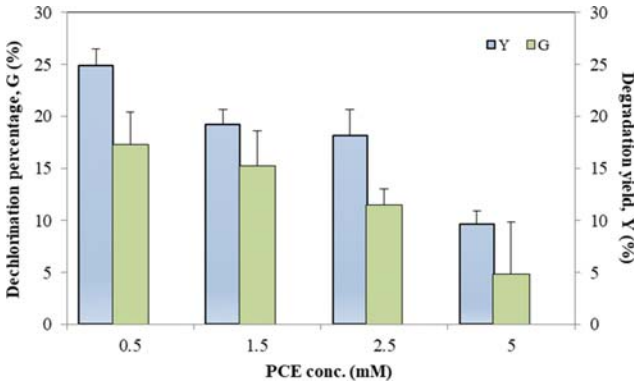

Fig. 1. Effect of the initial PCE concentration on Y and G by non-adapted *S. ummariensis VR13* cells after 48 h (T=30 °C).

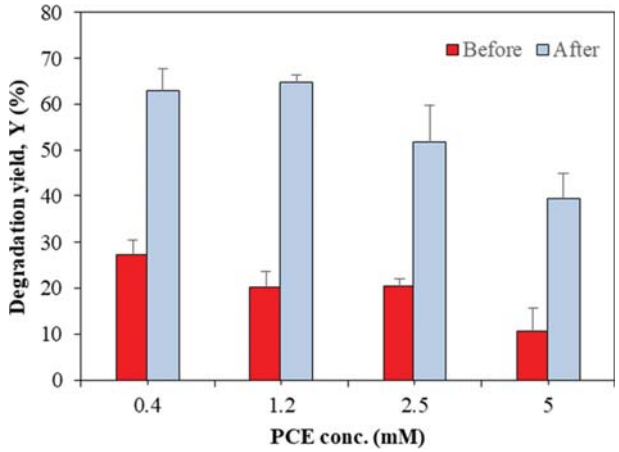

Fig. 2. PCE degradation yields at different initial concentrations after 48 h (T=30 °C) before and after adaptation.

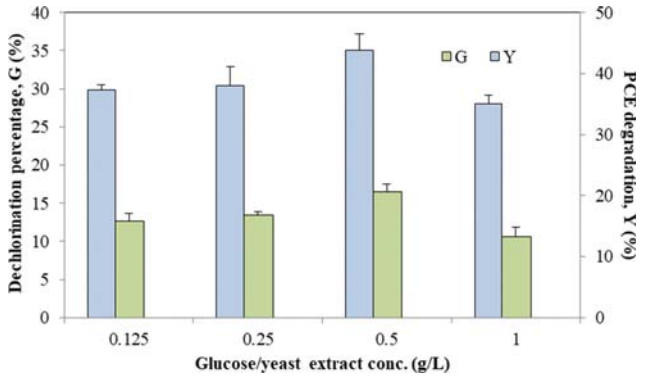

Fig. 3. Effect of the initial concentration of glucose/yeast extract (g/L) on PCE degradation by adapted *S. ummariensis VR13* after 32 h (initial PCE conc.=0.1 mM, T=30 °C).

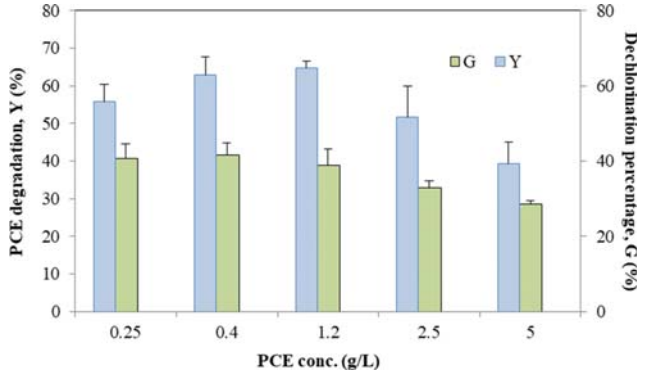

Fig. 4. Effect of the initial PCE concentration on Y and G by adapted *S. ummariensis VR13* in the presence of 0.5 g/L glucose/yeast extract after 32 h (T=30 °C).

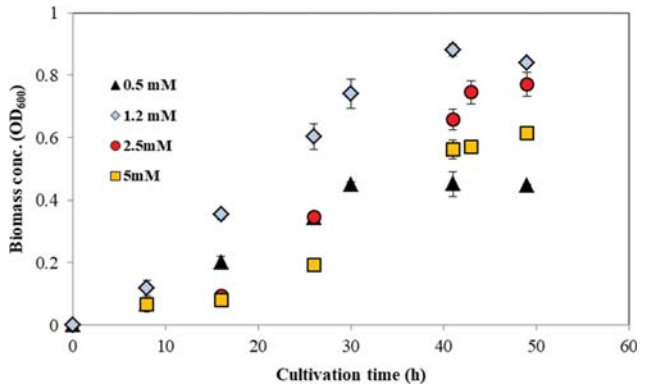

Fig. 5. The growth of adapted *S. ummariensis VR13* at different PCE concentrations (0.5 g/L glucose/yeast extract, T=30 °C).

represented an uptrend from 14.0% to 38.9% after adaptation.

The paired test also confirmed the significant difference in the results of adaptation ($p<0.05$). Before adaptation, Y continuously decreased with increasing PCE concentration, whereas afterward, Y initially increased then decreased.

The influence of the initial concentration of glucose/yeast extract co-substrate on Y and the G was investigated after cell adaptation (Fig. 3). The PCE degradation increased from 37.3 to 43.8% when the glucose/yeast extract concentration increased from 0.125 to 0.5 g/L, then declined to 35.0% at 1.0 g/L. The same trend was seen for the PCE dechlorination percentage. Therefore, the concentration of the glucose-yeast extract co-substrate was set to 0.5 g/L for further studies.

Fig. 4 shows the effect of different concentrations of PCE on the Y and G by the adapted cells. Y increased from 55.7 to 64.7%, while the initial PCE concentration increased from 0.25 to 1.2 mM in the adapted *S. ummariensis VR13* cell culture. By increasing the PCE concentration up to 5 mM, its biodegradation decreased to 39.4%. The highest Y was achieved in 1.2 mM by the adapted cells. However, the maximum G (41.6%) was measured in the lower concentration. These results showed that higher mineralization was expected in low PCE concentration.

The growth of the adapted *S. ummariensis VR13* was also investigated for different PCE concentrations (Fig. 5). The cell mass increased by increasing the initial PCE concentration to 1.2 mM, followed by a decline in 2.5 and 5 mM. Therefore, the optimum PCE concentration for both cell growth and Y was determined as 1.2 mM, and this was used for the kinetic studies.

3. Kinetic Studies of PCE Degradation

The kinetics of PCE degradation by the adapted *S. ummariensis VR13* was tested in the presence of glucose/yeast extract (0.5 g/l) for 50 h at 30 °C. The concentration of TCE and chloride ions in the culture media was also measured to follow up the biodegradation by-products. Fig. 6 shows the result of PCE degradation in the initial concentration of 1.2 mM, TCE and chloride ion concentrations by adapted *S. ummariensis VR13* in the presence of 0.5 g/L glucose/yeast extract at 30 °C. The PCE concentration decreased quickly from 1.2 to 0.56 mM in the initial 30 h, then the degradation slope decreased. The chloride ion concentration, as one of the products of the PCE biodegradation, increased at the same time interval. During the first 16 hours, the concentration of TCE increased from 0 to 0.23 mM, followed by a decline; then, it was totally removed from the solution after 40 hours. TCE is a by-product of PCE degradation. Further removal of TCE is also important due

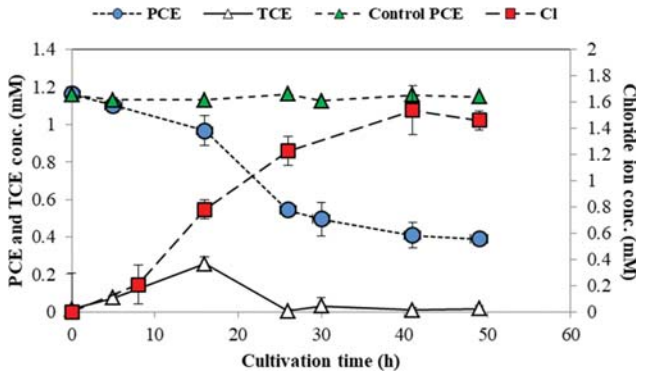

Fig. 6. The kinetics of PCE degradation (1.2 mM), TCE and chloride ion concentrations by adapted *S. ummariensis VR13* in the presence of 0.5 g/L glucose/yeast extract at 30 °C.

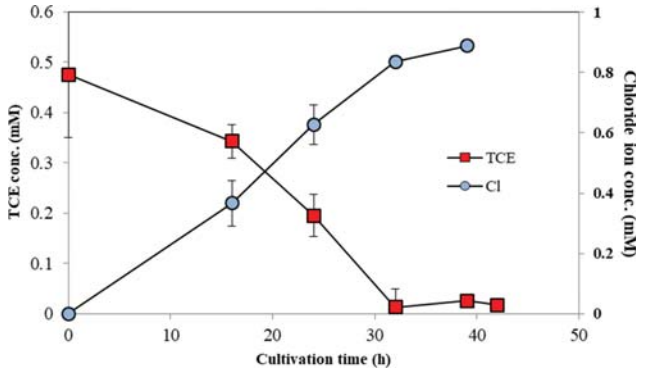

Fig. 7. The kinetics of TCE (0.47 mM) degradation and chloride ion concentrations by adapted *S. ummariensis VR13* in the presence of 0.5 g/L glucose/yeast extract at 30 °C.

to the assessment of the degradation ability of *S. ummariensis VR13*. Therefore, the kinetics of TCE biodegradation was also investigated in the initial concentration of 0.47 mM and is shown in Fig. 7. The bio-dechlorination of TCE by the adapted *S. ummariensis VR13* was similar to PCE, where the TCE concentration reached a very low value after 40 hours. In addition, the chloride ion concentration in the growth solution was increased by the reduction of TCE. These results showed that the *S. ummariensis VR13* was also able to decompose the by-products of the PCE degradation.

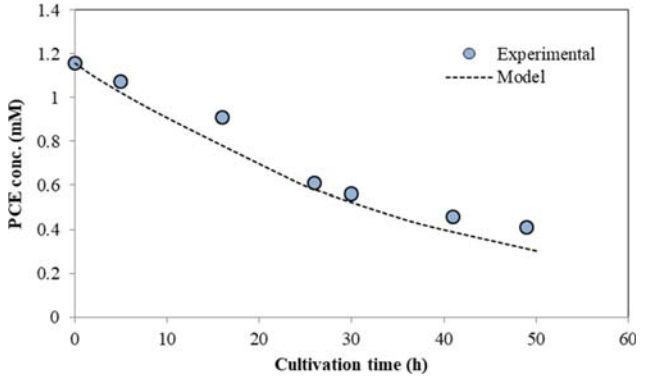

Fig. 8. Kinetic analysis of the PCE degradation by adapted *S. ummariensis VR13* in batch suspended system compared to the Monod-like model.

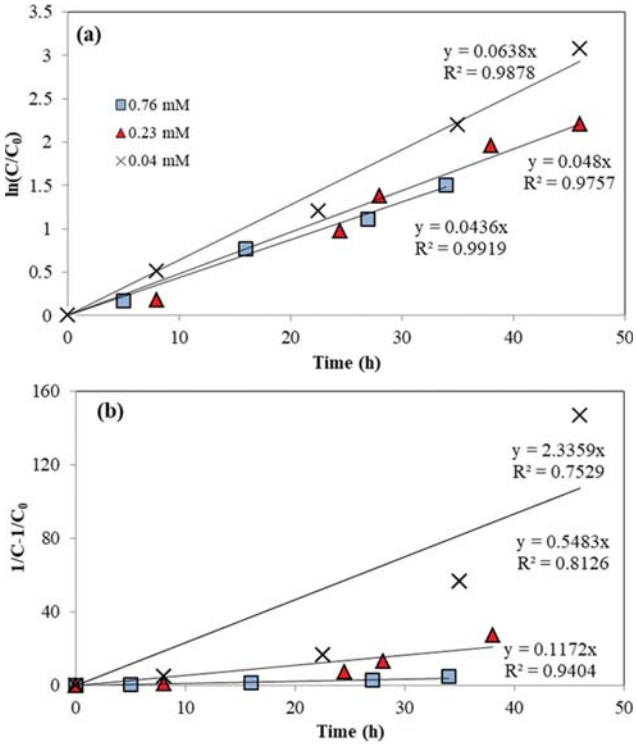

Fig. 9. Kinetic analysis of batch PCE biodegradation by adapted *S. ummariensis VR13* using first (a) and second-order (b) equations at different concentrations.

The results of different kinetic models fitted on experimental data are shown in Figs. 8 and 9. A Monod-like kinetic model was used to determine the kinetics of PCE biodegradation in batch mode (Fig. 8). The experimental data of this study was best fitted to this equation, where R^2 was 0.99. The first- and second-order kinetic formulas were also applied to fit the experimental data (Fig. 9). The results indicated that the first-order equation fitted the experimental data of PCE degradation better than the second-order equation.

DISCUSSION

The dechlorination and degradation of the PCE showed that the *S. ummariensis VR13* cells were capable of aerobic dechlorination of PCE (Fig. 1). There are few reports regarding the aerobic degradation of PCE [17,18,20]. The other studies demonstrated that the addition of a co-substrate may enhance the biodegradation yield of chlorinated hydrocarbons. The PCE dechlorination yield by *P. stutzeri OX1* increased when toluene was added as the co-substrate, where the bio-reductive dechlorination of PCE increased from 8.5 to 28% [17]. Zhang and Thy [30] utilized phenol as the co-substrate during TCE biodegradation. *P. fluorescens* degraded TCE when phenol and nutrient broth was added as the growth substrate and resulted in an increase in TCE degradation efficiency from 10% to about 66% [31]. The addition of a co-sub-

strate to the growth media was also carried out in the present study. Among the tested co-substrates, the glucose/yeast extract equal weight mixture showed the best result in PCE biodegradation. As Fig. 3 shows, the addition of the optimum concentration of glucose/yeast extract (0.5 g/L) to the growth media promoted PCE degradation yield. The reason behind this phenomenon is that the co-substrate enhanced the biomass concentration, which caused a higher degradation yield [31]. A further increase in the glucose/yeast extract concentration led to a reduction in the degradation yield. By increasing the biomass, more oxygen was needed, while the oxygen was limited because a closed system was used.

The results of cell adaptation showed an increased PCE biodegradation (Fig. 2). The method is effective to reduce lag phase [21]. The necessary enzymes for PCE degradation could effectively evolve from the adaptation procedure and enhance the PCE biodegradation efficiency. The same trend has been reported in the biodegradation of vinyl chloride [24]. The results for the adapted *S. ummariensis VR13* cells in the presence of co-substrates showed a 62.9-39.4% PCE degradation in the concentration range of 0.4-5 mM. These concentrations are in a relatively high range compared to the other studies. Ryoo et al. [17] observed that the aerobic removal and dechlorination of 0.002 mM PCE by *P. stutzeri OX1* in the presence of toluene was about 28 and 21%, respectively. In addition, 7.5% of PCE degradation occurred when the PCE was the sole substrate. In their study, the enzyme ToMO was successfully cloned and expressed in *E. coli*, which showed a higher degradation yield (9.7%) at the same PCE concentration. PCE degradation was reduced at a high concentration by the *P. stutzeri OX1* and enzyme cloned *in E. coli* for the binary mixtures of PCE/TCE in 100 μM (21±4 and 17±9%, respectively) [18]. Tabernacka et al. used activated sludge to remove PCE from the waste air. PCE concentration in the air was 0.14 mM, and the elimination efficiency was reported as 42% [20]. By comparing the results obtained in this study with other studies, it was concluded that *S.ummariensis VR13* showed better performance in the aerobic removal of PCE than other microorganisms reported up until today.

PCE would be environmentally safe if it could be totally dechlorinated. The higher concentration of chloride ions in the bacterial culture shows a better dissociation of PCE and the dissociated chlorinated by-products, which results in the higher mineralization of PCE [32]. In this regard, *P. stutzeri OX1* and the enzyme cloned *in E. coli* showed better results at low concentrations, where 71-100% of the chloride ions were released in 0.002 mM PCE solution after 29 hours [17]. In the present study, the maximum of G was 41.7% in 0.4 mM after 32 hours; however, a higher biomass growth might be needed for better PCE mineralization.

Taking into consideration Figs. 4 and 5, it is again shown that PCE degradation was associated with cell growth because the highest removal of PCE (64.7%) occurred in a higher cell density in the concentration of 1.2 mM. The biodegradation of PCE mainly occurred during the logarithmic bacterial growth phase (Figs. 5 and 6). These findings indicate that the PCE biodegradation is a growth-dependent process, which requires the optimization of the ratio of the non-growth (PCE) and growth (glucose/yeast extract) substrates [31,33,34]. The biodegradation linked to growth is important because the biodegradation rate is increased by an increase in the biomass. The same trend was found in all the reported studies regarding the biodegradation of chlorinated aliphatic hydrocarbons [18,35,36].

The TCE concentration increases by decreasing of PCE concentration during cultivation time (Fig. 6), which shows the degradation of PCE to TCE. TCE was also degraded in the exponential growth phase (Fig. 7). This is the reason for the further decline in TCE concentration during PCE degradation process [3].

The kinetics of PCE degradation is very important for industrial applications of microorganisms. Higher removal kinetics of chlorinated hydrocarbons leads to lower cost investment in wastewater treatment facilities. So far, the PCE biodegradation kinetics has not been reported under aerobic conditions; however, the rate of PCE anaerobic degradation at a concentration of 40 μM was

Table 1. An overview of the aerobic bacterial species used for removal of PCE and TCE

Chlorinated hydrocarbon	Strain	Energy source	G (%)	Y (%)	C_T^a (μM)	t (h)	Reference
TCE	*Rhodococcus erythropolis JE77*	Isoprene	100	100	1100	60	[39]
	Methylomonas sp. GD1, GD2, and GD3	Methane and air (1 : 4)	-	99	6	168	[40]
	Methylocystis parvus OBBP	Methane	-	-	6	-	[40]
	Methylosinus trichosporium OB3b	Methane and air (1 : 1)	-	-	60-1	-	[41]
	Consortium diazotrophs	Methane and air (1 : 5)	-	60	763	192	[42]
	Consortiu diazotrophs	-	-	100	1.5	1	[43]
	Microbial consortium	Phenol		80.6	933	288	[44]
	Mixed bacteria	-	-	-	Up to 400	-	[27]
	S. ummariensis VR13	Glucose/Yeast extract	-	96.4	475	40	This study
PCE	*P. stutzeri OX1*	Toluene 50 μM	21	33.5	2	21	[17]
	P. stutzeri OX1	-	65	8.0	2	24	[17]
	Mixed culture	-	-	42	142	19	[20]
	E-coli cloned by ToMO enzyme	Glucose 1%	100	9.7	2	22	[17]
	S. ummariensis VR13	Glucose/Yeast extract	38.9	64.7	1200	32	This study

[a]Initial concentration of PCE (mM)

described by a Monod-like equation with a specific PCE degradation rate of 1.23 μmol PCE (g biomass)$^{-1}$ d^{-1} [29]. The results of this study show that the dechlorination kinetics of PCE is best described by Monod-like (Fig. 8) and first-order (Fig. 9) equations. First-order kinetics was reported for the anaerobic PCE degradation by *Desulfitobacterium* and *Sulfurospirillum multivorans* [37].

The specific degradation rate in the Monod-like equation was measured as 7.2 mmol PCE (g biomass)$^{-1}$ d^{-1} (at 1.2 mM). The anaerobic dehalogenation rate of PCE by *Desulfitobacterium* was also reported as 7.2 μmol PCE (g biomass)$^{-1}$ d^{-1} at a concentration of 150 μM [38]. It can be concluded that the kinetics of the *S. ummariensis* cells in PCE degradation is comparable or even faster than the other reports.

Table 1 compares the aerobic results of PCE and TCE removal in the literature and the results of the present study, which shows high biodegradation by *S. ummariensis VR13* at elevated concentrations. However, the dechlorination percentage was lower than some other aerobic bacteria. For further experiments, molecular methods such as stable-isotope probing can be used for identification of specific functional groups of *S. ummariensis VR13* for degradation of PCE [45,46].

CONCLUSION

The biodegradation of PCE by aerobic strain of *S. ummariensis VR13* was studied in the presence of a glucose/yeast extract as co-substrate. The maximum degradation of PCE and the dechlorination percentage were 24.8% and 17.3% when the initial concentration of PCE and glucose/yeast extract were 0.5 mM and 0.2 g/L, respectively. By adaptation of the cells to higher PCE concentrations, the PCE degradation yield increased. The maximum biodegradation yield was 64.8% when the initial concentration of PCE was 1.2 mM and the glucose/yeast extract concentration was 0.5 mg/L. The use of the *S. ummariensis VR13* resulted in a high biodegradation yield and fast kinetics at relatively high PCE concentrations under aerobic conditions.

Although a number of anaerobic bacteria were introduced for PCE degradation, only a small number of species were isolated for aerobic conditions. The results of the study provide a promising technology for degrading of PCE aerobically. For this reason, the performance of the *S. ummariensis VR13* bacterium in the degradation of PCE in a bioreactor should be studied.

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