African Journal of Biotechnology Vol. 10(84), pp. 19551-19555, 26 December, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.2774
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Biodegradation of phenol by a newly isolated marine bacterial strain SM5

Cui Hong-xia[1,2]

[1]Key Laboratory of Applied Chemistry of HeBei Province, Yanshan University, No.438 Hebei street , Qinhuangdao 066004, P. R. China.
[2]College of Environmental and Chemical Engineering, Yanshan University, No.438 Hebei street , Qinhuangdao 066004, P. R. China, E-mail: cuihongxia2002@hotmail.com.

Accepted 25 November, 2011

15 bacterial strains were isolated from marine sources on the beef extract-peptone agar plates with 1500 mg/L phenol. Among them, the strain SM5 could tolerate 4500 mg/L phenol on solid beef extract-peptone plates and its phenol biodegradation rate was 96.4% in basal salt (BS) medium under the optimum conditions when the concentration of phenol was 1000 mg/L. These conditions were; initial pH 7.0, 37°C, 3 days, 20 ml medium/50 ml flask and inoculum biomass 12.5% (v/v). Rate of phenol biodegradation of the strain was up to 92.0% under the optimum conditions even when the phenol concentration was increased to 2500 mg/L. Also, the effects of supplement sodium chloride [NaCl (5 to 25 g/L)] on phenol biodegradation were very weak. In this study, a high-efficiency bacterial strain SM5 of phenol biodegradation was obtained from sea mud and could be used for treatment of phenolic water with salt.

Key words: Biodegradation, marine bacterial strain, phenol, isolation.

INTRODUCTION

Chemical and petroleum industrial activities produce multiform highly toxic organic wastes, which have resulted in accumulative dangerous effects on the environments. These wastes are resistant to natural biodegradation and persist in the environment. Among these, the aromatic compounds are of most serious concern (Chung TP et al., 2003). Phenol, a compound, was regarded as a priority contaminant by the U.S. Environmental Protection and Agency and China (Keith and Tclliard, 1979; Jin et al., 1990). It is an environmental pollutant because of its common presence in the effluents of many industrial processes, including oil refineries, petrochemical plants, steel plants, coal conversion processes, pharmaceuticals, pesticides, dyes, plastics, explosives, herbicides and phenolic resin industries (Santos and Linardi, 2004; Ha et al., 2000).

The presence of phenols in drinking and irrigation water presents a serious health risk for humans, animals, plants and microorganisms (Sharma et al., 1997; Abd-El-Haleem et al., 2002). Phenol is lethal to fish even at relatively low levels, e.g. 5 to 25 mg/L (Chung et al., 2003). Industrial wastewaters containing phenols therefore require proper treatment before being discharged into the environments. Classical methods such as solvent extraction, adsorption and chemical oxidation often suffer from serious drawbacks including high cost and formation of hazardous by-products (Atlow et al., 1984). Among various available methods, biodegradation is environmental friendly and cost effective. Biological treatment of phenol is therefore preferred (Loh and Tan, 2000).

The purpose of this investigation was to isolate and screen bacteria with potential for phenol degradation from sea water, mud and sand. The biodegradation capacity of the strain was studied.

MATERIALS AND METHODS

Sample collection

Seawater, sea mud and sea sand samples were collected by sterile method from the seashore along Qinhuangdao, East China. Samples were placed in sterile plastic tubes with tops, which were taken to the laboratory and stored at 4°C until use.

Isolation of strains

Various dilutions were made and spread on beef extract-peptone

Table 1. Growth of marine bacterial isolates in various concentration of phenol.

Isolate	Growth on beef extract-peptone agar plates with added phenol					
	Initial phenol concentrations (mg/L)					
	2000	2500	3000	3500	4000	4500
SM1	–[a]	–	–	–	–	–
SM2	+[b]	+	+	–	–	–
SM3	+	+	+	+	–	–
SM4	+	+	+	+	–	–
SM5	+	+	+	+	+	+
SM6	–	–	–	–	–	–
SM7	+	–	–	–	–	–
SS1	+	–	–	–	–	–
SS2	–	–	–	–	–	–
SS3	+	+	+	–	–	–
SS4	–	–	–	–	–	–
SS5	–	–	–	–	–	–
SW1	–	–	–	–	–	–
Sw2	+	+	+	+	–	–
SW3	+	+	+	+	–	–

[a]Absence of growth; [b]growth.

medium agar plates (1% peptone, 0.3% beef extract, 1.5% agar, pH 7.0 to 7.2 and 100% seawater) containing 1500 mg/L phenol, followed by incubation at 37°C for two days. Colonies were transferred onto beef extract-peptone agar plates for sub-culture to obtain pure strains by colony, cellular morphology and gram stain.

Tests of phenol tolerance

The pure strains were inoculated onto solid beef extract-peptone plates plus phenol at concentrations from 2000 to 5000 mg/L, at 500 mg/L intervals. The plates were inoculated at 37°C for five days with daily observation of growth. It showed that the strain could endure the corresponding concentration of phenol if it could grow on the plate.

Biodegradation experiments

The strains were inoculated into the lysogeny broth (LB) medium and flasks were incubated at 37°C under shaking (180 rpm) conditions for one day. LB medium (per liter) contained 10g of tryptone, and 5 g of yeast extract. The LB medium was prepared by seawater and adjusted to pH 7.0. The inoculation was transferred to basal salt medium (BS) [0.2% potassium di-hydrogen phosphate (KH_2PO_4), 0.01% magnesium sulphate ($MgSO_4$), 0.1% ammonium sulphate ($(NH_4)_2SO_4$), 0.01% calcium chloride ($CaCl_2$), 100% seawater, pH 7.0] supplemented with phenol (analytic grade) at a concentration of 1000 mg/L. Bacterial growth was at 37°C with shaking at 180 rpm for three days. The concentration of phenol was determined each day.

The effect of pH on the biodegradation of phenol was studied. The pH was adjusted with 1 M NaOH or HCl in the range 5.5 to 8.0. The effect of temperature was studied by incubation at 28 and 37°C. The effect of volume of medium in 50 ml flasks (10, 20 and 30 ml) and transfer inoculum biomass (2.5, 5, 7.5, 10, 12.5 and 15%, v/v) was also studied. The concentration of sodium chloride (NaCl) varied from (5 to 25 g/L) to determine the effect on the biodegradation of phenol.

Chemical determination

Phenol was determined quantitatively by the spectrophotometric method (722S visible-infrared spectrometer) using 4-aminoantipyrine as the colour reagent (λ_{max}: 510 nm) according to standard methods of analysis (Environmental Protection Agency of China, 1997).

RESULTS AND DISCUSSION

Isolation and tests of phenol tolerance

A total of 15 bacterial isolates able to grown in beef extract-peptone media supplemented with 1500 mg/L of phenol were isolated from the samples. Among them, seven, five and three strains were isolated from sea mud, sea sand and seawater, respectively. Among the 15 isolates, one grew on solid beef extract-peptone medium with phenol concentrations up to 4500 mg/L (Table 1) and was selected for the tests of phenol degradation in liquid BS medium. The capability of phenol tolerance was very high and the name of the strain was SM5. It is a red, long rod and gram-positive.

Phenol biodegradation by strain SM5

The time course of phenol biodegradation for the strain

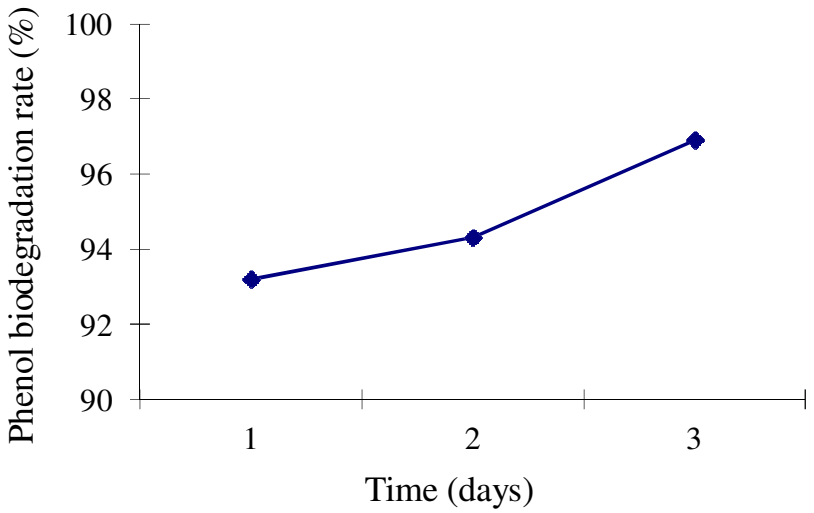

Figure 1. Phenol degradation by strain SM5 in the presence of phenol (1000 mg/L).

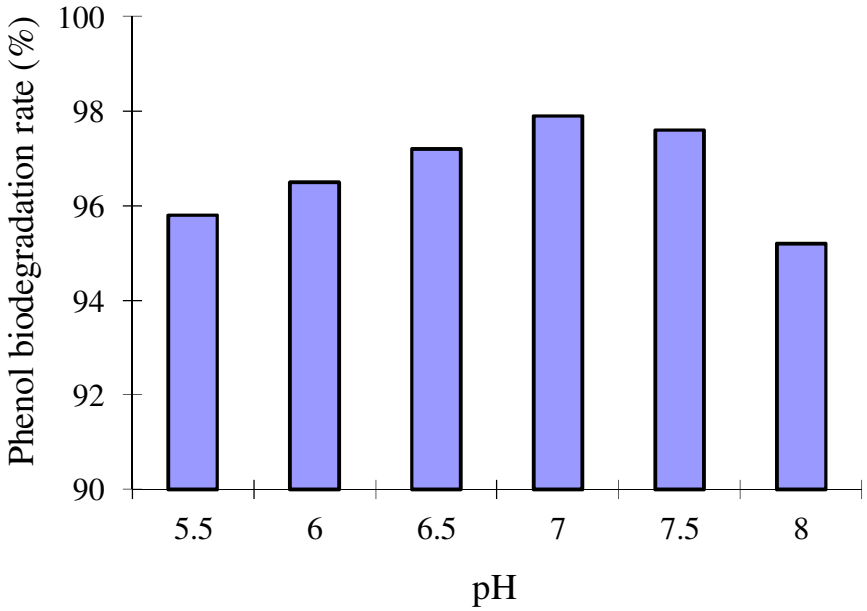

Figure 2. Effect of initial medium pH on phenol biodegradation.

SM5 is presented in Figure 1. Phenol was decomposed by the strain SM5 quickly. More than 93.0% of phenol biodegradation was obtained on day one. Moreover, phenol biodegradation rate was up to 96.9% on day three. Hence, the time parameter was selected for three days in order to de-compound completely.

Effect of initial pH and temperature on phenol biodegradation

The initial pH would affect the biodegradation rate as shown in Figure 2. The neutral pH (6.5 to 7.5) was the best, because the neutral pH was beneficial to strain SM5 growth. pH 7.0 was selected for the optimum pH. In the temperature tested, 37℃ was the optimum for phenol biodegradation up to 95.7% compared to 28℃ (93.8%).

Effect of volume of medium on phenol biodegradation

The strain was cultured in 50 ml flasks containing 10, 20 and 30 ml BS medium with 1000 mg/L phenol. And the

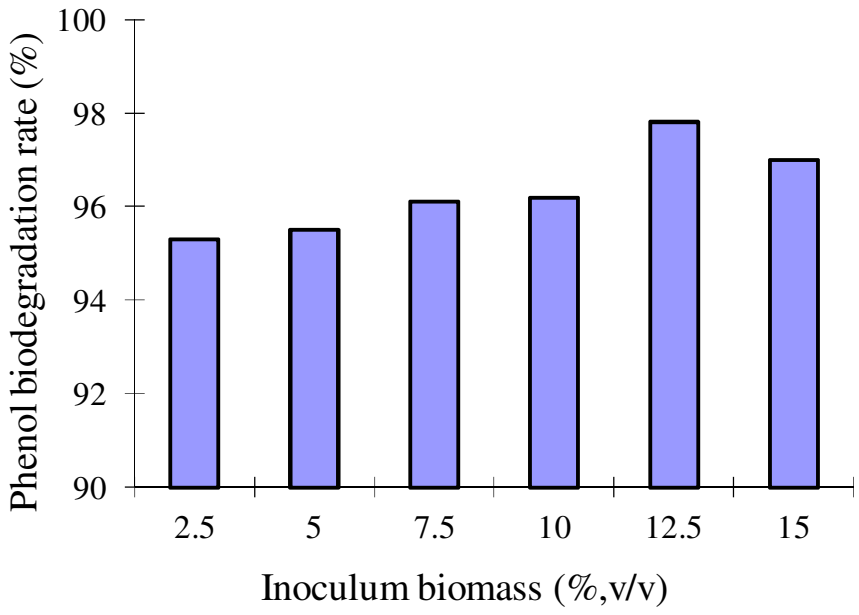

Figure 3. Effect of inoculum biomass on phenol biodegradation.

Table 2. Effects of salt concentration on phenol biodegradation.

Salt concentration NaCl (g/L)	Phenol biodegradation rate (%)
0	96.3
5	93.1
10	93.7
15	94.5
20	93.7
25	94.1

rates of phenol biodegradation are 97.3, 97.4 and 95.6%, respectively. There was no obvious difference on the first two values, but the phenol biodegradation rate decreased when the volume of medium was 30 ml. It was clear that the decrease of volume medium in the flasks increased the oxygen transfer. So dissolved oxygen may play a key role in the phenol biodegradation. Hence, 20 ml was selected for the volume of medium parameter considering efficiency.

Effect of inoculum biomass on phenol biodegradation

The transfer inoculum volume appears to be a very important parameter when incubation is carried out in poisonous compound media, such as phenol (Santos et al., 2001). For instance, in this study, phenol biodegradation rate increased with the biomass from 2.5 to 10% (v/v) (Figure 3), then biodegradation rate decreased above this range of biomass. The optimum inoculum biomass was selected as 12.5%.

Effects of salt concentration on phenol biodegradation

The effect of salt concentration of phenol biodegradation is shown in Table 2, after adding different concentration of NaCl into the reaction mixture. The phenol biodegradation rates were more than 93.0%, although they were restrained by NaCl from 5 to 25 g/L. This showed that the phenol biodegradation rate was not affected basically by NaCl and the strain of phenol biodegradation can tolerate high concentration of NaCl. This can be therefore be used for treatment of phenol with high concentration NaCl. It may also be used to treat the industrial wastewater and there is often high concentration sodium salt in industrial phenolic water (Liu, 1998).

Biodegradation of varied phenol concentration

Under optimum conditions, microbial capability of phenol biodegradation with different concentration of phenol was studied, and the data are shown in Table 3. Phenol biodegradation rate was up to a maximum of 96.4% when the concentration of phenol was 1000 mg/L. Rate of phenol biodegradation was also up to 92.0% when the concentration of phenol increased to 2500 mg/L. This showed that the strain SM5 can be used for treating the high concentration of phenolic water. It was also reported

Table 3. Effect of initial phenol concentration on phenol biodegradation.

Initial phenol concentration (mg/L)	Phenol biodegradation rate (%)
500	91.6
1000	96.4
1500	91.5
2000	91.6
2500	92.0

that 1504 mg/L phenol could be biodegraded by *Candida tropicalis* (Bastos et al., 2000). There is hardly strain possessing the ability of phenol biodegradation exceeding this concentration. Therefore, a potent phenol biodegradation strain was obtained in the study.

Conclusion

The strain SM5 isolated from sea mud was found to degrade phenol. Phenol-tolerance of the strain was up to 4500 mg/L on solid beef extract-peptone plate. The best phenol biodegradation conditions were initial pH 7.0, culture temperature 37°C, culture time three days, volume of medium 20 ml per 50 ml flasks, inoculum biomass 12.5% (v/v) and initial phenol concentration 1000 mg/L. Phenol biodegradation rate was up to 92.0% when the concentration of phenol was 2500 mg/L and was hardly affected by NaCl (5 to 25 g/L). The strain can therefore be used for the treatment of highly concentrated phenolic water with sodium salt.

ACKNOWLEDGEMENT

This study was supported by Hebei Provincial Natural Science Funds (D2010001149).

REFERENCES

Abd-El-Haleem D, Moawad H, Zaki EA, Zaki S (2002). Molecular characterization of phenol-degrading bacteria isolated from different Egyptian Ecosystems[J]. Microb Ecol, 43(2): 217-224.

Atlow SC, Bonadonna-Aaparo L, Klibanov AM (1984). Dephenolization of industrial wastewaters catalyzed by polyphenol oxidase[J]. Biotechnol Bioeng, 26(6): 599-603.

Bastos AER, Moon DH, Rossi A, Trevors JT, Tsai SM (2000). Salt-tolerant phenol-degrading microorganisms isolated from Amazonian soil samples[J]. Arch Microbiol, 174(5): 346-352.

Chung TP, Tseng HY, Juang RS, (2003). Mass transfer effect and intermediate detection for phenol degradation in immobilized *Pseudomonas putida* systems[J]. Proc Biochem, 38(10): 1497-1507.

Environmental Protection Agency of China (1997). Determination and analysis method of water and wastewater[M]. Beijing: Environmental Sciences of China Press, 408-410.

Ha SR, Vinitnantharat S, Ozaki H (2000). Biodegradation by mixed microorganism of granular activated carbon losded with a mixture of phenols[J]. Biotechnol Lett, 22(13): 1093-1096.

Jin XC, Cheng ZH, Xu NN (1990). contamination chemistry of organic compounds-contamination chemistry of poisonous organic compounds[M]. Beijing: Tsing Hua University Press, 22-24.

Keith LH, Tclliard WA (1979). Priority pollutants. I-A perspective view[J]. Environ Sci Technol, 13(4): 416-423.

Liu XW (1998). Current situations and developments of industrial phenolic water treatment techniques[J]. Industrial Water Treatment, 18(2): 4-6.

Loh KC, Tan CPP (2000). Effect of additional carbon sources on biodegradation of phenol[J]. Bull Environ Contam Toxicol, 64(6): 756-763.

Santos VL, Heilbuth NM, Linardi VR (2001). Degradation of phenol by *Trichosporon* sp. LE3 cells immobilized in alginate[J]. J Basic Microbiol, 41(3/4): 171-178.

Santos VL, Linardi VR (2004). Biodegradation of phenol by a filamentous fungi isolated from industrial effluents-identification and degradation potential[J]. Proc Biochem, 39(8): 1001-1006.

Sharma HA, Barber JT, Ensley HE, Polito MA (1997). A comparison of the toxicity and metabolism of phenol and chlorinated phenols by *Lemna gibba*, with special reference to 2,4,5-trichlorophenol[J]. Environ Toxicol Chem, 16(2): 346-350.