African Journal of Biotechnology Vol. 10(16), pp. 3143-3148, 18 April, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.2045
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Study on optimal biodegradation of terephthalic acid by an isolated *Pseudomonas* sp.

Zhi-Jiang Wang[1]*, Li-hua Teng[1], Jie-Zhang[1], Xu-Li Huang[1] and Jian-Fen Zhang[2]

[1]College of Biological and Environmental Science, Zhejiang Wanli University, Ningbo 315100, People's Republic of China.
[2]College of Biological and Environmental Engineering, Zhejiang Shuren University, Hanzhou 310015, People's Republic of China.

Accepted 14 March, 2011

The biodegradation of terephthalic acid (TA) by the *Pseudomonas* sp. was researched in this paper. The optimal nitrogen source was urea with its concentration of 1400 mg/l. The optimal conditions for TA degradation were optimized by orthogonal test. The test result showed the pH 7.0, temperature of 30°C and shaking speed of 140 rpm were the best condition for degradation. In that condition, terephthalic acid was almost degraded completely after 48 h cultivation of *Pseudomonas* sp. by inoculating 4% seed culture. Kinetics of degradation was performed at different initial TA concentrations. The degradation could be described with a first-order kinetics model. The half-life of degradation was about 12 h when the concentration of terephthalic acid was between 600 and 1000 mg/l.

Key words: Biodegradation, terephthalic acid, *Pseudomonas* sp.

INTRODUCTION

Terephthalic acid (TA) is the main raw material for production of polyester fiber and thin fiber and also widely used in coating, adhesive, dye, plasticizer and other production industries (Kleerbezem et al., 1997), So, TA is an important industrial chemical, which makes substantial increase in the demand for terephthalic acid. While, in terephthalic acid production process, a large number of high concentration wastewater would be produced. In China, terephthalic acid production capacity has reached 12 million tons/year and the wastewater emissions of about 30 million tons/year with discharge concentration over 1000 mg/l (Chen, 2006; Zhang et al., 2005). In polyester production process, its chemical dissolved oxygen (COD) of wastewater would reach 90 thousand mg/l, with terephthalic acid concentration of 70%. However, according to China industrial wastewater discharge standards, the discharge standard of COD should be under 200 mg/l. Microbial growth in water would be inhibited and some animals would have to get cancer or gene mutation, renal function would be impaired by TA (Wolkowski et al., 1982; Engela et al., 2007; Qi et al., 2002; Scholz, 2003). Terephthalic acid has been designated by the U.S. Environmental Protection Agency as a pollutant (USEPA, 1978). Research on terephthalic acid degradation is also widespread concerned (Ribbons and Evans, 1960; Park et al., 2003; Crepaldi et al., 2002).

Unfortunately, due to its chemical structure, terephthalic acid cannot be removed completely by natural processes. However, biodegradation of TA by microorganisms is considered to be one of the major routes for this widespread pollutant (Kleerebezem et al., 1997; Kleerebezem and Lettinga, 2000), while much more research was done on activated sludge (Manikavasagam et al., 2008).

In our laboratory, an aerobic bacterial strain was isolated from polyester wastewater. This strain identified as *Pseudomonas* sp. was recognized to have the ability to degrade terephthalic acid. In order to understand its microbial degradation of TA, fermentation process of this strain and the kinetics of biodegradation were investigated.

*Corresponding author. E-mail: wangzj1978@126.com. Tel: +860574-88222679.

Abbreviations: TA, Terephthalic acid; **CFU,** colony forming unit.

Table 1. Factors and levels for orthogonal test.

Variable	Level		
	1	2	3
Temperature(℃)	25	30	37
pH value	6	7	8
Shaking speed(rpm)	100	140	160

MATERIALS AND METHODS

Organism and growth

Pseudomonas sp. previously isolated from polyester wastewater, was preserved in our laboratory. Seed medium was contained (per liter) 1 g terephthalic acid, 1 g NH_4Cl, 0.25 g $MgSO_4$, 3 g KH_2PO_4, 0.5 g NaCl and 7 g Na_2HPO_4. Batch medium was contained (per liter) 1 g TA, 0.25 g $MgSO_4$, 3 g KH_2PO_4, 0.5 g NaCl and 7 g Na_2HPO_4 and 1 g various nitrogen. The microorganism *Pseudomonas* sp. was cultured on the seed medium for 12 h at 30℃ and then, was inoculated into a 500 ml conical flask containing 100 ml of sterilized batch medium by inoculating 4% seed culture with its concentration of 1.7×10^5 colony forming unit per ml (CFU/ml) and cultured at 30℃ for 48 h on an orbital shaker of 140 rpm.

Reagents

Terephthalic acid was purchased from Shanghai chemical reagent factory, China. Other chemicals used in this study were all of analytical grade.

Orthogonal test

Orthogonal experiment using L_9 $(3)^3$ table including three factors (temperature: A, pH value; B, shaking speed; C, three levels were designed to explore the optimal terephthalic acid degradation conditions (Table 1). *Pseudomonas* sp. was cultured on the batch medium, by inoculating 4% seed culture with its concentration of 1.7×10^5 CFU/ml.

Kinetics studies

The kinetics of terephthalic acid degradation in water at different initial concentrations was investigated on this strain during 48 h. The initial concentrations of the TA were 200, 600, 1000 and 1400 mg/l, respectively. *Pseudomonas* sp. was cultured on the batch medium containing the different respective terephthalic acid concentrations through inoculation 4% seed culture and cultured at pH 7.0, 30℃ and shaking speed 140 rpm for 48 h.

Analytical methods

To measure terephthalic acid concentrations, a HPLC-UV system equipped with a column (C_{18}, 25 cm × 4.6 mm) with packing size of 5 µm was used. The detection wavelength was 240 nm. The mobile phase consisted of methanol-water (80:20 v/v) and that water used contained KH_2PO_4 0.05 ml/l. The flow rate of the mobile phase was adjusted at 1 ml/min. The analysis was carried out at ambient temperature. TA retention time was at 2.15 to 2.17 min. Cell concentration was determined from optical density value obtained in a spectrophotometer at 600 nm.

RESULTS AND DISCUSSION

Effect of nitrogen sources

To investigate the effect of nitrogen on the biodegradation of terephthalic acid, *Pseudomonas* sp. was cultivated with various nitrogen sources for 24 h. As shown in Figure 1. Terephthalic acid degradation rate of inorganic nitrogen was significantly better than that of organic nitrogen, indicating the presence of organic matter can inhibit the degradation of the terephthalic acid, which is similar to previous studies (Cheng et al., 1997; Fajardo et al., 1997). It is possible that, organic nitrogen provides not only as the nitrogen source but also as the carbon source, such as amino acids. It provides amino as a nitrogen source and provides carboxyl as a carbon source. Carbon source from nitrogen source would be easier to be used by *Pseudomonas* sp. than terephthalic acid. When urea was used as nitrogen source, the cell concentration was lowest, but the specific activity of cell catalyst was the highest, its terephthalic acid biodegradation rate reached 91.3%. So, the urea was the best nitrogen sources for biodegradation of TA and urea was used as nitrogen source for further study.

Effect of urea concentration

The effect of urea concentration on the *Pseudomonas* sp. cell concentration and the degradation rate of terephthalic acid were researched. The *Pseudomonas* sp. was cultivated for 48 h in medium which contained 200, 600, 1000, 1400, 1800, 2200 mg/l urea, respectively. As shown in Figure 2, the cell concentration and biodegradation rate were increased largely when the urea concentration increased from 200 to 1400 mg/l and then, the cell concentration and biodegradation rate were decreased slightly, as the urea concentration was above 1400 mg/l. When the concentration of urea was 1400 mg/l, both the degradation rate and cell concentration reached the maximum. So, the optimum urea concentration was 1400 mg/l.

Effect of TA concentration

The effect of terephthalic acid concentration on the *Pseudomonas* sp. concentration and the degradation rate of TA were investigated. The *Pseudomonas* sp. was cultivated for 48 h in medium which contained 200, 600, 1000, 1400, 1800, 2200 mg/l TA, respectively, the result is shown in Figure 3. The cell concentration was increased and then decreased, when the terephthalic acid concentration was increased all along. It is possible that, the cell growth would be inhibited by the high concentration

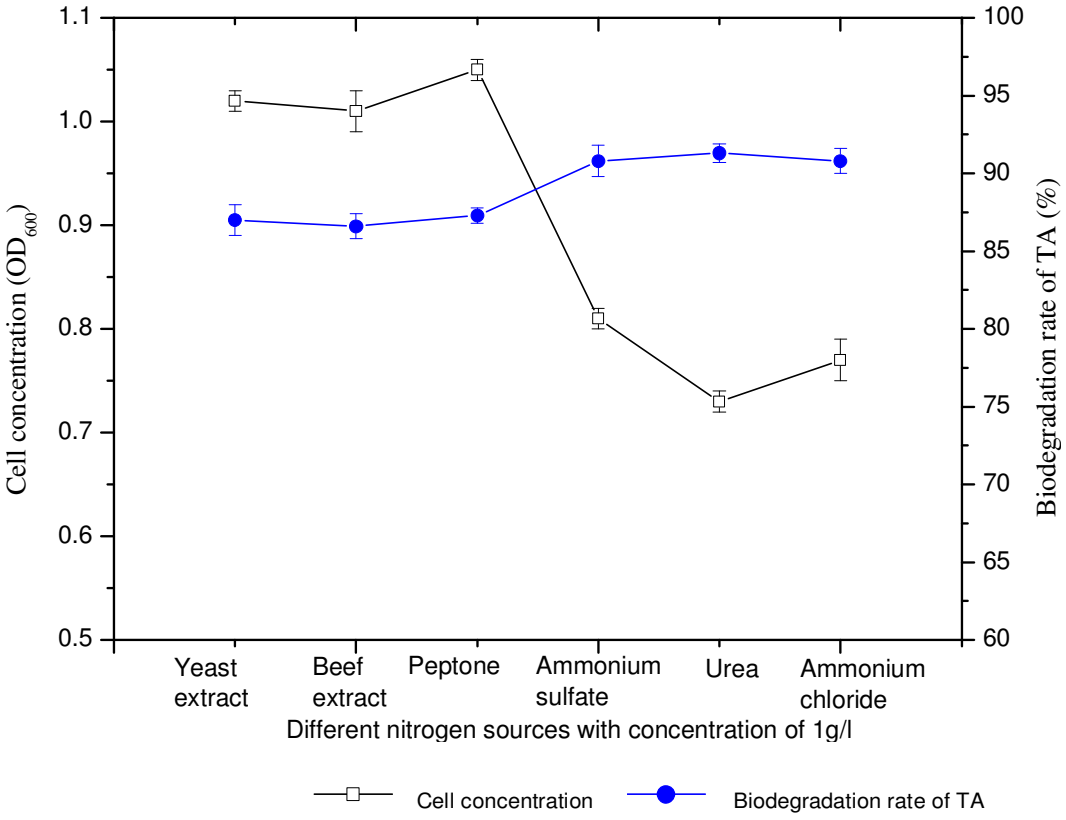

Figure 1. Effect of various nitrogen sources on *Pseudomonas* sp. cell concentration and TA biodegradation.

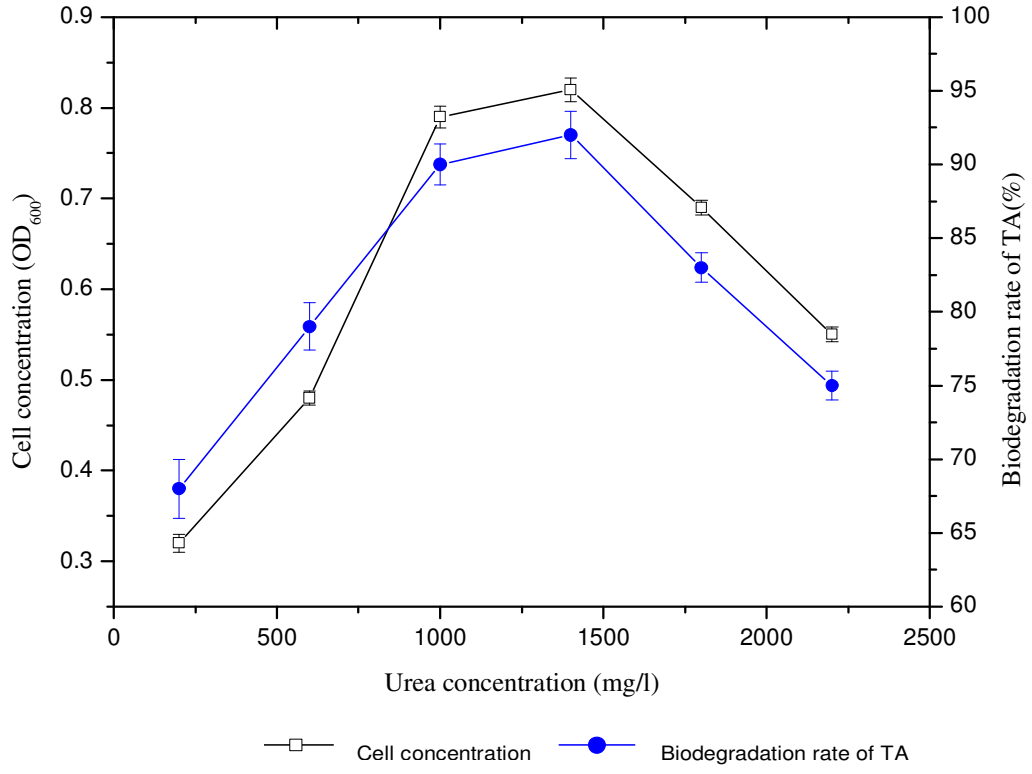

Figure 2. Effect of urea concentration on *Pseudomonas* sp. cell concentration and TA biodegradation.

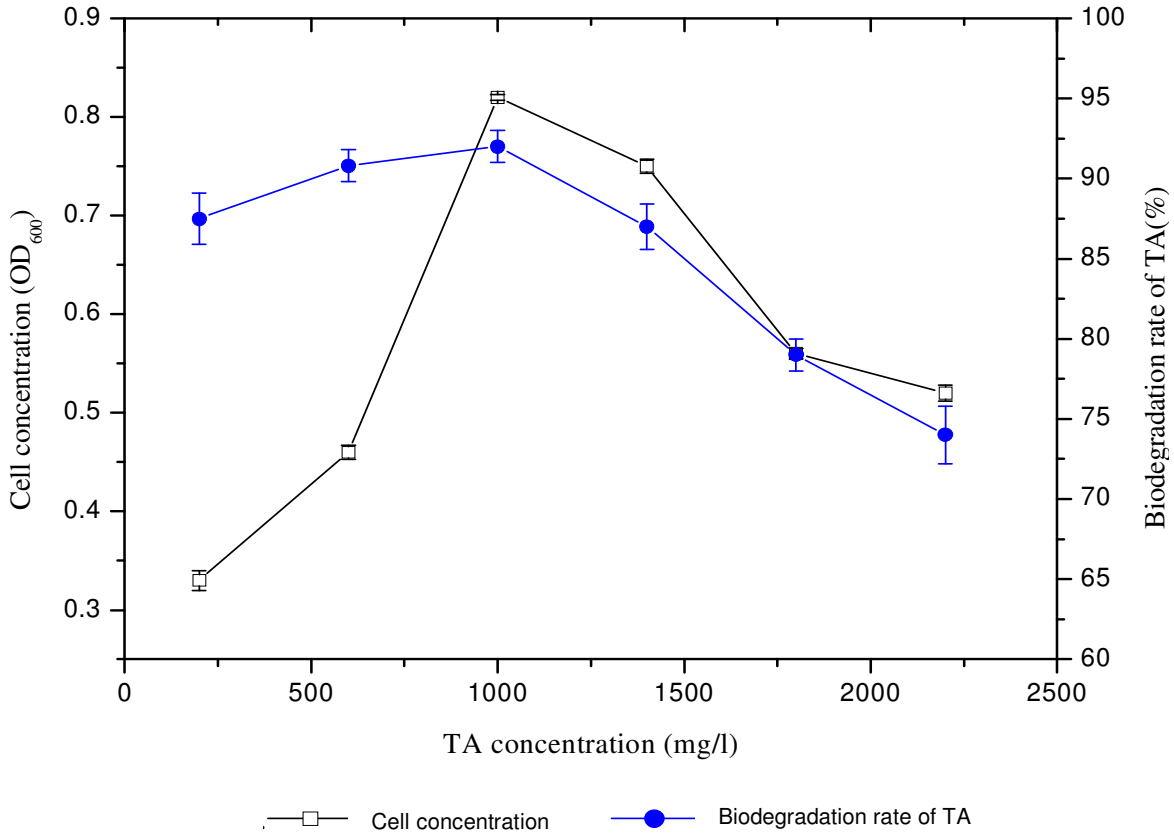

Figure 3. Effect of TA concentration on *Pseudomonas* sp. cell concentration and TA biodegradation.

of TA above 1000 mg/l (Kuang and Wang, 1993). From this figure, it is shows that the terephthalic acid concentration of 1000 mg/l is the best concentration for TA degradation. Considering the cell concentration and the degradation rate, 1000 mg/l TA concentration was used for fermentation.

Optimal external conditions for TA degradation

Orthogonal test was used to explore the optimal biodegradation conditions. In the orthogonal test, K_i (i= 1, 2, 3) was defined as average value of degradation rate of every level. The optimal level of factors can be confirmed by comparing the value of K_i. R value was used to estimate the effect of factors and R=max {K_1, K_2, K_3} – min {K_1, K_2, K_3}. High R value of factor means that this factor has strong effect on the results. The results and data analysis of orthogonal test were listed in Table 2. From orthogonal test, the influence of the factors to the degradation rate decreased in the order: B > C > A. pH was found to be the most important factor, which was prior to shaking speed and temperature. For degradation, pH 7.0, shaking speed 140 rpm, 30°C would be the optimal condition. The results of temperature 30°C, pH 7.0 were similar to previous research (Tong and Bai, 1990).

Terephthalic acid degradation rate of 97.6% was got by culturing *Pseudomonas sp.* in the final good condition of 30°C, pH 7.0, shaking speed of 1400 rpm, by inoculating 4% seed culture.

Kinetics studies on TA degradation

The terephthalic acid degradation by *Pseudomonas* sp. was very suitable to a first-order kinetics model, lnS =–Kt +A, where S is the initial concentration of TA (mg/l), K is the biodegradation rate constant, t is the reaction time, A is the constant. The degradation half life ($t_{1/2}$) of terephthalic acid is ln2/k.

The tendency of degradation curve of the different terephthalic acid concentrations was shown in Figure 4 and the kinetics equations were shown in Table 3. From the results, the half-life of degradation was about 12 h and the inhibition of terephthalic acid to *Pseudomonas* sp.'s catalytic ability was not strong. No obvious lag time was observed. The half-life was bigger than 12 h when the terephthalic acid concentration was lower than 200 mg/l and higher than 1400 mg/l, which indicated the degradation rate of the terephthalic acid became slower. It means the production of cells in contact with the

Table 2. Results of orthogonal test of *Pseudomonas* sp. on TA biodegradation.

Number	Temperature (℃)	pH value	Shaking speed(rpm)	Biodegradation rate (%)
1	25	6	100	25.7
2	25	7	140	95.8
3	25	8	160	80.7
4	30	6	140	29.6
5	30	7	160	95
6	30	8	100	80.7
7	37	6	160	27.5
8	37	7	100	92.7
9	37	8	140	84.5
K_1	67.4	27.6	66.4	
K_2	68.4	94.5	70.0	
K_3	68.2	82.0	67.7	
R	1.0	66.9	3.6	

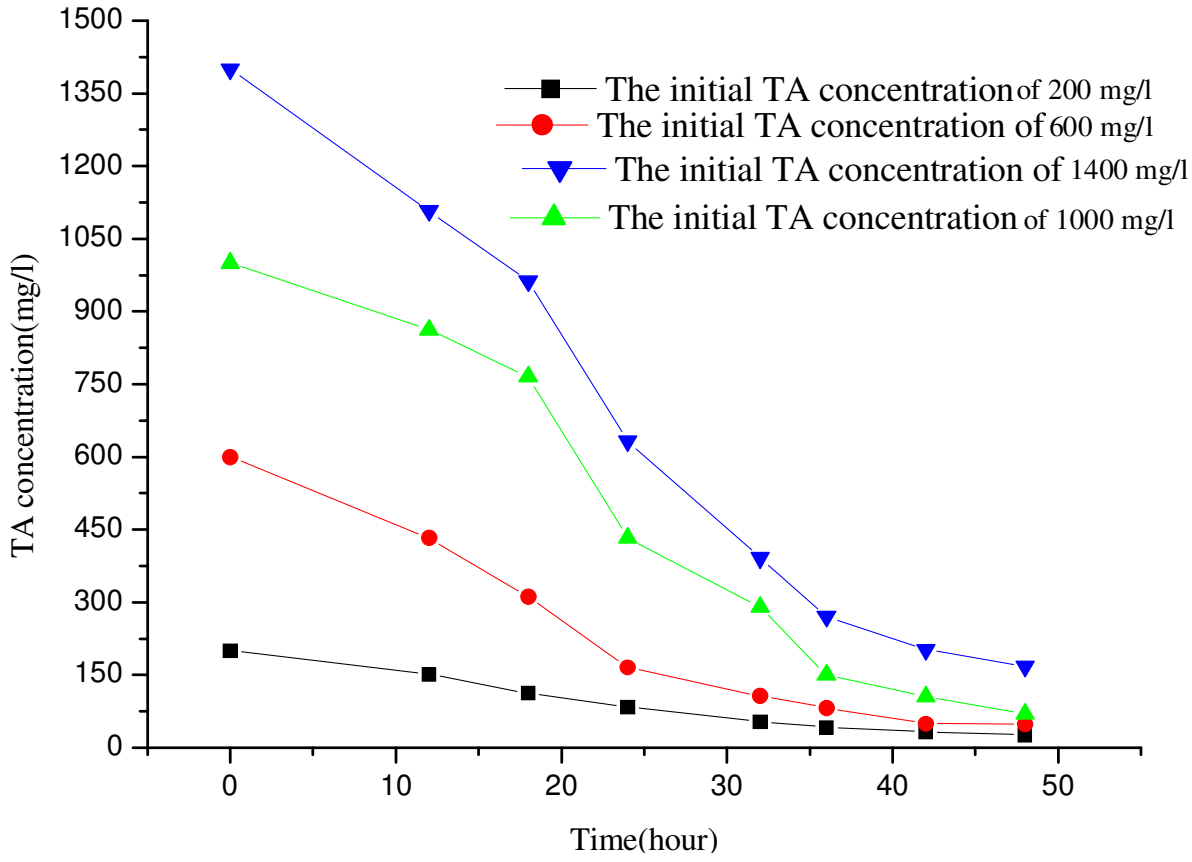

Figure 4. The degradation curve of the TA concentration.

substrate of TA would be reduced, resulting in slower reaction rate when the cells were in a concentration of terephthalic acid less than 200 mg/l. On the contrary, cells growth in a concentration of terephthalic acid more than 1400 mg/l would be inhibited and that caused the half-life to become extended.

Conclusions

In conclusion, the final batch medium contained (per liter) 1 g terephthalic acid, 0.25 g $MgSO_4$, 3 g KH_2PO_4, 0.5 g NaCl and 7 g Na_2HPO_4, urea 1.4 g, and the biodegradation conditions were pH 7.0, temperature 30℃ and

Table 3. TA degradation kinetics equation.

Initial concentration of TA (mg/l)	Kinetics equation	Half life (hour)	Biodegradation rate constant	Correlation coefficient
200	lnS = -0.0457x + 5.452	15.167	0.0457	0.9851
600	lnS = -0.0595x + 6.5966	11.65	0.0595	0.9723
1000	lnS = -0.0598x + 7.305	11.59	0.0598	0.9394
1400	lnS = -0.0494x + 7.5065	14.03	0.0494	0.9584

shaking speed 140 rpm. A first-order kinetics model could fit the degradation curve of TA; the half-life of degradation was about 12 h by curve fitting.

ACKNOWLEDGEMENTS

This study was supported by the Program of Education Department of Zhejiang Province (No. Y200907155) and the Natural Sciences Foundation of Ningbo City (2010A-610093).

REFERENCES

Chen J (2006). Preparation of highly efficient PTA degradation bacteria and biological treatment of PTA wastewater. Environ. Sci. Technol. 10: 75-76.

Cheng SS, Hu CY, Wu JH (1997). Pilot study of UASB process treating PTA manufacturing wastewater. Water Sci. Technol. 36: 75-82.

Crepaldi EL, Tronto J, Cardoso LP (2002). Sorption of terephthalate anions by calcined and uncalcined hydrotalcite-like compounds. Colloid Surf. A: Physicochem. Eng. Aspects, 211: 103-114

Engela P, Brandta KK, Rasmussenb LH, Ovesenb RG, Sørensen J (2007). Microbial degradation and impact of bracken toxin ptaquiloside on microbial communities in soil. Chemosphere, 67: 202-209.

Fajardo C, Guyot JP, Macarie H, Monroy O (1997). Inhibition of anaerobic digestion by terephthalic acid and its aromatic by products. Water Sci. Technol. 36: 83-90

Kleerebezem R, Mortier J, Hulshoff Pol LW, Lettinga G (1997). Anaerobic pretreatment of petrochemical effluents: terephthalic acid wastewater. Water Sci. Technol. 36: 237-248.

Kleerebezem R, Lettinga G (2000). High-rate anaerobic treatment of purified terephthalic acid wastewater. Water Sci. Technol. 42: 259-268.

Kuang X, Wang JS (1993). A study on the biodegradability of terephthalic acid (TA) under anaerobic condition. Environ. Chem. 12: 179-186.

Manikavasagam K, Nishant D, Pradyumna P, Tapas N (2008). Biodegradability enhancement of purified terephthalic acid wastewater by coagulation-flocculation process as pretreatment. J. Hazard. Mater. 154: 721-730.

Park TJ, Lim JS, Lee YW (2003). Catalytic supercritical water oxidation of wastewater from terephthalic acid manufacturing process. J. Supercrit. Fluid. 26: 201-213.

Qi S, Wang X, Xu X, Xiang Q (2002). Study on the bladder calculi and bladder cancer induced by terephthalic acid in rats. J. Hyg. Res. 31: 10-12.

Ribbons DW, Evans WC (1960). Oxidative metabolism of phthalic acid by soil pseudomonads. Biochem. J. 76: 310-318.

Scholz N (2003). Ecotoxicity and biodegradation of phthalate monoesters. Chemosphere, 53: 921-926.

Tong H, Bai YQ (1990). Degradation of TA by microorganism. Acta Scientiae Circumstantiae, 10(4): 464-470.

USEPA (1978). Hazardous waste guidelines and regulations. Fed. Reg. 43: p. 58947.

Wolkowski TR, Chin TY, d'A Heck H (1982). Chemical urolithiasis. β-pharmacokinetics and transplacental transport of terephthalic acid in fischer-344 rats. Drug Metab. Dispos. 10: 486-490.

Zhang XX, Wan YQ, Cheng SP, Sun SL, Zhu CJ, Li WX, Zhang XC, Wang GL, Lu JH, Luo X, Gu JD (2005). Purified terephthalic acid wastewater biodegradation and toxicity. J. Environ. Sci. 17: 876-880.