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Comparative Studies on Simultaneous Biodegradation of Phenol and Cyanide Using Different Strains

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Abstract
Removal of pollutants like phenol and cyanide is a serious environmental concern. Widespread studies on the biodegradation of phenol and cyanide have been carried out to overcome the environmental problems. This study provides an overview on the biological degradation of phenol and cyanide by isolated strain *S.odorifera*. For comparison three strains namely, *A. chroococuum, E. coli* and *P. putida* were also used for the degradation of phenol and cyanide. In this study, the effect of initial concentration of phenol and cyanide on their removal and biomass concentration was studied. It was observed that amongst these four bacteria percentage removal of phenol and cyanide, was found to be maximum for *S. odorifera*. The maximum tolerance level of phenol and cyanide for *S. odorifera* was found to be 1500 mg/l and 150 mg/l respectively. It was also concluded from this study that, the bacteria *S. odorifera* was capable simultaneous removal of phenol and cyanide i.e., 88.26% and 99.85% respectively.

Keywords: Biodegradation, Environmental concern, Phenol, Cyanide, Simultaneous removal

I. INTRODUCTION

A huge source of water pollution is industrial waste; industries produce large amount of waste water which is harmful to environment and human health. Phenol and cyanide compounds are produced as waste products of many industrial processes; coke plant being one of them. Waste water from coke plant also contains toxic xenobiotics: phenol derivatives (pyrocatechol, pyrogallol, problems. The concentration of phenol, cyanide and their derivatives in coke waste water is generally higher than the standard limits set for their release in to the environment. The Maximum Contaminant limit (MCL) of phenol and cyanide in drinking water as defined by the US Environmental Protection Agency (USEPA) and the Minimum National Standards (MINAS) of the Central Pollution Control Board (CPCB) in India are 0.5 mg/l and 0.2 mg/l respectively [1,2].

Phenol and cyanide contamination in water has posed severe health effects around the world. Long-term exposure of cyanide at levels above the MCL, can cause weight loss, thyroid effects, nerve damage and even death and phenol exposure can lead to gastrointestinal disorders, skin and eyes injuries, lung kidney, liver and heart damage, collapse, coma and other serious mental disturbances [3,4,5].

Several routes for phenol and cyanide removal from the environment are under investigation, including the use of biodegradation. Biodegradation requires the use of living microorganisms for degradation or adsorption of pollutant. The microorganisms that can be used for biological treatment method must have the ability to utilize waste material and converting them into simple compounds by natural metabolism. Biological removal of pollutants provides a cost effective and eco- friendly solution for the pollution deterioration from the industrial effluent. Widespread studies on the biodegradation of phenol and cyanide have been carried out in order to overcome the environmental problems. During the past two decades, a variety of bacteria capable of degrading phenol and cyanide, in single substrate system have been isolated and characterized. Some of the bacteria having phenol degrading capacity are *Rhodococcus arythropolis, Gordonia sputa, Pseudomonas putida, Streptomyces, Phormidium valderianum* BDU 30501, *Arthrobacter sp., Bacillus cereus, Citrobacter freundii, Micrococcus agilis* etc. [6,7,8,9,10,11]and cyanide degrading capacity are *Klebsiella oxytoca, Citrobacter sp., Pseudomonas sp., Cryptococcus humicolus* MCN2, *Pseudomonas pseudoalcaligenes, Azotobacter chroococcum* 446 etc,[12,13].

A very few studies have also investigated the phenol removal in the presence of cyanide. However no study has been reported where simultaneous removal of phenol and cyanide by bacterial degradation takes place. Hence the focus of this study was on the simultaneous biodegradation of phenol and cyanide from coke waste water. For this purpose, the bacterial strain capable of co-

fermentation of phenol and cyanide as sole source of carbon and nitrogen has been utilized as described elsewhere [14]. Biodegradation capacity of three other strains, namely *E. coli* BA07, *Pseudomonas putida* MTCC 1194, and *Azotobacter chroococcum* 446 was also studied. It is a well known fact that the initial concentration of the pollutant influences its percentage removal as well as biomass concentration[15,16].Therefore, the effect of initial concentration of phenol and cyanide on their removal and biomass concentration was also studied.

II. MATERIALS AND METHODS

2.1 Chemicals

All the chemicals used in this study were of analytical reagent grade and obtained from Himedia Laboratories Pvt. Ltd. Mumbai India. All the solutions were prepared in Milli-Q Water (Q-H_2O, Millipore Corp. with resistivity of 18.2 MΩ-cm). Stock solution of 100 mg/l cyanide was prepared by dissolving 0.256 g of KCN in 1 L of water whose pH was pre-adjusted to 10. Stock solution containing 1000 mg/L of phenol was prepared by dissolving 1 g of pure phenol crystal in 1 L of water.

2.2 Bacteria strains and medium

Pseudomonas putida MTCC 1194 and *Azotobacter chroococcum 446,* used in this study, were supplied by Microbial Type Culture Collection, Chandigarh, India. The strains were revived according to the instructions given by MTCC [MTCC guidelines]. Cultures were stored on agar plates till further use and were sub cultured after every 30 days. All inoculations were performed in aseptic conditions in laminar air flow unit (Rescholar Equipment, INDIA). The strains *E. coli* BA07 and *S. odoriferra* MTCC 5700 were isolated from coke waste water in laboratory by [14]. The composition of growth media specific to above mentioned strains is given in Table 1.

2.3 Acclimatization

The acclimatization of all four strains in phenol and cyanide environment was performed as follows:

The cultures were sub-cultured from agar plate in 100 ml of steam sterilized prescribed media (Table 1) in 250 ml flasks. The media was supplemented with 10 mg/l of phenol and 1 mg/l of cyanide. The conical flasks were agitated/ incubated in an incubator shaker (Metrex, MO-250, INDIA) at 30 °C with agitation speed of 120 rpm for 24 h. After 24 h the synthetic medium in the flasks turned turbid indicating significant bacterial growth in the flasks. Thereafter, phenol and cyanide were periodically added in increments of 5 mg/l till their concentration in the growth media reached 1500 mg/l and 150 mg/l respectively.

2.4 Batch Biodegradation Experiments

Batch experiments for simultaneous biodegradation of phenol and cyanide were carried out in 250 ml round flasks with working volume of 100 ml and incubated in an incubator cum Orbital shaker, at 29±1 ^{0}C with agitation speed of 150 rpm. The flasks were covered with both cotton plug and aluminium crimp cap. All the flasks containing growth medium were steam sterilized in autoclave at 121±1 ^{0}C for 20 minutes at 15 psi pressure. Phenol and cyanide were added after steam sterilization to avoid oxidation. The initial phenol concentration was ranged from 100 to 1500 mg/l and cyanide concentrations were ranged from 10 to 150 mg/l in the ratio of 10:1. During the biodegradation experiments, the pH of the medium was set between 7 and 8. The batch experiments were conducted in triplicate and lasted for a maximum of 96 h.

2.5 Analytical Methods

For biodegradation studies, appropriate volumes of samples were withdrawn and centrifuged using Remi Lab Centrifuge at 9000 rpm for 10 min. The supernatant was analyzed for phenol and cyanide by 4-aminoantipyrene method at 510 nm and colorimetric picric acid method at 520 nm, respectively [17]. The bacterial growth was measured as optical density by UV-Vis spectrophotometer (Lasany International) at 600 nm after 96 h and was expressed in terms of biomass concentration (mg dry weight /l) [18].

III. RESULTS AND DISCUSSION

3.1 Studies on cyanide biodegradation

Cyanide biodegradation was followed over a period of 96 h in all the selected strains and analysed for reduction of concentrations of cyanide over the incubation period. Suitable dilutions of the samples were made to make sure the values detected were within the linear range of the cyanide standard curve. Though all of the four bacteria consumed cyanide as a source of energy, percentage removal of cyanide was found to be maximum for *S. odorifera*. It was observed that the percentage removal of cyanide decreases with increase in initial concentration of cyanide (Fig. 1). With *S. odorifera* percentage removal remained constant up to initial concentration of 20 mg/l and decreased thereafter. However all other strains showed a decrease in removal with increase in initial concentration. The cyanide degradation by *S. odorifera, A. chroococuum, P. putida and E. coli* was found to be 99.85 %, 96.34%, 96.67% and 25.56 %, respectively at 20 mg/l of

cyanide. It was found that concentration above 50-60 mg/l

Table. 1 Composition of media for microorganisms

Micro-organisms	Media Compositions (g/l)	References
Serratia odoriferra MTCC 5700	Na_2HPO_4 KH_2PO_4 NaCl NH_4Cl $MgSO_4.7H_2O$ Glucose	[14]
Azotobacter chroococuum 446	$MgSO_4.7H_2O$ $Na_2MoO_4.2H_2O$ $CaCl_2$.$2H_2O$ KH_2PO_4 $FeSO_4.7H_2O$ Glucose	[19]
Pseudomonas putida MTCC 1194	Na_2HPO_4 KH_2PO_4 NaCl $MgSO_4.7H_2O$ Glucose $FeSO4.7.H_2O$ $CaCl2.2H_2O$ $MgCl2.6\ H_2O$ $Na_2MoO_4.2H_2O$ $MnCl_2.4H_2O$ $(NH_4)_2SO_4$	[11]
E. coli BA07	Peptone Yeast extract NaCl Glucose	[20]

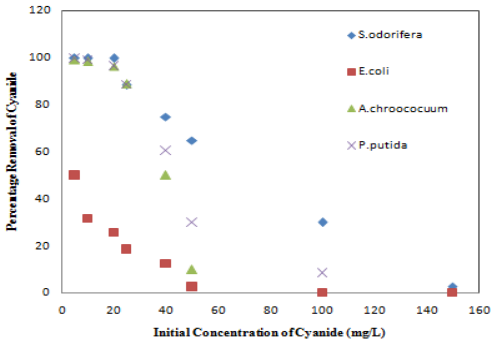

Fig1. Effect of initial concentration of cyanide on its percentage removal by *S. odorifera, E. coli, A. chroococuum and P. putida*

was toxic to microorganism and biodegradation rate of both *A. chroococuum and P. putida* decreased to 40-50% at 50 mg /l. However *S. odorifera* was able to remove 35.6% of 100 mg/l cyanide and 7.56 % removal 150 mg/l cyanide. This is due to the fact that as initial concentration of cyanide increased the toxicity of cyanide to microorganism also increased thereby reducing the percentage removal of cyanide [15].

3.2 Studies on phenol degradation

Phenol biodegradation was also followed over a period of 96 h in all the selected strains and analyzed for reduction of concentrations of phenol over the incubation period. Suitable dilutions of the phenol samples were carried out to make sure the values were detected within the linear range of the phenol standard curve. The percentage removal of phenol was observed to decrease with increase in initial concentration of phenol as shown in Fig.2. The removal was found to be constant after 1000 mg/l of phenol for *S. odorifera, P. putida and E. coli*. However 500 mg/ l was found to be the maximum tolerance limit for *A. chroococuum*. At low phenol concentration i.e., 50 mg/l, *S. odorifera, E.coli, A. chroococuum* and *P. putida* showed 88.265%, 83.179 %, 65.643% and 69.99%

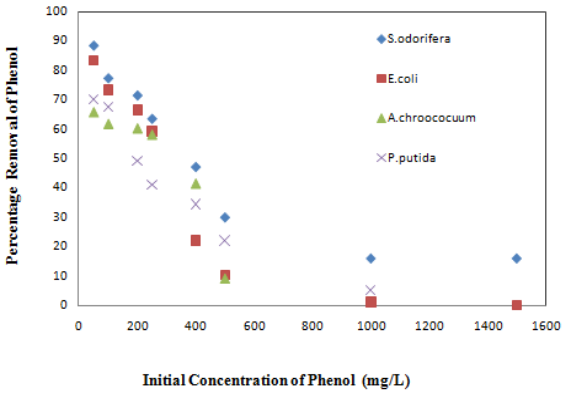

Fig2.Effect of initial concentration of phenol on its percentage removal by *S. odorifera, E. coli, A. chroococuum and P. putida*

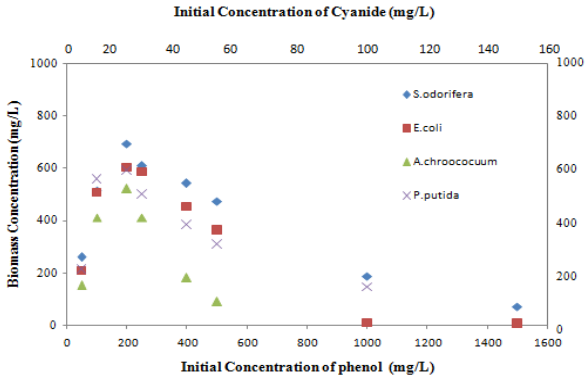

Fig 3. Effect of initial concentration of phenol and cyanide on biomass concentration by *S. odorifera, E. coli , A. chroococuum and P. putida*

removal, respectively. However at high phenol concentration of 400 mg/l, *S. odorifera*, *E. coli*, *A. chroococuum* and *P. putida* showed only 47.13 %, 22 %, 41.34 %, and 34.36 % removal of phenol, respectively. Maximum tolerance limit of *P. putida* was found to be 1000 mg/l however biodegradation rate at such high concentration was negligible, whereas *E.coli* was able to survive till 1500 mg/L of phenol but showed no removal. So it was observed from Fig. 2 that *S. odorifera* provided maximum removal capacity of phenol even at 1500 mg/l of phenol.

3.3 Effect on Biomass concentration

The biomass concentration was found to be increasing with the increase in phenol and cyanide concentration up to 200 mg/l of phenol and 20 mg/l of cyanide (Fig. 3). This is due to the fact that with the increase in phenol and cyanide concentration, microorganism utilizes more phenol and cyanide as carbon and nitrogen source, hence there is an increase in the biomass concentration [14]. However, above 200 mg/l of phenol and 20 mg/l of cyanide, substrate inhibition starts to play the inhibitory role; as a result biomass concentration starts to decrease. The biomass concentration becomes negligible at a phenol concentration of 1500 mg/l and cyanide concentration of 150 mg/l for *S. odorifera*. The maximum tolerance level where the biomass concentration became negligible for *E. coli and P. putida* was found to be 1000 mg/l of phenol and 100 mg/l of cyanide. However growth of *A. chroococuum* was inhibited at much lower concentration (500 mg/l of phenol and 50 mg/l of cyanide).

IV. CONCLUSIONS

In the present study, the biodegradation capacity of *S. odorifera* strain is compared with three other strains, namely *E. coli* BA07, *Pseudomonas putida* MTCC 1194, and *Azotobacter chroococcum* 446. It was concluded from this study that, the bacteria *S. odorifera* was capable of almost complete removal of phenol and cyanide i.e., 88.26% and 99.85% respectively and survive till 1500 mg/l of phenol and 150 mg/l of cyanide concentration, whereas *E. coli* BA07, *Pseudomonas putida* MTCC 1194, and *Azotobacter chroococcum* 446 gives 83.179 %, 65.643% and 69.99% removal for phenol, respectively and 25.56 %,96.67% and 96.34% removal for cyanide, respectively.

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REFERENCES

[1] A. Akcil, "Destruction of cyanide in gold mill effluents: Biological versus chemical treatments", Biotechnol. Adv., **21**. 501–511,2003.

[2] G. Busca, S.Berardinelli, C.Resini, and L.Arright, "Technologies for removal of phenol from fluid streams: a short review on recent developments", J. Hazard. Mater., **160(2-3)**, 265-288,2008.

[3] ATSDR, Agency for toxic substances and Disease Registry, Public Health Statement, Cyanide.http://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=72&tid=19,2006.

[4] ATSDR, Agency for toxic substances and Disease Registry, Toxicological profile for phenol, US, Department of Health and Human Service.<http://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=148&tid=27>,2008.

[5] A.Mittal, , L. Krishnan, and V. K. Gupta, "Use of waste materials—Bottom Ash and De-Oiled Soya, as potential adsorbents for the removal of Amaranth from aqueous solutions", J. of Hazard. Mater., **117**, 171-178,2005.

[6] M.Prieto, A.Hidalgo J. L.Serra, M.J. Llama, "Degradation of phenol by *Rhodococcus erythropolis* UPV-1 immobilized on Biolite in a packed-bed reactor", J. Biotechnol., **97**, 1–11,2002.

[7] K.Przybulewska, A.Wieczorek, A.Nowak, and M.Pochrzaszcz, "The isolation of microorganism capable of phenol degradation", Polish J. of Microbiol., **55(1),** 63-67,2006.

[8] T. S.Chung, H. Y. Tseng, and R.S. Juang, "Mass transfer effect and intermediate detection for phenol degradation in immobilized *Pseudomonas putida* systems", Process Biochem., **38**, 1497–1507,2003.

[9] A.Mordocco, C.Kuek, and R. Jenkins, "Continuous degradation of phenol at low concentration using immobilized *Pseudomonas putida"*, Enzyme Microb. Tech., **25**, 530–536,1999.

[10] G. Spigno, M. Zilli, and C. Nicolella, "Mathematical modeling and simulation of phenol degradation in biofilters", Biochem. Eng. J., **19**, 267–275,2004.

[11] A. Kumar, S. Kumar, and S. Kumar, "Biodegradation kinetics of phenol and catechol using *Pseudomonas putida* MTCC 1194", Biochem. Eng. J., **22**,151–159,2005.
[12] Y. B. Patil, and K. M. Paknikar, "Biodetoxification of silver cyanide from electroplating industry waste water", Lett. App. Microbial., **30(1),** 33-37,2001.
[13] V. M.Luque-Almagro, R.Blasco, M.J. Huertas, M.Martinez-Luque, C.Moreno-Vivian, F.Castillo, and M.D.Roldan, "Alkaline cyanide biodegradation by *Pseudomonas pseudoalcaligenes* CECT 5344", Biochem. Soc. Trans., **33**, 168-9,2005.
[14] B. Agrawal, and C.Balomajumder, " Coke waste water: A source of phenol and cyanide assimilating bacteria", J. of Sci. Tech. Manegement, **5(1)** ,2012.
[15] R. R. Dash,. C. B.Majumder,. and A. Kumar, "Treatment of metal cyanide bearing wastewater by simultaneous adsorption and biodegradation (SAB)". J. of Hazard. Mater., **152(1)**, 387–396,2008.
[16] P.Wu, G. Zhang, J. Li, H. Lu, and W. Zhao, "Effects of Fe^{2+} concentration on biomass accumulation and energy metabolism in photosynthetic bacteria wastewater treatment", Bioresource Tech., **119**, 55–59,2012.
[17] APHA,."Standard methods for the examination of water and waste water" ,20^{th} ed, American Public Health Association, Washington, DC,2001.
[18] M. Afzal, S. Iqbal, S. Rauf, and M. K.Zafar,"Characteristics of phenol biodegradation in saline solutions by monocultures of *Pseudomonas aeruginosa* and *Pseudomonas pseudomallei",* J. of Hazard. Mater., **140**, 60-66,2007.
[19] N. Gupta, "Simultaneous biodegradation and biosorption of cyanide from industrial waste water". Ph.D Thesis, I.I.T. Roorkee.2013.
[20] MTCC Guide lines, http://www.imtech.res.in/mtcc/bacteria.htm.