Kinetics of chromium (VI) reduction and phenol biodegradation by *Pseudomonas sp.* JF122

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Abstract: Kinetics of chromium (VI) reduction and phenol biodegradation by a pure culture of *Pseudomonas sp.* JF122 was studied. High inoculum (volume) increased both chromium (VI) reduction and phenol biodegradation velocity, which are ascribable to shorter acclimation period requirement for cell growth. Haldane's kinetics model adequately described the substrate kinetics with kinetic constants μ_{max1}=0.113 h^{-1}, K_{s1}=0.4009mM, K_{i1}=5.165mM for chromium (VI) reduction and μ_{max2}=0.3081 h^{-1}, K_{s2}=7.411mM K_{i2}=2.511mM for phenol biodegradation. Further, the growth yield for phenol biodegradation and chromium (VI) reduction were 30.6mg cell/mmol phenol and 8880.2mg cell/mmol Cr (VI), respectively.

Keywords: Kinetics; Haldane's kinetics model; Chromium (VI) reduction; phenol biodegradation

INTRODUCTION

Hexavalent chromium and its organic copollutants including phenol, naphthalene and trichloroethylene (TCE) are often encountered together in many industrial wastewaters, such as those coming from wood preservation, car manufacturing, photographic-film making, petroleum refineries and agriculture activity (Nkhalambayausi-chirwa and Wang, 2001), coexistence of phenol and hexavalent chromium can also occur as wastewaters from different industries (Tziotzios *et al*., 2008). The co-contamination of hexavalent chromium and phenol poses severe threats to the environment since both phenol and hexavalent chromium are dangerous pollutants, and any effort to propose solution for simultaneous removal of the two pollutants would be of value. Conventional processes for simultaneously removing phenol and chromium (VI) include sorption process (Gładysz-Płaska *et al*., 2012; Lach *et al*., 2008; Aksu and Gönen 2006), photocatalytic process (Xie *et al*., 2006; Lee *et al*., 2003), electrochemical process (Liu, 2009) and biological process (Lin *et al*., 2009; Chirwa and Wang 2000; Nkhalambayausi-chirwa and Wang 2005), etc. In recent years, enormous efforts have been made to simultaneously remove chromium (VI) and phenol from aqueous solutions using microorganisms, which represents an efficient and prevalent process. Nkhalambayausi-Chirwa and Wang reported the use of coculture consisting of Pseudomonas putida DMP-1 and Escherichia coli ATCC 33456 for phenol degradation and chromium reduction. In this cases, metabolites formed from phenol (as the sole carbon and energy source) degradation by Ps. putida were utilized by E. coli for growth and Chromium (VI) reduction (Nkhalambayausi-chirwa and Wang 2001). Liu *et al*. Studied simultaneous removal of phenol and Chromium (VI) using phenol-degrading organism Pseudomonas putida Migula CCTCC AB92019 and Chromium (VI)-reducing organism Bacillus sp., similarly, the electron donors for Chromium (VI) reduction by Bacillus sp. were intermediate products of phenol degradation by Ps. putida Migula (Liu *et al*., 2008). Song *et al*. Studied simultaneous removal of phenol and Chromium (VI) using Pseudomonas aeruginosa CCTCC AB91095, The result indicates the ability of Ps. aeruginosa to simultaneously remove phenol and chromium (VI) (Song *et al*., 2009).

However, until recently the information about the growth kinetics, associated with chromium (VI) reduction and phenol biodegradation by a pure culture is scarce. Therefore, chromium (VI) reduction and phenol biodegradation using a pure culture of *Pseudomonas sp.* JF122 was studied in the present research. Aim of the work is elucidation of kinetics of chromium (VI) reduction and phenol biodegradation for this particular strain, which will help scientists or engineers who are working on design and operation of biological processes for removal the two pollutants.

MATERIAL AND METHODS

Microorganism and medium

The strain Pseudomonas sp. JF122 was used in this study. It was grown on minimal medium (MM) prepared with desired concentrations of phenol and chromate. The components of the MM were K_2HPO_4 (2.87mmol), NaH_2PO_4 (4.17mmol), $(NH_4)_2SO_4$ (7.58mmol), NaCl (3.42mmol), $CaCl_2$ (1.8mmol), $MgSO_4 \cdot 7H_2O$ (0.81 mmol), and $Fe_2(SO_4)_3 \cdot 7H_2O$ (0.019mmol) dissolved in 1000mL distilled water. The primary inocula in this study was obtained by inoculating 150mL of MM supplemented with 3.19mmol/kg phenol and strain JF122, on a rotary shaker at 150 rpm and 30°C for 24 h. The optical density of inocula was maintained at 0.2 (OD_{600}).

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Experimental

Effect of inoculum volume on chromium (VI) reduction and phenol biodegradation were investigated using a series of 250ml sterile flasks, Each conical flask containing 150mL mineral medium with inoculum volume varied from 0.15 to 0.3% (v/v%), at 30°C, 6.5 pH, the chromium (VI) concentration of 0.038mM and the initial phenol concentration of 6.38mM. To estimate the kinetic parameters of chromium (VI) reduction and phenol biodegradation by strain JF122, 1mL of inoculums was inoculated into 250mL Erlenmeyer flasks. Each flask contained 150 ml sterile MM with desired phenol concentration and chromate (K_2CrO_4) and incubated on rotary shaker at 150 rpm, 6.5 pH and 30°C for 72h. The optical density, biomass and residual concentrations of chromium (VI) and phenol were all measured at 12h interval during the incubator period.

Analytical methods

The optical density, phenol and chromium (VI) concentrations were all measured with a UV-vis spectrophotometer (UV 754N Shanghai, China). The liquid cultures samples of 10mL were taken from each flask and the optical density was measured at OD_{600}, the dry weight of cell mass density (mg/L) was correlated to optical density with the following equation: X (mg/L) = -7.6153+2567.3 (OD_{600}). For Measuring phenol and chromium (VI) concentrations, samples were centrifuged at 4472×g for 10min and the supernatants were used for phenol and Chromium (VI) determination. Phenol was determined by using 4-aminoantipyrine colorimetric test at 510 nm, chromium (VI) concentration was measured by diphenyl carbazide method at 540nm.

RESULTS

Effect of inoculum volume

Fig. 1a indicate the effect of inoculum volume on chromium (VI) reduction. It was observed that there was no significant difference in reduction performance for inoculum volume of 0.3 and 1%, in which 0.038mM Chromium (VI) was almost reduced completely within 72 h, with much higher chromium-reducing velocity than that of inoculum volume 0.15%. Similar results were shown in fig. 1b. During the course of biodegradation experiment, when inoculum volume was between 0.3% and 1%, the biodegradation velocity of phenol was improved and complete biodegradation occurred within 72 h, but for the inoculum volume of 0.15%, the process was delayed. The reasons can be attributed to shorter acclimation period requirement for both phenol biodegradation and Chromium (VI) reduction with the increase in cell concentrations.

Estimation of specific growth rate

To determine the specific growth rate for different phenol concentrations, batch studies were conducted by varying initial phenol concentration from 0-7.45mM at the initial chromium (VI) concentration of 0.023mM. In addition, the specific growth rate for different chromium (VI) concentrations was investigated by varying initial concentration of chromium (VI) from 0.008 to 0.046mM at the initial phenol concentration of 6.38mM. Biomass from each concentration of phenol/chromium (VI) against time were plotted on a semi-logarithmic graph. The specific growth rate was calculated from the slope of such linear plots during the exponential growth phase (Monteiro *et al*., 2000). Results of these studies are presented in figs. 2 and 3.The values of specific growth rates at the different phenol concentrations were presented in fig. 2. It showed the value of specific growth rate increased from 0 to 0.0716h^{-1} with initial phenol concentration increased from 0-6.38mM, whereas it started decreasing with the increase in phenol concentration from 6.38-7.45mM. which indicated that the phenol was an inhibitory compound at higher concentrations (Bajaj *et al*., 2009).

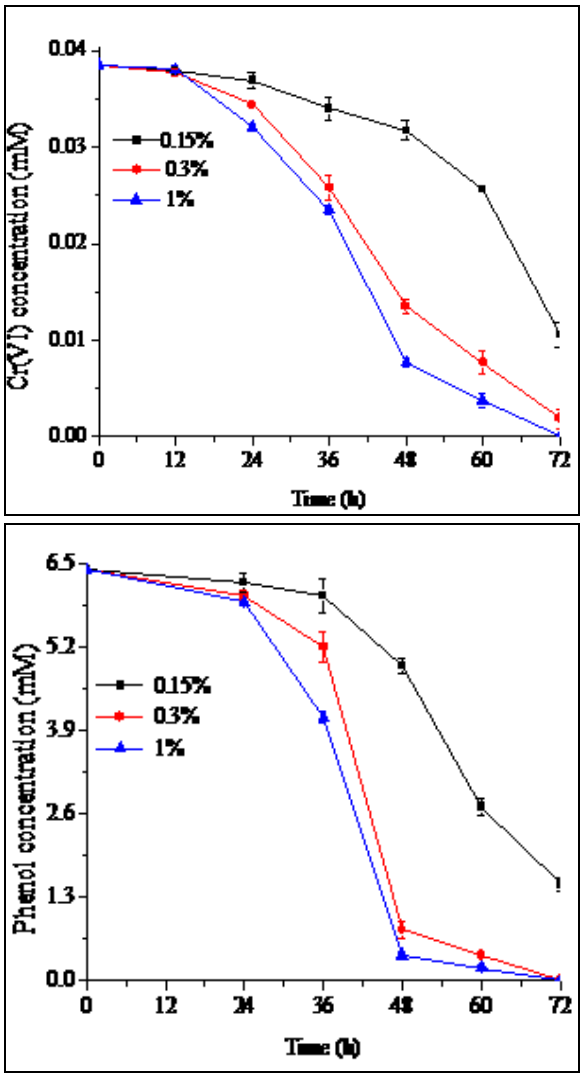

Fig. 1: Effect of inoculum (volume) on chromium (VI) reduction (a) and phenol biodegradation (b)

Table 1: Growth kinetics constants of Haldane's model for phenol degradation and chromium reduction by JF122

Substrate	$\mu_{max,\,j}$ (h^{-1})	$K_{s,\,j}$ (mM)	$K_{i,\,j}$ (mM)	R-square	RMSE
Chromium (VI) (j=1)	0.113	0.4009	5.165	0.9835	0.004172
Phenol (j=2)	0.3081	7.411	2.511	0.9827	0.003778

As shown in fig. 3. The value of specific growth rate at different chromium (VI) concentrations increased from 0.0561 to 0.0763 h^{-1} with the initial chromium (VI) concentration teaming increased from 0.008-0.03mM, then it started decreasing with the increase in Chromium (VI) concentration from 0.03-0.046mM. These results demonstrate the inhibitory effect of Chromium (VI) on JF122 growth. Similar observations have already been reported by other reseachers (Somasundaram *et al*., 2009; Viamajala *et al*., 2004; Stasinakis *et al*., 2002).

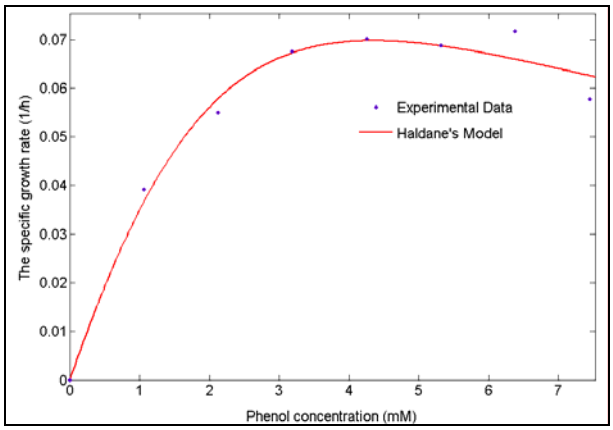

Fig. 2: The specific growth rates for different phenol concentrations

Determination of kinetic parameters

To estimate the dynamic behaviovr of JF122 grown on chromium (VI) and phenol, Haldane's growth model was used to describe phenol degradation and chromium reduction respectively. The growth kinetics equation is as follows:

$$\mu = \frac{\mu_{max,\,j} S}{K_{s,\,j} + S + (S^2 / K_{i,\,j})} \quad \text{for } j = 1,2 \qquad (1)$$

Where μ is the specific growth rate (h^{-1}), $\mu_{max,\,j}$ is the maximum specific growth rate (h^{-1}), S is the substrate concentration (mM), $K_{s,}$ is the half saturation constant (mM), $K_{i,\,j}$ is the inhibition constant (mg /L) (j=1 for chromium (VI); j=2 for phenol).

The model parameters were obtained by using the non-linear regression and fitting the experimental specific growth rate vs phenol/chromium (VI) concentration with Matlab 7.0 based on Windows XP. The comparison between experimental and predicted specific growth rates for each concentration of phenol/chromium (VI) are shown in figs. 2 and 3. The values of the model parameters for both phenol degradation and chromium reduction are given in table 1.

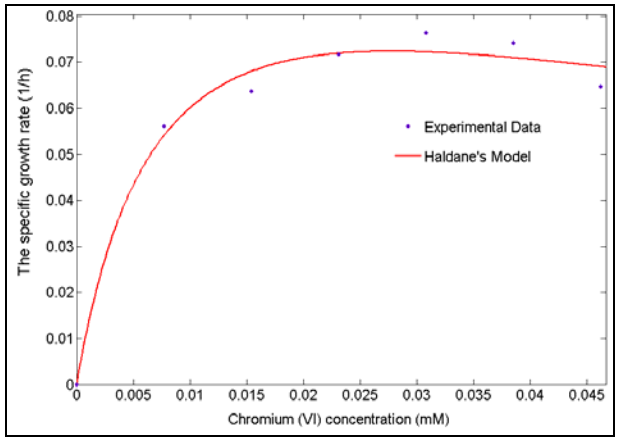

Fig. 3: The specific growth rates for different chromium (VI) concentrations

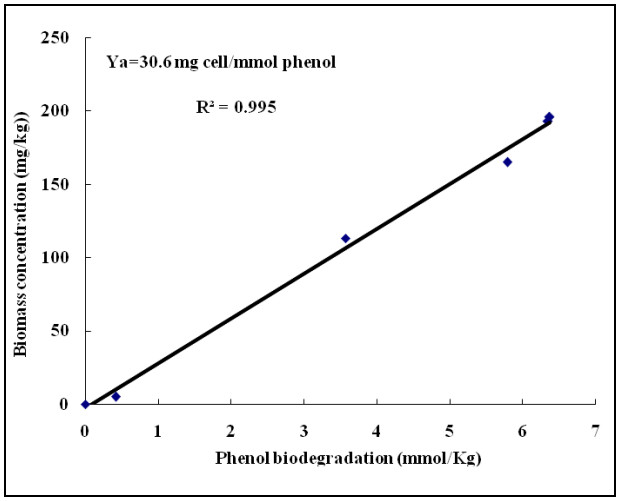

Fig. 4: The growth yield of JF122 as a function of phenol biodegradation.

The coefficient of determination R^2 for both phenol degradation and chromium reduction are more than 0.98, which demonstrate that the dynamic behaviovr of JF122 grown on phenol and Chromium (VI) could be represented by growth kinetics model (Eq.1) very well.

The growth yield

To determine yield coefficient for phenol and chromium (VI), batch experiments were conducted with 6.38 mM phenol and 0.023mM chromium (VI). Biomass produced versus phenol and Chromium (VI) consumption were plotted on a linear graph respectively. The linearized plot slope is yield coefficient on the following equation:

$$Y = \frac{X - X_o}{S - S_o} \quad (2)$$

where X is the maximum value of biomass concentration (mg/L), X_0 is the initial biomass concentration (mg/L), S_0 is the initial concentration of phenol or chromium (VI) (mM), and S is the phenol or chromium (VI) concentration when biomass concentration reached maximum (mM). As presented in figs. 4 and 5. The growth yield for phenol biodegrading and Chromium (VI)-reducing were 30.6mg cell/mmol phenol and 8880.2 mg cell/mmol Chromium (VI), respectively. The yield coefficient value of Chromium (VI) is greater than that of phenol, the possible reasons may be JF122 use phenol as sole carbon for cell growth and Chromium (VI) were the terminal electron acceptor (Nkhalambayausi-chirwa and Wang 2001).

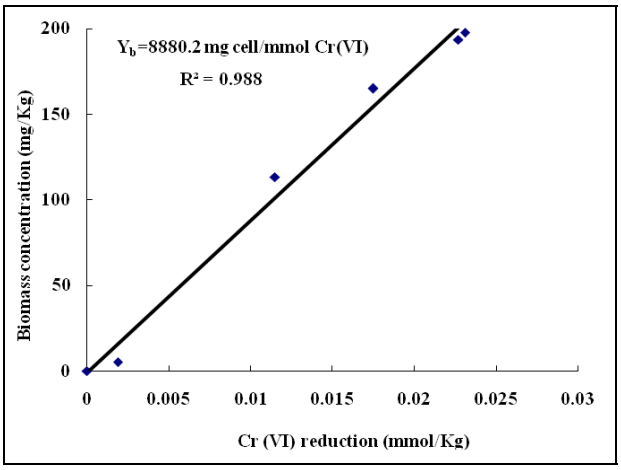

Fig. 5: The growth yield of JF122 as a function of chromium (VI) reduction

CONCLUSIONS

The present study elucidated the kinetics of chromium reduction and phenol degradation by Pseudomonas sp. JF122. Chromium (VI) reduction experiments carried out with chromium (VI) concentration varied from 0.008 to 0.046mM, the temperature of 30°C, the pH of 6.5 and the initial phenol concentration of 600 mg/L, Haldane kinetic model constants were $\mu_{max,1}$=0.113h^{-1}, $K_{s,1}$=0.4009mM, $K_{i,1}$=5.165mM. Phenol biodegradation experiments carried out with phenol concentration varied from 0 to 7.45mM, at 30°C, 6.5pH and the initial chromium (VI) concentration of 0.008mM. Haldane kinetic model constants were $\mu_{max,2}$=0.3081 h^{-1}, $K_{s,2}$=7.411mM, $K_{i,2}$=2.511 mM. For batch experiments carried out with phenol concentration of 6.38mM, at 30°C, 6.5pH and the initial chromium (VI) concentration of 0.023mM. The yield coefficient value of Chromium (VI) reduction and phenol biodegradation were 8880.2mg cell/mmol Chromium (VI) and 30.6mg cell/mmol phenol, respectively.

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REFERENCES

Aksu Z and Gönen F (2006). Binary biosorption of phenol and chromium (VI) onto immobilized activated sludge in a packed bed: Prediction of kinetic parameters and breakthrough curves. *Sep. Purif. Technol.,* **49**: 205-216.

Bajaj M, Gallert C and Winter J (2009). Phenol degradation kinetics of an aerobic mixed culture. *Biochem. Eng. J.,* **46**: 205-209.

Chirwa EN and Wang YT (2000) Simultaneous chromium (VI) reduction and phenol degradation in an anaerobic consortium of bacteria. *Water Res.,* **34**: 2376-2384.

Gładysz-Płaska A, Majdan M, Pikus S and Sternik D (2012). Simultaneous adsorption of chromium (VI) and phenol on natural red clay modified by HDTMA. *Chem. Eng. J.,* **179**: 140-150.

Lach J, Okoniewska E, Neczaj E and Kacprzak M (2008). The adsorption of Cr (III) and Cr (VI) on activated carbons in the presence of phenol. *Desalination,* **223**: 249-255.

Lee SM, Lee TW, Choi BJ and Yang JK (2003). Treatment of Chromium (VI) and phenol illuminated TiO_2. *J. Environ. Sci. Health A.,* **38**: 2219-2228.

Lin Y, Wu C, Hsu C and Li H (2009). Biodegradation of phenol with chromium (VI) reduction in an anaerobic fixed-biofilm process-Kinetic model and reactor performance. *J. Hazard. Mater.,* **172**: 1394-1401.

Liu Y (2009). Simultaneous oxidation of phenol and reduction of Cr (VI) induced by contact glow discharge electrolysis. *J. Hazard. Mater.,***168**: 992-996.

Liu YG, Pan C, Xia WB, Zeng GM, Zhou M, Liu YY, Ke J and Huang C (2008). Simultaneous removal of Cr (VI) and phenol in consortium culture of Bacillus sp. and *Pseudomonas putida* Migula (CCTCC AB92019). *Trans. Nonferrous Met Soc China,* **18**: 1014-1020.

Monteiro AAMG, Boaventura RAR and Rodrigues AE (2000). Phenol biodegradation by Pseudomonas putida DSM 548 in a batch reactor. *Biochem. Eng. J.,* **6**: 45-49.

Nkhalambayausi-chirwa EM and Wang YT (2001). Simultaneous chromium (VI) reduction and phenol degradation in a fixed-film coculture bioreactor: Reactor performance. *Water Res.,* **35**: 1921-1932.

Nkhalambayausi-Chirwa EM and Wang YT (2005). Modeling chromium (VI) reduction and phenol degradation in a coculture biofilmreactor. *J. Environ. Eng.,* **131**: 1495-1506.

Somasundaram V, Philip L and Bhallamudi SM (2009). Experimental and mathematical modeling studies on Cr (VI) reduction by CRB, SRB and IRB, individually and in combination. *J. Hazard. Mater.,* **172**: 606-617.

Song H, Liu Y, Xu W, Zeng G, Aibibu N, Xu L and Chen B (2009). Simultaneous Cr (VI) reduction and phenol degradation in pure cultures of *Pseudomonas aeruginosa* CCTCC AB91095. *Bioresource. Technol.,* **100**: 5079-5084.

Stasinakis AS, Mamais D, Thomaidis NS and Lekkas TD (2002). Effect of chromium (VI) on bacterial kinetics of heterotrophic biomass of activated sludge. *Water Res.,* **36**: 3341-3349.

Tziotzios G, Dermou E, Politi D and Vayenas DV (2008). Simultaneous phenol removal and biological reduction of hexavalent chromium in a packed-bed reactor. *J. Chem. Technol. Biotechnol.,* **83**: 829-835.

Viamajala S, Peyton BM, Sani RK, Apel WA and Petersen JN (2004) Toxic effects of chromium (VI) on anaerobic and aerobic growth of *Shewanella oneidensis* MR-1. *Biotechnol. Prog.,* **20**: 87-95.

Xie B, Zhang H, Cai P, Qiu R and Xiong Y (2006). Simultaneous photo catalytic reduction of Chromium (VI) and oxidation of phenolover monoclinic $BiVO_4$ under visible light irradiation. *Chemosphere,* **63**: 956-963.