Desalination and Water Treatment
www.deswater.com
doi: 10.1080/19443994.2014.982956

57 (2016) 3059–3064
February

Taylor & Francis
Taylor & Francis Group

Biodegradation of p-cresol by *Pseudomonas* spp.

A. Hamitouche[a,b,*], Z. Bendjama[a], A. Amrane[c,d], F. Kaouah[a]

[a]*Laboratoire des Sciences du Génie des Procédés Industriels, Faculté de Génie Mécanique et de Génie des Procédés, Université des Sciences et de la Technologie Houari Boumediene, BP 32,El-Alia, 16111 Bab Ezzouar, Algiers, Algeria, emails: ahamitouche2@yahoo.fr (A. Hamitouche), zbendjama@yahoo.fr (Z. Bendjama)*
[b]*Centre de Recherche scientifique et technique en Analyses Physico-Chimiques, BP 248, CRAPC, Algiers, Algeria*
[c]*Ecole Nationale Supérieure de Chimie de Rennes, Université Rennes 1, CNRS, UMR 6226, Avenue du Général Leclerc, CS 50837, 35708 Rennes Cedex 7, France*
[d]*Université européenne de Bretagne, 35000 Rennes, France*

Received 27 October 2013; Accepted 22 October 2014

ABSTRACT

Phenols are toxic to several biochemical reactions. However, biological transformation of phenols to nontoxic entities exists in specialized microbes, owing to enzymatic potential involving enzymes of aromatic catabolic pathways. In this study, a series of experiments were performed to examine the effects of the mineral medium composition and the pH on p-cresol removal. In this purpose, p-cresol biodegradation was carried out in a batch reactor containing mixed bacteria; the temperature (30°C), the stirring velocity (200 rpm), the KH_2PO_4 concentration (1.5 g/L), the K_2HPO_4 concentration (2 g/L), and p-cresol concentration (100 mg/L) were kept constants. The initial pH was varied in the range 5–9 and the mineral components were tested in the following concentration ranges: 0–2 g/L for Nitrogen sources (NH_4Cl, KNO_3, and NH_4NO_3), 0–0.5 g/L for NaCl, and 0–0.2 g/L for $MgSO_4$. Their effects on p-cresol biodegradation and specific growth rate were examined. The shorter biodegradation time of p-cresol was 30.5 h for NH_4Cl, NaCl, and $MgSO_4$ concentrations of 1, 0.3, and 0.1 g/L, respectively. Maximum specific growth rate (0.34 h^{-1}) and total p-cresol removal were recorded for an optimal pH value of 8.

Keywords: Biodegradation; p-Cresol; *Pseudomonas* spp.; Kinetics

1. Introduction

With the development of industrialization, more and more industrial wastewater containing phenolic compounds are discharged from industrial processes such as oil refineries, chemical plants, and coke ovens [1–5]. A number of these industrial processes produce phenolic effluents in which phenol may be present at a level as high as several grams per liter. Because of their toxicity and persistence, inadequate handling of the effluents will lead to serious pollution problem [6–10]. Biological treatment of industrial effluents and bioremediation of the contaminated environment by exploiting the capability and potential of microorganisms have been considered the most promising methods [11–17].

The main goal of this paper was to investigate the biodegradation of phenol by mixed bacteria; the effect of pH and the mineral medium composition were optimized to determine the best conditions for

*Corresponding author.

phenol removal, especially the mineral salts supplementation and the optimal mineral nitrogen concentration.

2. Materials and methods

2.1. Micro-organisms cultivation

The mixed bacteria of Pseudomonas genus (*Pseudomonas aeruginosa*, *Pseudomonas putida*, *Pseudomonas fluorescens*) used in this work were obtained from activated sludge from the hazardous wastewater station of Boumerdès (Algeria). The stock cultures were stored at 4°C. The mixed bacteria were activated for 24 h at 30°C in the nutrient medium (NB) containing (g/L): peptone, 15, yeast extract, 3, sodium chloride, 6, and (D+)-glucose, 1. After 24 h, when cells were grown, the biomass was harvested by centrifugation. The micro-organisms collected after centrifugation (3,000 rpm) for 30 min were suspended in NaCl 0.5% and re-centrifuged. After the third washing, The micro-organisms collected after centrifugation were re-suspended in NaCl 0.5% to determine the concentration of the mixed bacteria. This solution (mixed bacteria and NaCl 0.5%) was analyzed by measuring OD at 600 nm using a Vis spectrophotometer (HACH DR2800); the OD value was then converted to dry cell mass using a dry weight calibration curve. The dry cell mass density (g/L) was found to follow the following regression equation $x\ (g/L) = 1.044 \times OD_{600}$. Specific growth rate was determined in the exponential growth phase. For each flask, the specific growth value was determined from linear semi-logarithmic plot of cell concentration vs. time during the exponential growth phase, i.e. when specific growth rate became nearly constant [18].

2.2. Biodegradation experiments

As the OD value of adapted cells reached 2.7–2.9, an aliquot of the culture was centrifuged at 3,000 rpm for 30 min. To wash the biomass, it was re-suspended in NaCl 0.5% and centrifuged. The cells (1 mL) were then transferred and inoculated in Erlenmeyer flasks (250 mL) to yield an initial OD of 0.078, and containing 100 mL of medium containing nitrogen source (NH_4Cl) and the following mineral salt supplementation (MSS), namely KH_2PO_4, KH_2PO_4, and $MgSO_4$ at the required concentrations, and 125 mg/L of p-cresol. The cells were cultivated at 30°C and 200 rpm. Samples were withdrawn at suitable time intervals and the concentration of cells was deduced from optical density measurement and p-cresol was measured as described below.

2.3 Residual p-cresol determination

p-Cresol was colorimetrically estimated using a Vis spectrophotometer (HACH DR5000), absorbance read at 288 nm.

3. Results and discussion

3.1. Optimal salt concentrations

At pH 7, the effect of mineral salt supplementation of culture medium on p-cresol degradation was shown for instance for NaCl (Fig. 1).

Fig. 1 shows that the residual p-cresol concentration decreases vs. the time; on the other side, the evolution of biomass concentration increases vs. the time.

The lag phase was at least 20 h, and in the range of NaCl concentrations tested, total p-cresol removal (100 mg/L) was recorded in less than 33.5 h. The shorter time of biodegradation of p-cresol is equal to 30.5 when NaCl concentration is equal to 0.1 g/L.

Table 1 shows the effect of culture medium components on specific growth rate. Table 1 shows that the specific growth rate increases with the increase in NaCl concentration, reaching a maximum and then decreases; this decrease indicates that bacterial growth is slowed down beyond this inhibitory concentration. Maximum specific growth rate was 0.21 h^{-1} recorded for 0.1 g/L NaCl.

This amount was in agreement with the mineral supplementation considered by other workers, since Luo et al. [19] and Nakano et al. [20] supplemented with 0.1 and 0.21 g/L NaCl to biodegrade phenol, while 0.4 g/L was used to biodegrade chlorophenols (2-chlorophenol, 4-chlorophenol, 2,4-dichlorophenol, and 2,4,6-trichlorophenol) [21].

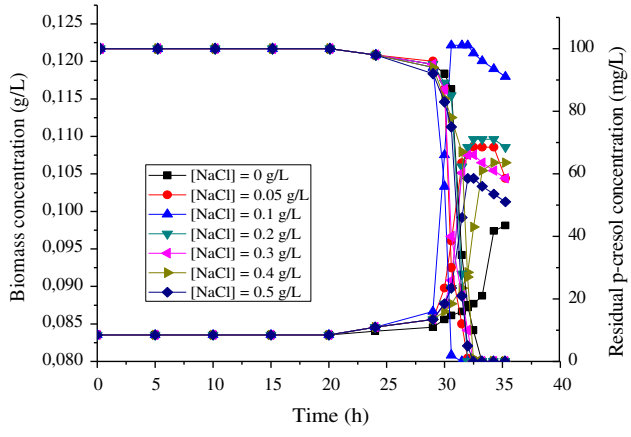

Fig. 1. Time courses of the residual p-cresol and biomass concentrations for different initial NaCl concentration.

Table 1
Effect of culture medium components and pH on the specific growth rate

Parameters (g/L)	Value of parameters	Specific growth rate (h^{-1})
[NaCl]	0	0.093
	0.05	0.11
	0.1	**0.21**
	0.2	0.18
	0.3	0.158
	0.4	0.128
	0.5	0.1
[$MgSO_4$]	0.05	0.149
	0.1	**0.21**
	0.15	0.157
	0.2	0.127

At 30°C and pH 7, a series of degradation batch test at different $MgSO_4$ concentration were conducted with mixed culture. The effect of $MgSO_4$ concentration on p-cresol degradation was observed on Fig. 2.

Fig. 2 shows that a mixed bacterium degrades a 100 mg/L of p-cresol within 35.25 h; the shorter time is equal to 30.58 h when $MgSO_4$ concentration is equal to 0.1 g/L. The evolution of cell concentration (Fig. 2) shows that a lag phase is equal to 20 h.

The evolution of specific growth rate vs. $MgSO_4$ concentration (Table 1) shows that the maximum value is equal to 0.21 h^{-1} when $MgSO_4$ concentration is equal to 0.1 g/L.

Table 1 shows that the specific growth rate increases with the increase in MgSO4 concentration, reaching a maximum and then decreases; this decrease indicates that bacterial growth is slowed down beyond this inhibitory concentration.

The same concentration of $MgSO_4$ in mineral salt medium was used by Hamitouche et al. [11,12], Qui et al. [22] to biodegrade phenol and p-cresol, respectively.

3.2. Optimal nitrogen source concentration

A series of degradation batch test at different nitrogen concentration sources were conducted with *Pseudomonas* spp. culture to see the effect of the three nitrogen sources selected on biodegradation of p-cresol. The results were regrouped in Figs. 3–5.

Figs. 3–5 clearly indicate the affinity of *Pseudomonas* spp. to the three nitrogen sources in p-cresol degradation process. A 100 mg/L of p-cresol was entirely degraded within 48.5 h; the time shorter for p-cresol biodegradation is equal to 30.58 h when the optimal concentration is equal to 1 g/L. The evolution of cell

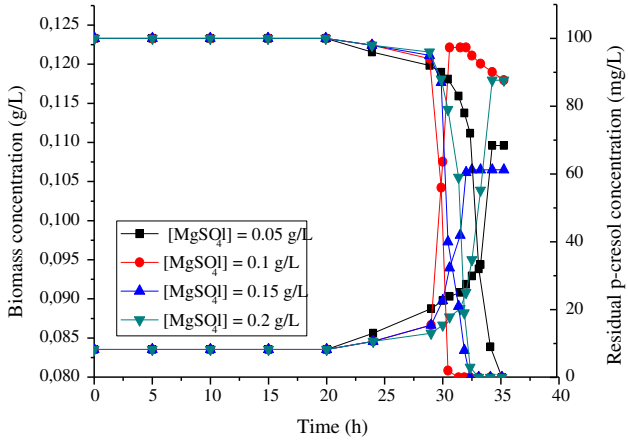

Fig. 2. Time courses of the residual p-cresol and biomass concentrations for different initial $MgSO_4$ concentrations.

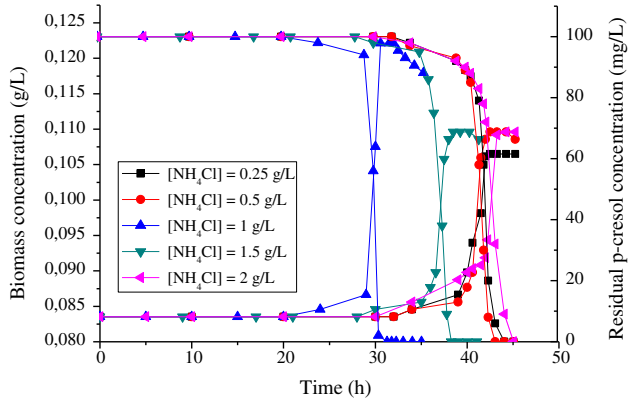

Fig. 3. Time courses of the residual p-cresol and biomass concentrations for different initial NH_4Cl concentrations.

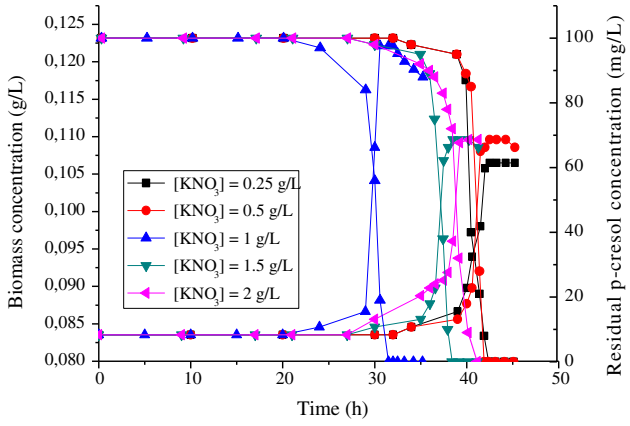

Fig. 4. Time courses of the residual p-cresol and biomass concentrations for different initial KNO_3 concentrations.

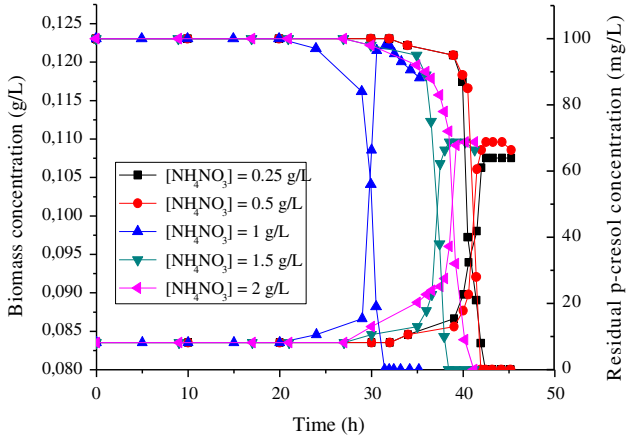

Fig. 5. Time courses of the residual p-cresol and biomass concentrations for different initial NH_4Cl concentrations.

concentration shows that the time for lag phase is between 20 and 32 h for different nitrogen sources concentration.

Table 2 shows that the effect of evolution of nitrogen sources concentration on specific growth rate, and the specific growth rate increases with the increase in nitrogen concentration, reaching a maximum and then decreases; this decrease indicates that bacterial growth is slowed down beyond this inhibitory concentration.

The maximum value of specific growth rate is equal to 0.21, 0.203, and 0.194 h^{-1} when the concentration of NH_4Cl, KNO_3, and NH_4NO_3, respectively, is equal to 1 g/L.

The same optimal concentration of nitrogen sources was obtained by Hamitouche et al. [11–13] to biodegrade phenol and p-cresol, with another mixed culture composed of four bacteria with different genus.

3.3. Optimal pH

Batch cultures of mixed bacteria of Pseudomonas genus were conducted in an optimal condition of salt and nitrogen concentration with initial p-cresol concentration of 100 mg/L and initial pH ranging from 5 to 9. The evolution of residual p-cresol concentration and cell concentration vs. the time for different value of initial pH were represented in Figs. 6–8.

A p-cresol (100 mg/L) was entirely degraded within 45.25 h; the shorter time for p-cresol biodegradation is equal to 30.58 h when pH is equal to 7. The evolution of cell concentration shows that a lag phase is equal to 20 h to 32 h for different pH value. Fig. 9 shows the effect of pH on specific growth rate. The

Table 2
Effect of nitrogen sources on the specific growth rate

Parameters (g/L)	Value of parameters	Specific growth rate (h^{-1})	Time of lag phase (h)
[NH_4Cl]	0.25	0.156	32
	0.5	0.18	32
	1	**0.21**	20
	1.5	0.178	28
	2	0.146	30
[KNO_3]	0.25	0.151	32
	0.5	0.201	32
	1	**0.203**	20
	1.5	0.188	27
	2	0.171	27
[NH_4NO_3]	0.25	0.161	32
	0.5	0.181	32
	1	**0.194**	20
	1.5	0.18	28
	2	0.16	28

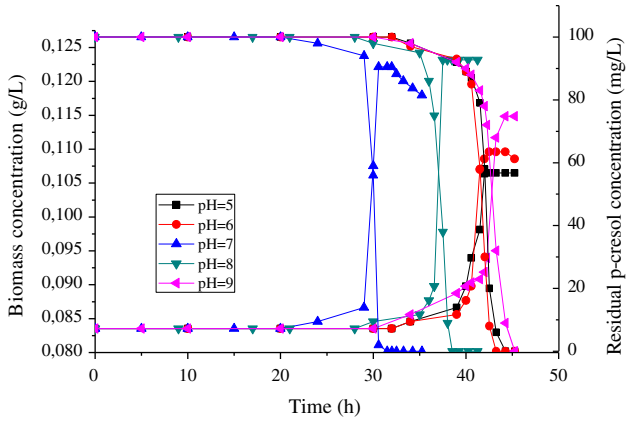

Fig. 6. Time courses of the residual p-cresol and biomass concentrations for different initial pH value with NH_4Cl as nitrogen source used.

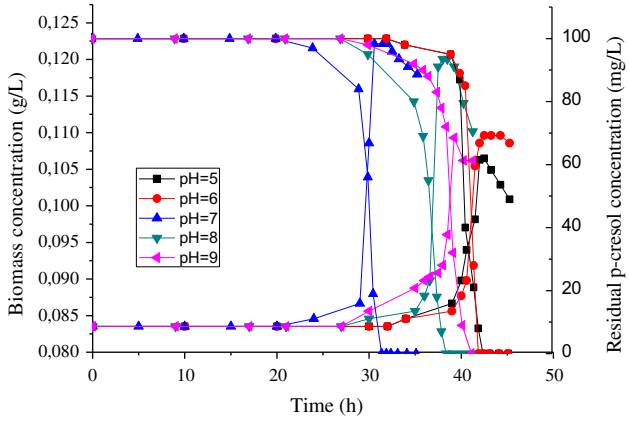

Fig. 7. Time courses of the residual p-cresol and biomass concentrations for different initial pH value with KNO_3 as nitrogen source used.

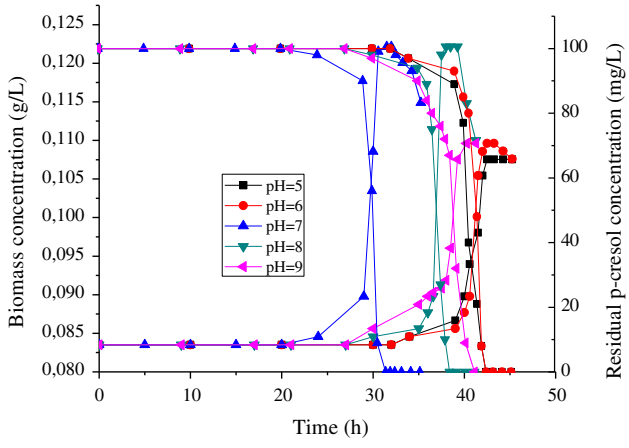

Fig. 8. Time courses of the residual p-cresol and biomass concentrations for different initial pH value with NH_4NO_3 as nitrogen source used.

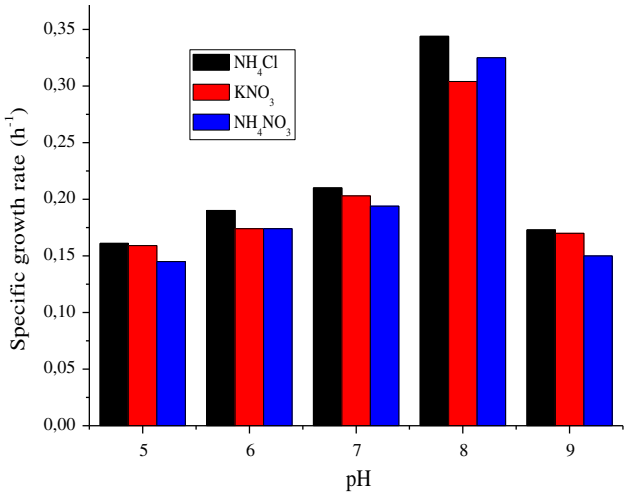

Fig. 9. Evolution of specific growth rate vs. pH for different nitrogen sources.

maximum value of specific growth rate is equal to 0.344, 0.304, and 0.313 h^{-1} for NH_4Cl, KNO_3, and NH_4NO_3, respectively, when pH is equal to 8. The study of Ho et al. [1], Qiu et al. [22], and Ying et al. [23] obtained the same optimal pH with a phenol and p-nitrophenol. The optimal values of specific growth rate are much higher than those found in the literatures, whereas the specific growth rate of mixed culture obtained has been reported to be in the range of 0.13–0.36 h^{-1} [1,18–23].

4. Conclusion

Growth kinetics of mixed bacteria and their degradation nature for p-cresol were investigated.

The following conclusions were drawn:

(1) As the mixed culture has a high biodegradation activity, specific growth rate raises until 0.34 h^{-1}, with the time of biodegradation equal to 32.67 h.
(2) A 100 mg/L of p-cresol is completely degraded for each batch experience and for different parameter studied, whereas time of biodegradation range is 20–32 h.
(3) The optimal mineral medium concentrations (g/L) are 0.1, 0.1, and 1 for NaCl, $MgSO_4$, and nitrogen sources, respectively.
(4) The optimal value of pH is equal to 8.

References

[1] K.-L. Ho, B. Lin, Y.-Y.u. Chen, D.-J. Lee, Biodegradation of phenol using *Corynebacterium* sp. DJ1 aerobic granules, Bioresour. Technol. 100 (2009) 5051–5055.

[2] P. Saravanan, K. Pakshirajan, P. Saha, Biodegradation kinetics of phenol by predominantly *Pseudomonas* sp. in a batch shake flask, Desalin. Water Treat. 36 (2011) 99–104.
[3] W. Al Hashemi, M.A. Maraqa, M.V. Rao, Md.M. Hossain, Characterization and removal of phenolic compounds from condensate-oil refinery wastewater, Desalin. Water Treat. (in press), doi: 10.1080/19443994.2014.884472.
[4] S. Mohammadi, A. Kargari, H. Sanaeepur, K. Abbassian, A. Najafi, E. Mofarrah, Phenol removal from industrial wastewaters: A short review, Desalin. Water Treat. (in press), doi: 10.1080/19443994.2014.883327.
[5] S. See, J.-W. Lim, P.-E. Lim, C.-E. Seng, S.-L. Ng, R. Adnan, Enhancement of o-cresol removal using PAC and acclimated biomass immobilized in polyvinyl alcohol hydrogel beads, Desalin. Water Treat. (in press), doi: 10.1080/19443994.2013.833874.
[6] A. Hussain, S.K. Dubey, Specific methanogenic activity test for anaerobic treatment of phenolic wastewater, Desalin. Water Treat. (in press), doi: 10.1080/19443994.2013.823116.
[7] J.-W. Lim, H.-M. Gan, C.-E. Seng, Bioremediation of wastewaters containing various phenolic compounds by phenol-acclimated activated sludge, Desalin. Water Treat. 51 (2013) 7018–7024.
[8] Y.C. Kuo, S.F. Cheng, P.W.G. Liu, H.Y. Chiou, C.M. Kao, Application of enhanced bioremediation for TCE-contaminated groundwater: A pilot-scale study, Desalin. Water Treat. 41 (2012) 364–371.
[9] A. Karakaya, G. Bozkoyunlu, Y. Laleli, S. Takaç, Development of pH adjustment-based operational strategy to increase total phenol removal rate in biodegradation of olive mill wastewater by *Rhodotorula* glutinis, Desalin. Water Treat. (in press), doi: 10.1080/19443994.2013.823357.
[10] Y.J. Liu, P. Kuschk, A.N. Zhanga, X.C. Wang, Characterisation of phenol degradation by *Acinetobacter* sp. XA05 and *Sphingomonas* sp. FG03, Chem. Ecol. 25(2) (2009) 107–117.
[11] A. Hamitouche, A. Amrane, Z. Bendjama, F. Kaouah, Effect of the ammonium chloride concentration on the mineral medium composition—Biodegradation of phenol by a microbial consortium, Int. J. Environ. Res. 4(4) (2010) 849–854.
[12] A. Hamitouche, A. Amrane, Z. Bendjama, F. Kaouah, Phenol biodegradation by mixed culture in batch reactor—Optimization of the mineral medium composition, Desalin. Water Treat. 25 (2011) 20–24.
[13] A. Hamitouche, A. Amrane, Z. Bendjama, F. Kaouah, Biodegradation of p-cresol by mixed culture in batch reactor—Effect of the three nitrogen sources used, Procedia Eng. 33 (2012) 458–464.
[14] A. Hamitouche, Z. Bendjama, A. Amrane, F. Kaouah, D. Hamane, Relevance of the Luong model to describe the biodegradation of phenol by mixed culture in a batch reactor, Ann. Microbiol. 62 (2012) 581–586.
[15] Q. Zhang, Z.F. Jin, Z.C. Hu, Z.Y. Pan, Biodegradation of m-cresol in alkaline wastewater by resting photosynthetic bacteria, Desalin. Water Treat. (in press), doi: 10.1080/19443994.2014.913992.
[16] R.-H. Toh, C.-E. Seng, P.-E. Lim, R. Adnan, A.N.A. Sujari, Biodegradation of o-cresol by polyvinyl alcohol–alginate immobilized mixed culture, Desalin. Water Treat. 51 (2013) 7216–7222.
[17] F. Lassouane, S. Amrani, H. Ait-Amar, Evaluation of o-cresol degradation potential by a strain of *Pseudomonas aeruginosa* S8, Desalin. Water Treat. 51 (2013) 7577–7585.
[18] P.D. D'Adamo, A.F. Rozich, A.F. Gaudy Jr., Analysis of growth data with inhibitory carbon sources, Biotechnol. Bioeng. 26 (1984) 397–402.
[19] H. Luo, G. Liu, R. Zhang, S. Jin, Phenol degradation in microbial fuel cells, Chem. Eng. J. 147(2) (2009) 259–264.
[20] Y. Nakano, W. Nishijima, E. Soto, M. Okada, Relationship between growth rate of phenol utilizing bacteria and the toxic effect of metabolic intermediates of trichloroethylene (TCE), Water Res. 33(4) (1999) 1085–1089.
[21] G. Zhao, L. Zhou, Y. Li, X. Liu, X. Ren, X. Liu, Enhancement of phenol degradation using immobilized microorganisms and organic modified montmorillonite in a two-phase partitioning bioreactor, J. Hazard. Mater. 169 (2009) 402–410.
[22] X. Qiu, P. Wu, H. Zhang, M. Li, Z. Yan, Isolation and characterization of *Arthobacter* sp. HY2 capable of degrading a high concentration of p-nitrophenol, Bioresour. Technol. 100 (2009) 5243–5248.
[23] W. Ying, T.T. Ye, H. Bin, H.-B. Zhao, J.-N. Bi, B.-L. Cai, Biodegradation of phenol by free and immobilized *Acinetobacter* sp. strain PD12, J. Environ. Sci. 19 (2007) 222–225.