African Journal of Microbiology Research Vol. 6(11), pp. 2686-2693, 23 March, 2012
Available online at http://www.academicjournals.org/AJMR
DOI: 10.5897/AJMR11.1177
ISSN 1996-0808 ©2012 Academic Journals

Full Length Research Paper

Biodegradation of an endocrine-disrupting chemical di-n-butyl phthalate by *Serratia marcescens* C9 isolated from activated sludge

Chuang Li[1#], Xiuli Tian[1#], Zhengsuo Chen[1], Dong Yu[1], Jiayu Deng[2], Heng Xu[1]*

[1]Key Laboratory for Bio-resources and Eco-environment of Education Ministry, College of Life Science, Sichuan University, Chengdu, 610064, China.
[2]Longquanyi Aro-technical Extension Centre, Chengdu, 610100, China.

Accepted 23 February, 2012

In this study, an aerobic bacterial strain capable of utilizing di-*n*-butyl phthalate (DBP) as sole carbon source and energy was isolated from activated sludge collected from a plastic molding plant. Based on its morphology, physiochemical characteristics, and 16S rDNA sequence, the strain was identified as *Serratia marcescens* C9. The Taguchi method was used for determining the optimum condition of DBP degradation from aqueous solution by *S. marcescens* C9. An orthogonal array experimental design L_9 (3^3) was chosen to employ the experiment and L_9 (3^3) is consisted of three control factors (Temperature, pH and initial DBP concentration) and each factor has three different levels. DBP degradation rate was investigated as the quality characteristic to be optimized. In order to determine the optimum levels of the control factors precisely, range analysis and analysis of variance were performed. The optimum condition for DBP degradation was found to be temperature =37°C, pH= 7, C_0= 50 mg/L. In addition, first-order kinetic model was used to evaluate the degradation process of DBP at different initial concentrations in liquid medium. The results showed that the degradation half-life was about 1.35 d when the concentration of DBP was lower than 100 mg/L. This work suggested that the isolated strain C9 may have potential for use in bioremediation of DBP contaminated water.

Key words: Degradation, Taguchi method, first-order kinetic model, di-*n*-butyl phthalate, *Serratia marcescens*.

INTRODUCTION

Phthalate esters (PAEs) are a class of refractory organic compounds, which are widely used in plastics, coatings, and cosmetics (Chen et al., 2007). They may constitute up to 50% of the total weight of PVC plastics (van Wezel et al., 2000). The worldwide annual production of phthalates is approximately 2.7 million metric tons (Bauer and Herrmann, 1997). As they are not chemically bounded to the plastic resin, PAEs can be released into environment during production, distribution and waste disposal. PAEs have been found in marine water (Fatoki and Ogunfowokan, 1993), river water (Fatoki and Vernon, 1990), soil (Hunter and Uchrin, 2000) and sediment (Xu et al., 2005a). A number of studies show that some PAEs can cause cancer and interfere with the reproductive systems and development of animals and humans (Gray et al., 2000; McKee et al., 2004; Lottrup et al., 2006; Mo et al., 2008). The United States Environmental Protection Agency and China National Environmental Monitoring Center have classified most of PAEs as priority pollutants and as endocrine disrupting compounds (EDCs) (Xu et al., 2007; Lu et al., 2009).

*Corresponding author. E-mail: xuheng64@sina.com. Tel: 86-28-85414644. Fax: 86-28-85418262.

# Author contributed equally to the work

Di-n-butyl phthalate (DBP) belongs to the family of PAEs and is considered to be the most commonly used plasticizer in China. DBP, like other PAEs, could be detected in river water, soil, lake sedimen and municipal solid waste (Xu et al., 2005b; Fang et al., 2009). All of these pollutional sources can easily lead to a variety of negative effects on aquatic organism and indirectly threaten human health through food chain transmission. Therefore, it has increased the pubilc concern to establish a safe, efficient, and cost-effective method to removal or detoxify DBP residues in contaminated environment. Due to its chemical structure and low water solubility, DBP can't be easily removed by hydrolysis, photolysis and volatilization. Metabolic breakdown of DBP by microorganisms is considered to be one of the major routes of environmental degradation for this widespread pollutant (Wolfe et al., 1980). Lee et al. (2004) reported that DBP can be efficiently degradated by white rot fungi (*Trametes versicolor* and *Daldinia concentrica*) after 6 days of incubation. Recently, Wu et al. (2011) constructed the optimal condition of DBP degradation by *Agrobacterium* sp. using different kinds of incubation factors such as temperature, initial pH, and substrate concentration. These bacterial strains with the ability of degrading DBP have been isolated from different sources such as activated sludge, mangrove sediment and soil collected from landfill (Liao et al., 2010; Li et al., 2006).

The objectives of this study were to isolate the DBP-degrading bacteria from the activated sludge and characterize its degradation potential. We conducted the experiment to evaluate the optimum condition for removing DBP residues by C9 in pure cultures using orthogonal experimental design. Three factors, including temperature, pH, DBP initial concentration and their respective three levels were examined (Table 1). In addition, the kinetics of biodegradation at different concentrations were also investigated.

MATERIALS AND METHODS

Chemicals

DBP with 99.0% analytical standards was obtained from Dr. Ehrenstorfer, Germany. DBP (A.P), HPLC-grade dichloromethane and other reagents were purchased from Kelong Chemical Reagent Factory, Chengdu, Sichuan province, China. Stock solution of DBP was dissolved in methanol at a concentration of 10 $g \cdot l^{-1}$. All glassware were soaked overnight in K_2CrO_7/H_2SO_4 solution, washed with tap water and redistilled water, baked at 200°C for 4 h. After cooling, the glassware was rinsed with acetone and air-dried for use.

Culture enrichment and isolation

Microorganisms using DBP as sole source of carbon and energy were isolated by the enrichment-culture technique. Activated sludge was collected at a plastic molding plant of Chengdu, the province of Sichuan, China. The mineral salt medium (MSM) contained 1.0 g/L NH_4NO_3, 0.5 g/L $MgSO_4$, 0.5 g/L $(NH_4)_2SO_4$, 0.5 g/L KH_2PO_4, 0.5 g/L NaCl, 1.5 g/L K_2HPO_4. DBP was added as sole carbon and energy source and the concentration of DBP was increased gradually from 50 to 300 mg/L. The pH of the culture medium was adjusted with HCl or NaOH to7.0±0.1. The initial enrichment culture was established by inoculating 100 ml sterile mineral salt medium (MSM) with 5 g fresh activated sludge with DBP (50 mg/L) in a 250 ml Erlenmeyer flask. Enrichments were incubated for 7 days at 37°C on a rotary shaker operated at 180 rpm, and portions of the enrichments were then transferred to fresh medium. The final enrichment was streaked on to plates containing 1.0% peptone, 0.5% malt extract, 1.5% agar and inorganic salts. Each isolated bacterial strain was put into MSM medium containing DBP in order to screen dominant bacterium. After 1 week degradation, the dominant DBP-degrading bacterium could be confirmed by degradation rate. After screening, we obtained a strain named strain C9 showing higher DBP-degradation efficiency than other strains.

Table 1. Taguchi method experimental parameters and their levels.

Variable		Level		
		1	2	3
(A)	Temperature (°C)	15	25	37
(B)	pH	5	7	9
(C)	DBP initial concentration（mg/L）	50	100	150

Identification

The identification of strain C9 was based on its morphology, physiochemical characteristics, and analysis of the 16S rDNA gene. The primers which were used to amplify 16S rDNA gene were Pf: 5'-AGAGTTTGATCCTGGCTCAG-3' and Pr: 5'-ACGGCTACCTTGTTACGACT-3' corresponding to 8–27 and 1495–1514 bases of the *Escherichia coli* 16S rDNA gene, respectively (Weisburg et al., 1991). PCR amplification conditions for each PCR mixture (50 µl) were composed of buffer solution (10X) 5 µl, $MgCl_2$ (25 mM) 3 µl, dNTPs (2.5 mM each) 4 µl, primer (10 µM) 2 µl each, Tag (5 U/µl) 0.5 µl, DNA (56 µg/ml) 1 µl, and H_2O 32.5 µl. The amplification program consisted of one cycle of 94 ℃ for 3 min; 30 cycles of 94°C for 30 s, 54°C for 30 s, and 72°C for 90 s; and finally one cycle of 72°C for 5 min. The PCR products were purified by agarose gel electrophoresis and ligated into pMD 19-T vector (TaKaRa, Dalian, China), then sequenced in Invitrogen Shanghai, China. The similarity of the nucleotide sequence was determined by BLAST search in NCBI (National Center for Biotechnology information Databases).

Taguchi method

A full factorial design that we need to test every possible combination of two or more factors is a common technique to evaluate the effects of each environmental factors. However, if we consider these factors simultaneously, the work load will be so large that the process could be impractical. On the contrary, the Taguchi method calling for a much smaller combination of factors and levels is a powerful problem-solving technique for improving work efficiency and productivity. It saves time and reduces manufacturing costs due to its scientific design in the process (Kaminari et al., 2007). Therefore, the Taguchi method was chosen for carrying out this study to help researchers determine the possible combinations of factors and identify the best combination.

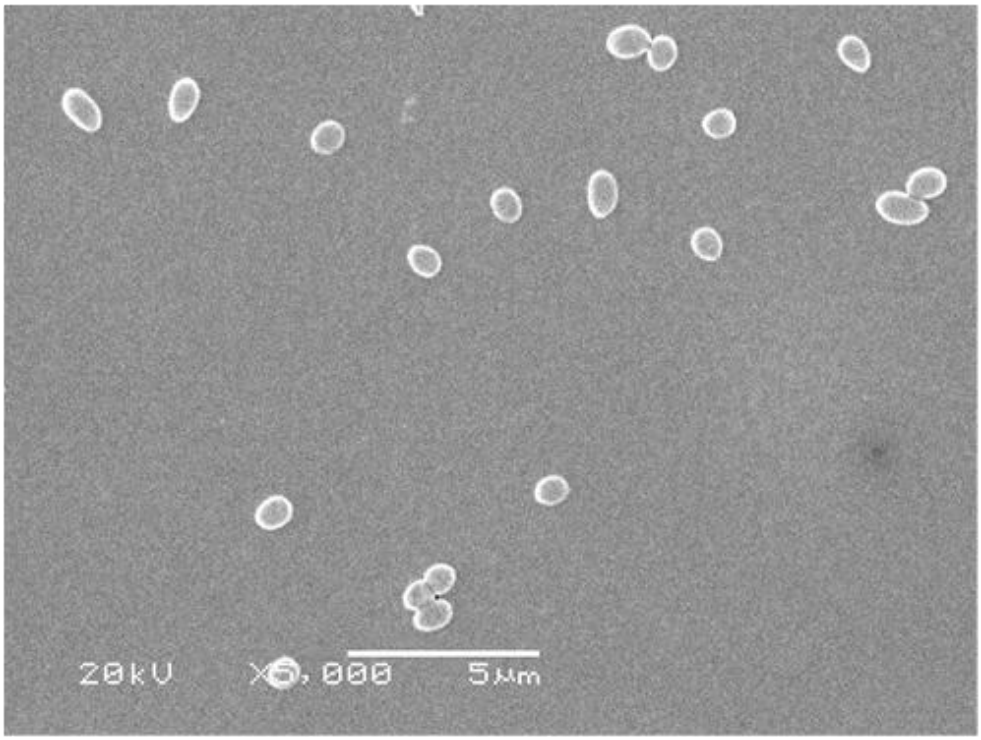

Figure 1. SEM micrograph of bacterial strain capable of degrading DBP: *Serratia marcescens* C9.

The Taguchi method experiment design L_9 (3^3), which is consisted of three factors (temperature: A; pH value: B; DBP concentration: C) having three levers separately, was designed to explore the optimal degrading conditions of DBP by strain C9 (Table 1). The degrading experiments were performed in a 150 ml stoppered glass Erlenmeyers flasks containing 50 ml of MSM and incubated at the constant temperature shaker (SUKUN) with 180 rpm for 7 days. The Erlenmeyer flasks were heated by digital heating circulating water bath at 65°C and then shaked with speed of 200 rpm for 20 min to evaporate the methanol. Each flask was inoculated with 2.5 ml (5%, v/v) degrading bacteria C9, which was collected from a 15 h LB culture and washed twice with 0.9% (w/v) sterile NaCl solution. Each experimental group designed in Table 1 was conducted in duplicate. Blank control was set up as above but with uninoculated media.

Kinetics studies

Based on the optimal pH and temperature detected from the Taguchi method experiment, the DBP degradation kinetics of strain C9 was also studied. An inoculum (5%, v/v) was inoculated into sterile 150 ml Erlenmeyers flasks containing 50 ml of MSM with different concentrations of DBP (50, 100,150 mg/L). Residual DBP was determined at 0, 1, 2, 3, 4, 5, and 6 d and all experiments were performed in duplicate.

Analytical methods

Samples were analyzed for DBP concentration by GC-MS (SHIMADZU, Japan) equipped with Rtx-5 ms capillary column (30 m× 0.25 mm× 0.25 μm). The conditions were as follows: carrier gas, high pure helium gas (1 ml/min); injector temperature, 250°C; column temperature, 100°C; programmed temperature started at 100°C with 2 min and was ramped up to 260°C at 20°C/min before being held for 5 min; injection volume, 1μl.

Statistical Package for the Social Sciences (SPSS) 16 statistical software was used for the analysis of variance (ANOVA) of the data in the Taguchi method experimental design.

RESULT AND DISCUSSION

Identification and characterization of the bacterial strain C9

Among the bacterium isolated, the strain C9 was capable of degrading DBP quickly as the sole carbon and energy source (Figure 1). The strain with cell size of 0.5 -0.7 μm × 0.5-1.1 μm was Gram-negative and it was found to be nonspore forming, rod-shaped with flagella. After cultured for 18–24 h at 28°C, this strain C9 formed red, opaque and irregular morphological colonies on LB agar. The strain of this common water and soil, Gram-negative bacterium produced the characteristics red pigment prodigiosin (Trias et al., 1988). Methyl red, indole prodution and starch hydrolysis tests were negative. Positive results were obtained in Voges Proskauer, citrate liquefaction, catalase, cytochrome oxidase assays. In addition, this strain utilized α-D-Glucose, D-Galactose, D-Fructose, Maltose, D-sorbitol, and also reduced nitrate to nitrite. The other physiochemical properties of strain C9 were shown in Table 2. The strain C9 was identified

Table 2. Physiochemical properties of the *Serratia marcescens* C9.

Characteristics	Strain C9
Aerobic growth	+
α-D-Glucose	+
D-Galactose	+
D-Fructose	+
Lactulose	-
L-Arabinose	W
Maltose	+
D-Mannose	+
D,L-Lactic Acid	+
α-Hydroxybutyric Acid	W
Citric Acid	+
Itaconic Acid	-
Formic Acid	+
Uridine	+
D-Alanine	+
L-Alanine	+
L-Proline	+
L-Phenylalanine	-
D-Serine	+

Positive (+); negative (−); weakly positive (w).

Table 3. Results of orthogonal test of *Serratia marcescens* C9 on DBP biodegradation.

Experiment no.	A	B	C	DBP degradation rate (%)[a]
1	1	1	1	18.5
2	1	2	3	13.3
3	1	3	2	10.7
4	2	1	2	25.6
5	2	2	1	61.4
6	2	3	3	9.2
7	3	1	3	63.8
8	3	2	2	92.4
9	3	3	1	85.6

a-Degradation rate (%)=($C_{initial} - C_{final}$) / $C_{initial}$

as *S. marcescens* by its morphology, physiochemical characteristics and 16S rDNA sequence. According to previous reports, *S. marcescens can* also degrade other pollutants, such as hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) (Young et al., 1997), dichlorodiphenyltrichloroethane (Bidlan and Manonmani, 2002), dyes (Verma and Madamwar, 2003), diesel (Rajasekar et al., 2007), hexachlorobutadiene (Li et al., 2008), fatty amines (Araujo et al., 2010), and benzo[a]pyrene (Machín-Ramírez et al., 2010).

Table 4. Range analysis for the L_9 (3^3) orthogonal array experiment.

	DBP degradation rate (%)		
	A	B	C
K1	14.2	36.0	55.2
K2	29.1	55.7	42.9
K3	80.6	35.2	28.8
R[a]	66.4	20.5	26.4

a-R value refer to the result of range analysis, R = max(K_1, K_2, K_3) – min(K_1 ,K_2, K_3). K1, K2 and K3 refer to average values of each level.

Taguchi method experiment

The results of Taguchi method experiment and its range analysis

In this study, we conducted only 9 experiments by using the Taguchi experimental design L_9 (3^3) to determine the optimal biodegradation condition of DBP-degrader strain C9. K1, K2 and K3 were the average values of each level of each variable in the Taguchi method experiment [for example, A (K1) listed in Table 4 represents the average values of the results of Experiments 1, 2 and 3 reported in Table 3], and the optimal level of variable can be determined by comparing the value of Ki (i=1, 2, 3). R =max {K1, K2 . . . Ki} − min {K1, K2 . . . Ki}, and *R* value was used to estimate the effect of variables. During the range analysis, variable with higher R value has stronger effect on the results than other variables. The results of Taguchi method experiment are shown in Table 3. From the results presented in Table 3, we can find out DBP degradation rate was maxima for the combination of three factors: temperature 37°C, pH 7.0 and initial DBP concentration 100 mg/L. Under this condition, the degradation performance can reach 92.4%. The results of range analysis were also shown in Table 4. Based on the R value, we can reach the conclusion that the influence on the degradation rate decreased following the order: *A* > *C* > *B*. Temperature was found to be the most important factor in DBP degrading process, which was prior to pH value and initial DBP concentration.The relationship between factorial level and the degrading rates in orthogonal experiment was shown in Figure 2. It was observed that the degradation rate increased with increasing temperature (within the range of bacteria growth), and decreased with increasing initial DBP concentration, indicating the best levels for each single factor: A_3, B_2, C_1. Bacteria growth is sensitive to environment temperature and the best level in our study was 37°C in liquid. The result match those reported in the previous study on DBP degradation in soil (Li et al., 2006). PH has been suggested as one of the important factors affacting DBP biodegradation and Fang et al. (2010) indicated that *Enterobacter* sp. was preferred to degrade DBP in a neutral condition especially at pH 7 as

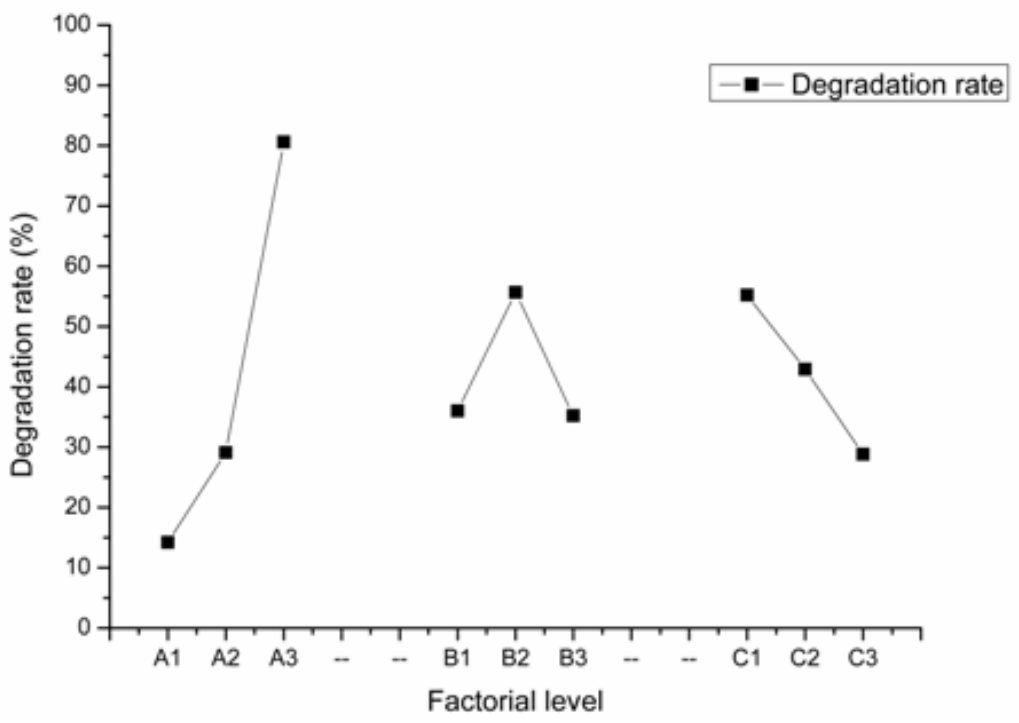

Figure 2. The relationship between factorial level and the degrading rates in orthogonal experiment.

our result suggested. Wu et al. (2011) showed that *Agrobacterium* sp. could degrade DBP completely in 48 h when the initial concentration of DBP not higher than 200 mg/L, suggesting that a possible toxic effect of this compound on the strain.

Control experiments were carried out in the autoclaved culture medium containing DBP without bacterial inoculation. The results showed no obvious reduction which suggests that abiotic losses can be neglected in this study. The recovery rates of DBP after sample pretreatment were between 96–104%.

Analysis of variance

A statistical ANOVA was performed to see which process factors significantly affect the process responses. The variance is the amount of deviation degree, which is obtained by finding the sum of squares of D-value between a set of data and actual value, then dividing the degree of freedom. The Sig means the significance, which is calculated from the experimental results. Then it was compared to the given critical significance level 0.05 representing significance level at a 95% confidence interval. If the Sig calculated is less than 0.05, it is an indication that the statistical test is significant at the confidence level selected. If not, it indicates that the statistical test is not significant at the confidence level. The results of ANOVA for the Taguchi method experiment are tabulated in Table 5. It can be seen that the temperature and initial DBP concentration have significant influences on the degradation rate, and pH shows no significance for the range tested here. The result was accorded with the result of range analysis.

In our study, if a full factorial design was applied to detect the effects of 3 factors and each with 3 levels, 27 experiments (3^3) would be needed. Instead, by using the Taguchi experimental design, only 9 experiments were conducted to determine the optimal biodegradation condition of DBP by strain C9. In light of the previous reports, the Taguchi method has been widely applied in the same domains, such as biodegradation of phenolic wastewater (Hsien and Lin, 2005), decomposition of polycyclic aromatic hydrocarbons in sediment slurries (Chen et al., 2008) and heavy metals recovery from industrial wastewater (Kaminari et al., 2007).

Kinetics of DBP degradation

First-order kinetics is very important both for the prediction of DBP's fate and for the design of remediation, so we used it to describe the biodegradation process of DBP in water. Different initial DBP concentration (50, 100, 150 mg/L) was assessed. As shown in Figure 3, the time course of degradation rate was recorded.

The calculated results of DBP degradation rate by *S. marcescens C9* indicated that the first-order model gave a good fit. A first-order kinetics model could be

Table 5. ANOVA for degradation rate in the $L_9(3^3)$ orthogonal array experiment.

Source	SSD	dof	Mean Square	F	Sig.
Corrected Model	8948.127[a]	6	1491.354	67.286	0.015
Intercept	16086.690	1	16086.694	725.788	0.001
A	7089.282	2	3544.641	159.925	0.006
B	811.662	2	405.831	18.310	0.052
C	1047.182	2	523.591	23.623	0.041
Error	44.329	2	22.164		
Total	25079.150	9			
Corrected Total	8992.456	8			

a-R Squared= 0. 995(Adjusted R Squared= 0. 980), SSD-sum of squares of deviations, dof: degree of freedom, F-a ratio of the mean of the squared deviations to the mean of the squared error.

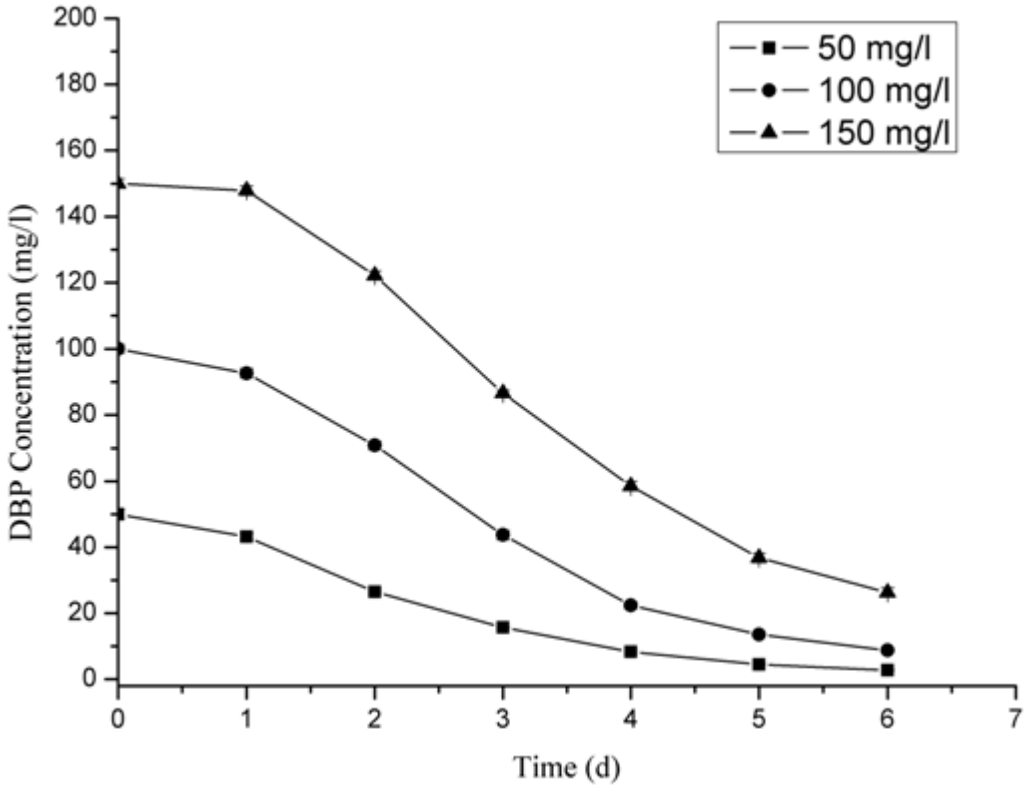

Figure 3. Degradation curves of DBP by the strain C9.

constructed by logarithmic transformation, with the form:

$$\ln C = -k t + A \quad (1)$$

Where C is the DBP concentration, K the first-order kinetic rate constant, t time, and A the constant. The biodegradation half-life of the DBP first-order reaction can be expressed as:

$$t_{1/2} = \ln 2/k \quad (2)$$

The different DBP degradation kinetics equations at different DBP initial concentration were shown in Table 6. From the results, it can be found that the half-life of degradation was about 1.35 days when the concentration of DBP was lower than 100 mg/L. The half-life would be longer as the initial concentration increased. When the

Table 6. DBP degradation kinetics equation under different initial concentrations.

Initial DBP concentration (mg/L)	Kinetics equation	Half life (days)	Correlation coefficient (R^2)
50	ln C = -0.5118 t + 4.1582	1.35	0.9833
100	ln C = -0.4391 t + 4.8971	1.58	0.9633
150	ln C = -0.3122 t + 5.2535	2.22	0.9519

initial concentration of DBP was higher than 100 mg/L, the first-order kinetic rate constant (k) decreased gradually, suggesting that the higher initial DBP concentration may constrain biodegradation ability. According to previous studies, first-order kinetics has been frequently employed to describe the biodegradation dynamics of DBP (Lu et al., 2009; Fang et al., 2010).

Conclusion

A strain capable of degrading DBP, isolated from activated sludge of a plastic molding plant was identified as *S. marcescens* through analysis of morphology, physiochemical properties and 16S rDNA sequence. The effects of multifactors on degradation of DBP in pure cultures by *S. marcescens* were investigated using the Taguchi experimental design L_9 (3^3). The optimal condition for DBP biodegradation in pure cultures was temperature in 37°C, initial DBP concentration 50 mg/L, pH=7, respectively. In addition, range analysis and ANOVA results indicate that temperature and initial DBP concentration statistically inferred to have significant influences on degradation rate. As to degradation kinetics analysis, the DBP degradation process in water by *S. marcescens C9* was considered to fit to the first-order kinetic equation. Based on these findings, future work will be attempted to define more factors that influence the degradation performance. These findings in the present study also indicated the isolated bacteria C9 may have potential for use in bioremediation of DBP-containing wastes.

ACKNOWLEDGEMENT

This study financially supported by the NSFC (No.41171253), the National 863 High Technology Research and Development Program of China (No. 2006AA06Z361), Science and Technology Supportive Project of Sichuan Province, China (No. 2009SZ0204), Science and Technology project of Chengdu (10GGYB472SF-023) and Science and Technology Development Project of Longquanyi District Aro-technical Extension Centre, Chengdu.

REFERENCES

Araujo DM, Yoshida MI, Takahashi JA, Carvalho CF, Stapelfeldt F (2010). Biodegradation studies on fatty amines used for reverse flotation of iron ore. Int. Biodeterior Biodegradation 64:151-155.

Bauer MJ, Herrmann R (1997). Estimation of the environmental contamination by phthalic acid esters leaching from household wastes. Sci. Total Environ. 208:49-57

Bidlan R, Manonmani HK (2002) Aerobic degradation of dichlorodiphenyltrichloroethane (DDT) by *Serratia marcescens* DT-1P. Process Biochemistry 38:49-56.

Chen J, Wong MH, Wong YS, Tam NF (2008) Multi-factors on biodegradation kinetics of polycyclic aromatic hydrocarbons (PAHs) by *Sphingomonas* sp. a bacterial strain isolated from mangrove sediment. Mar. Pollut. Bull. 57: 695-702

Chen JA, Li X, Li J, Cao J, Qiu Z, Zhao Q, Xu C, Shu W (2007). Degradation of environmental endocrine disruptor di-2-ethylhexyl phthalate by a newly discovered bacterium, Microbacterium sp. strain CQ0110Y. Appl. Microbiol. Biotechnol. 74: 676-682

Fang CR, Long YY, Shen DS (2009). Comparison on the removal of phthalic acid diesters in a bioreactor landfill and a conventional landfill. Bioresour. Technol. 100: 5664-5670.

Fang CR, Yao J, Zheng YG, Jiang CJ, Hu LF, Wu YY, Shen DS (2010). Dibutyl phthalate degradation by *Enterobacter* sp. T5 isolated from municipal solid waste in landfill bioreactor. Int. Biodeterior Biodegradation. 64: 442-446.

Fatoki OS, Ogunfowokan AO (1993). Determination of phthalate ester plasticizers in the aquatic environment of southwestern Nigeria. Environ. Int. 19: 619-623.

Fatoki OS, Vernon F (1990). Phthalate esters in rivers of the greater Manchester area, U.K. UK Sci. Total Environ. 95: 217-232.

Gray LE, Jr., Ostby J, Furr J, Price M, Veeramachaneni DN, Parks L (2000). Perinatal exposure to the phthalates DEHP, BBP, and DINP, but not DEP, DMP, or DOTP, alters sexual differentiation of the male rat. Toxicol. Sci., 58:350-365.

Hsien TY, Lin YH (2005). Biodegradation of phenolic wastewater in a fixed biofilm reactor. Biochem. Eng. J., 27: 95-103.

Hunter JG, Uchrin CG (2000). Adsorption of phthalate esters on soil at near saturation conditions. J. Environ. Sci. Heal, 35: 1503-1515

Kaminari NMS, Schultz DR, Ponte MJJS, Ponte HA, Marino CEB, Neto AC (2007). Heavy metals recovery from industrial wastewater using Taguchi method. Chem. Eng. J., 126:139-146

Weisburg WG, Barns SM, Pelletier DA, Lane DJ (1991). 16S ribosomal DNA for phylogenetic study. J. Bacteriol., 173:697-703.

Lee SM, Koo BW, Lee SS, Kim MK, Choi DH, Hong EJ, Jeung EB, Choi IG (2004). Biodegradation of dibutylphthalate by white rot fungi and evaluation on its estrogenic activity. Enzyme Microbial Technol., 35: 417-423

Li J, Chen JA, Zhao Q, Li X, Shu W (2006). Bioremediation of environmental endocrine disruptor di-n-butyl phthalate ester by *Rhodococcus ruber*. Chemosphere, 65: 1627-1633

Li MT, Hao LL, Sheng LX, Xu JB (2008). Identification and degradation characterization of hexachlorobutadiene degrading strain *Serratia marcescens* HL1. Bioresour. Technol., 99: 6878-6884.

Liao CS, Chen LC, Chen BS, Lin SH (2010). Bioremediation of endocrine disruptor di-n-butyl phthalate ester by Deinococcus radiodurans and *Pseudomonas stutzeri*. Chemosphere, 78: 342-346.

Lottrup G, Andersson AM, Leffers H, Mortensen GK, Toppari J, Skakkebaek NE, Main KM (2006). Possible impact of phthalates on infant reproductive health. Int. J. Androl., 29: 172-180.

Lu Y, Tang F, Wang Y, Zhao J, Zeng X, Luo Q, Wang L (2009). Biodegradation of dimethyl phthalate, diethyl phthalate and di-n-butyl phthalate by *Rhodococcus* sp. L4 isolated from activated sludge. J. Hazard. Mater., 168: 938-943.

Machín-Ramírez C, Morales D, Martínez-Morales F, Okoh AI, Trejo-Hernández MR (2010). Benzo[a]pyrene removal by axenic- and co-cultures of some bacterial and fungal strains. Int. Biodeterior Biodegradation, 64: 538-544

McKee RH, Butala JH, David RM, Gans G (2004). NTP center for the evaluation of risks to human reproduction reports on phthalates: addressing the data gaps. Reprod. Toxicol. 18: 1-22.

Mo CH, Cai QY, Li YH, Zeng QY (2008). Occurrence of priority organic pollutants in the fertilizers, China. J. Hazard. Mater., 152: 1208-1213.

Rajasekar A, Babu TG, Pandian ST, Maruthamuthu S, Palaniswamy N, Rajendran A (2007). Role of *Serratia marcescens* ACE2 on diesel degradation and its influence on corrosion. J. Ind. Microbiol. Biotechnol. 34(9): 589-598.

Trias J, Vinas M, Guinea J, Loren JG (1988). Induction of yellow pigmentation in *Serratia marcescens*. Appl. Environ. Microbiol., 54: 3138-3141.

van Wezel AP, van Vlaardingen P, Posthumus R, Crommentuijn GH, Sijm DT (2000). Environmental risk limits for two phthalates, with special emphasis on endocrine disruptive properties. Ecotoxicol. Environ. Saf. 46: 305-321.

Verma P, Madamwar D (2003). Decolourization of synthetic dyes by a newly isolated strain of *Serratia marcescens*. World J. Microbiol. Biotechnol., 19(6): 615-618.

Wolfe NL, Steen WC, Burns LA (1980). Phthalate ester hydrolysis: linear free energy relationships. Chemosphere, 9: 403-408.

Wu XL, Wang YY, Liang RX, Dai QY, Jin DC, Chao WL (2011). Biodegradation of an endocrine-disrupting chemical di-n-butyl phthalate by newly isolated *Agrobacterium* sp. and the biochemical pathway. Process Biochem., 46(5): 1090-1094.

Xu XR, Gu JD, Li HB, Li XY (2005a). Kinetics of di-n-butyl phthalate degradation by a bacterium isolated from mangrove sediment. J. Microbiol. Biotechnol. 15: 946-951.

Xu XR, Li HB, Gu JD (2005b). Biodegradation of an endocrine-disrupting chemical di-n-butyl phthalate ester by *Pseudomonas fluorescens* B-1. Int. Biodeterior Biodegradation, 55: 9-15.

Xu XR, Li HB, Gu JD, Li XY (2007). Kinetics of n-butyl benzyl phthalate degradation by a pure bacterial culture from the mangrove sediment. J. Hazard. Mater., 140: 194-199

Young DM, Unkefer PJ, Ogden KL (1997). Biotransformation of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) by a prospective consortium and its most effective isolate *Serratia marcescens*. Biotechnol. Bioeng., 53: 515-522.