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RESEARCH ARTICLE

BIODEGRADATION OF PHENOL AND CYANIDE COMPOUNDS BY USING HALOBACTERIA

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ABSTRACT

The present study was made an attempt to degrade the phenolic compounds along with co-toxicants by halobacteria isolated from the coconut retting water. Thirty two morphologically different bacterial strains were isolated, of which 3 strains *Pseudomonas putida, Bacillus* sp. and *Pyrococcus horikoshii* showed better degradation. However, *Pseudomonas putida* showed maximum degradation of phenol up to 2000 $mg.l^{-1}$ without any supplement, but the maximum phenol removal efficiency was observed with the addition of 60 $mg.l^{-1}$ of cyanide at 96 hrs by *Pseudomonas putida* and 600 $mg.l^{-1}$ of thiocyanate at 144 hrs by *Bacillus* sp. It is concluded from the present study that, the isolated bacterial strains *viz., Pseudomonas putida, Bacillus* sp. and *Pyrococcus horikoshii* could be effectively used to degrade the phenolic compounds along with cyanide and thiocyanate under saline environment.

INTRODUCTION

Phenol and its derivatives are one of the major toxic compounds derived from industrial processes, such as oil refineries, cooking plants and petroleum based processing plants (Santos and Linardi, 2004). These compounds are toxic by ingestion, contact or inhalation even at low concentration as because the vapour of phenol can be easily absorbed through the skin and it affects the metabolic activity of the living system (Chung *et al.,* 2003). These problems were occurred due to an improper treatment of the industrial products which contaminated the soil and ground water. Likewise, cyanide and thiocyanate compounds are also toxic and carcinogenic pollutants (Chena and liu, 1999; Yanase *et al.,* 2000) that have been widely used in industries *viz.,* metal-plating, pharmaceuticals, synthetic fibers, coal gasification, coal coking, ore leaching, gold mining and electroplating (Knowles and Bunch, 1986; White *et al.,* 1988; Yanase *et al.,* 2000). Several methods such as precipitation, coagulation, ion exchange and ultra filtration have been developed for the phenol degradation along with co-toxicants, but these methods are more expensive and inefficient because, the byproducts of the above methods are more toxic in nature. Biodegradation of phenolic wastes by bacteria has been studied extensively and a large number of phenol degrading bacteria has been isolated and characterized at the physiological and genetic levels (Heinaru *et al.,* 2000; Hinteregger *et al.,* 1992; Kivisaar *et al.,* 1989; Powlowski and Shingler, 1994; Qureshi *et al.,* 2001; Stephen *et al.,* 2005). But studies on the phenol degradation by halotolerant bacteria are too limited. The phenolic compounds also entered into the aquatic environment through the retting of coconut husk. Generally, the coconut retting water contains high amount of phenol with the additional toxic compounds such as, cyanide and thiocyanate. Fish and aquatic invertebrates are particularly sensitive to cyanide exposure. Concentrations of free cyanide and thiocyanate in the aquatic environment ranging from 5.0 to 7.2 $\mu g.l^{-1}$ which inhibit the reproduction in many species of fish and excess of 200 $\mu g.l^{-1}$ leads to adverse effects include delayed mortality, pathology and susceptibility to predation, disrupted respiration, osmoregulatory disturbances and altered growth patterns of the organisms. The sensitivity of aquatic organisms to cyanide and thiocyanate are highly species specific and is also affected by water pH, temperature and oxygen content (Remani *et al.,* 1989). To overcome the above problems, the present study was made an attempt to find out the potential halotolerant bacteria to be used for the biodegradation of phenol along with cyanide and thiocyanate in coconut retting water.

MATERIALS AND METHODS

Water samples were collected from coconut retting ponds in Rajakkamangalam coast (Lat. 8° 07'N; Long. 77° 23' E), Kanyakumari District, Tamil Nadu, India, by using sterile

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Table 1. Morphological Characteristics of the isolated halobacteria

Morphological Characteristics	*Pseudomonas putida*	*Bacillus sp.*	*Pyrococcus horikoshii*
Configuration	Round	Round	Round
Margin	Entire	Wavy	Wavy
Elevations	Convex	Convex	Flat
Surface	Smooth	Rough	Rough
Pigments	-	-	-
Gram-reaction	Negative	Positive	Positive
Shape	Rod	Rod	Cocci
Motility	+	+	+

(+) indicates positive; (-) indicates negative

Table 2. Biochemical Characteristics of the isolated halobacteria

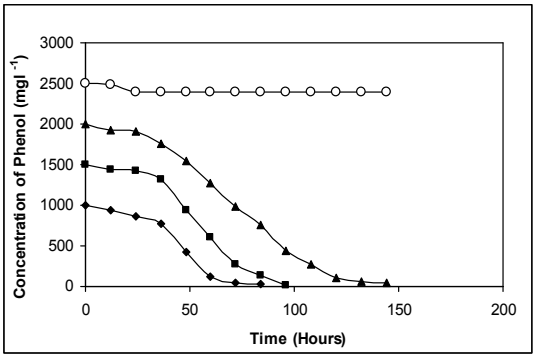
Biochemical Characteristics	*Pseudomonas putida*	*Bacillus sp.*	*Pyrococcus horikoshii*
Growth on MacConkey Agar	+	-	-
Indole Test	-	-	-
Methyl Red Test	-	-	-
Voges Proskauer Test	+	+	-
Citrate Utilization	+	+	+
Gas Production from Glucose	-	+	+
Casein Hydrolysis	-	+	-
Starch Hydrolysis	-	+	+
Urea Hydrolysis	-	-	-
Nitrate Reduction	+	+	-
Nitrite Reduction	-	-	-
H_2S Production	-	-	-
Cytochrome Oxidase	+	-	-
Catalase Test	+	-	+
Gelatin Hydrolysis	-	+	-
Oxidation/Fermentation	-	-	-
Arginine dihydrolase	+	+	-
Lysine decarboxylase	-	-	-
Ornithine decarboxylase	-	-	-
Acid Production			
Adonitol	+	-	+
Arabinose	+	+	-
Cellobiose	+	+	+
Dextrose	+	+	+
Dulcitol	+	-	+
Fructose	+	+	-
Galactose	+	-	+
Inositol	+	-	-
Lactose	+	+	+
Maltose	+	+	+
Mannitol	+	+	-
Melibiose	+	+	+
Raffinose	+	-	-
Rhamnose	+	+	+
Salicin	+	+	-
Sorbitol	+	-	-
Sucrose	+	+	+
Trehalose	+	-	+
Xylose	+	+	-

(+) indicates positive; (-) indicates negative

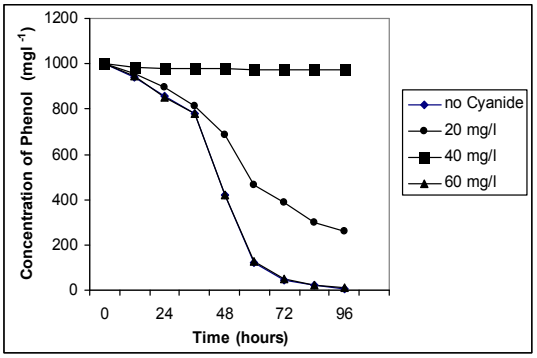
plastic container. Water samples were serially diluted and plated in sterilized nutrient agar media. Phenol (100 $mg.l^{-1}$) was added as a substrate for the isolation of phenol degrading bacteria. After 24 hrs of incubation at 34˚C, colonies appeared on the solid media were counted and the colonies were selected based on the morphological characters and further restreaked thrice on the respective medium for purification and species level identification was performed by following the method of Holt *et al.,* (1994). Biodegradation of phenol was carried out by using shake flask method. A loopful of 32 isolated bacterial strains were inoculated into 100 ml basal minimal medium [$(NH_4)_2SO_4$-325 mg; $MnSO_4$-8.5 mg; $MgSO_4.7H_2O$-65 mg; $FeCl_3.6H_2O$-2 mg; $CaCl_2.2H_2O$-9 mg; K_2HPO_4-2.62 g; and KH_2PO_4-1.43 g; dissolved in one litre of dis.H_2O] containing various concentration of phenol in 250 ml Erlenmeyer flasks. These flasks were incubated at room temperature with continuous shaking at 150 rpm in a thermostat incubator. The biodegradation of phenol was assessed by the following the method of Yang and Humphrey, (1975).

RESULTS AND DISCUSSION

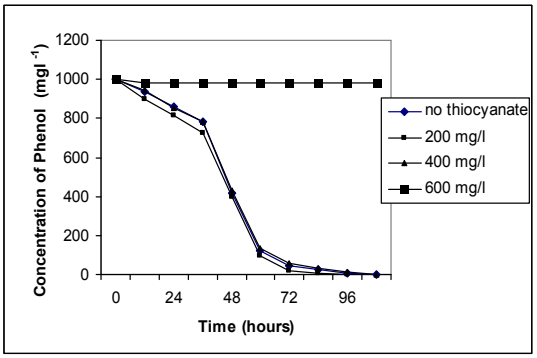
The present study reveals that, 32 morphologically different halophilic bacterial strains were isolated from the coconut retting water. Of which, 3 strains *viz., Pseudomonas putida,*



(a) Phenol alone

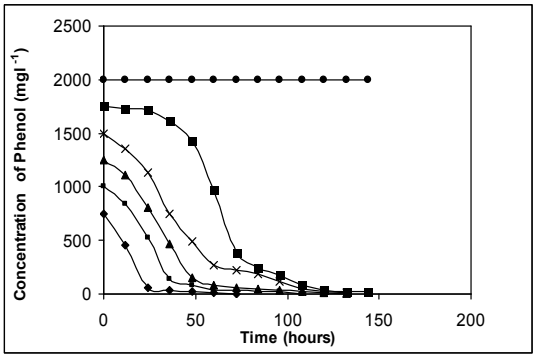


(b) Phenol with cyanide

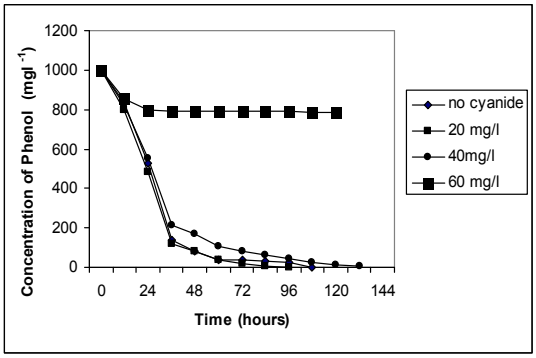


(c) Phenol with thiocyanate

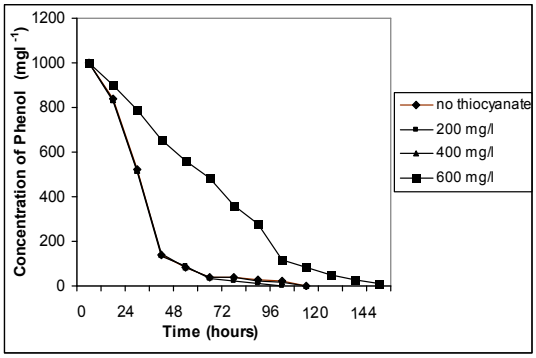
Fig. 1. Biodegradation of phenol by *Pseudomonas putida*



(a) Phenol alone



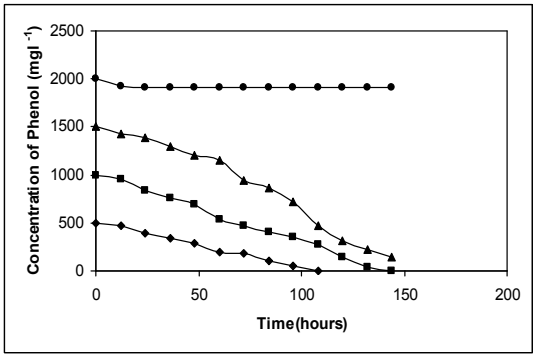
(b) Phenol with cyanide



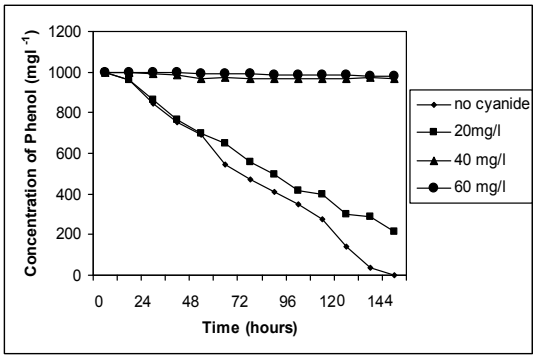
(c) Phenol with thiocyanate

Fig. 2. Biodegradation of phenol by *Bacillus* sp.

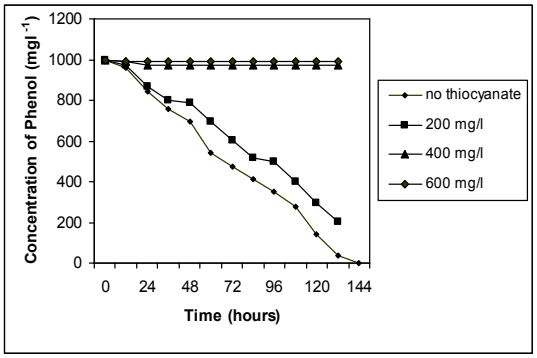
Bacillus sp. and *Pyrococcus horikoshii* which showed better degradation (Table 1 & 2). Among them, *Pseudomonas putida* showed the maximum degradation of phenol up to 2000 mg.l^{-1} within 144 hrs, followed by *Bacillus* sp. and *Pyrococcus horikoshii* [Fig. 1 (a), 2 (a) & 3(a)]. An interesting observation was noted that, the isolated strain *Pseudomonas putida* showed maximum phenol degradation in short time and it might be due to the production of different enzymes including oxygenases and hydroxylases. Generally, the purified enzymes oxidized the phenolic compounds very effectively (Indu Nair *et al.,* 2008). The resonance structure of the aromatic compounds is broken down by microbes is too critical. However, in the aerobic system, phenol is first converted into catechol through meta fission because most of the halogenated compounds undergoes through ortho pathway. Koutny *et al.,* (2003) isolated phenol degrading bacteria *P. putida* from Siberia soils. Similarly, Paraskevi and Euripides (2005) reported that, *Pseudomonas* sp. isolated from the petroleum contaminated soil capable of growing at the concentration of 1200 mg.l^{-1}. Moreover, *Pseudomonas aeruginosa* degrades the phenol up to 1300 mg.l^{-1} within 156 hrs (Kotresha and Vidyasagar, 2008). Chung *et al.* (2003) reported that, *Pseudomonas putida* showed maximum degradation of phenol up to 2000 mg.l^{-1} within 156 hrs. The phenol removal efficiency was investigated with the addition of different concentration of cyanide as a co-toxicant. It reveals that, *Pseudomonas putida* degrade the maximum amount of phenol up to 1000 mg.l^{-1} with the addition of 60 mg.l^{-1} cyanide at 96 hrs followed by *Bacillus* sp. and *Pyrococcus horikoshii* respectively [Fig. 1 (b), 2 (b) & 3 (b)]. Phenol removal was more effective at a low concentration of cyanide, but decreased with increase of cyanide concentration. The reason



(a) Phenol alone



(b) Phenol with cyanide



(c) Phenol with thiocyanate

Fig. 3. Biodegradation of phenol by *Pyrococcus horikoshii*

behind that, cyanide at high concentration inhibits one of the enzymes that participate in the phenol degradation metabolism and this leads to reducing the phenol degradation. Moreover, the cyanide inhibits phenol transportation into the cells, thus affecting the phenol degradation (Arutchelvan *et al.,* 2005; Fujita *et al.,* 1993). The mechanism of cyanide reduction could be associated with the N_2-fixing enzymes of *Pseudomonas putida.* Nevertheless, additional organisms with the capacity for cyanide biodegradation are still being reported (Adjei and Ohta, 1999; Kwon *et al.,* 2002a; Sexton and Howlett, 2000; Yanase *et al.,* 2000). Generally, four pathways for the biodegradation of cyanide and they are hydrolytic, oxidative, reductive and substitution/transfer. More than one pathway can be utilized for cyanide biodegradation in some organisms (Ezzi-Mufaddal and Lynch James, 2002; Raybuck, 1992). The pathway used is dictated by the external conditions such as oxygen, pH and cyanide concentration. The phenol degradation was also investigated with the addition of thiocyanate at various concentrations. The result reveals that, 3 bacterial species which showed excellent phenol degradation of which *Pseudomonas putida* degrade the phenol up to 1000 $mg.l^{-1}$ with the addition of 400 $mg.l^{-1}$ of thiocyanate at 96 hrs followed by *Bacillus* sp. (600 $mg.l^{-1}$) at 144 hrs and *Pyrococcus horikoshii* (200 $mg.l^{-1}$) at 144 hrs respectively [Fig. 1 (c), 2 (c) & 3 (c)]. The removal of thiocyanate might be due to the production of thiocyanate hydrolase enzyme (Kwon *et al.,* 2002b). Generally, thiocyanate is a biodegradable compound (Usha Rani, 2001). Thiocyanate can be produced from the reaction of cyanide with pyretic materials in waste streams and *in vivo* by the action of thiosulfate-cyanide sulfurtransferase enzymes (Du Plessis *et al.,* 2001; Kwon *et al.,* 2002b; Sorokin *et al.,* 2001; Yamasaki *et al.,* 2002). Biodegradation of thiocyanate can occur by at least two pathways. The formation of sulfate is consistent with the proposed cyanate pathway (Du Plessis *et al.,* 2001; Sorokin *et al.,* 2001). In a second pathway, thiocyanate is converted to ammonia and carbonyl sulfide. Kwon *et al.,* (2002b) reported that, the fungi *Acremonium strictum* produced ammonia and sulfate from thiocyanate without the production of cyanate. Moreover, the combination of thiocyanate and phenol is not usually degraded effectively by most of the organisms in the single stage process. But, the bacterial strains isolated from coconut retting water degraded phenol along with thiocyanate at certain level very effectively. Similarly, Stafford and Callely (1969) reported that, removal of phenol along with thiocyanate by heterotrophic bacterium isolated from activated sludge tank. *Klebsiella oxytoca* isolated from industrial waste waters has the potency to degrade thiocyanate at certain level (Kao *et al.,* 2003). Industrial activities are likely to increase thiocyanate levels in the environment; the biodegradation pathways for these compounds are highly warranted.

CONCLUSION

It is concluded from the present study that, the bacterial strains *viz., Pseudomonas putida*, *Bacillus* sp. and *Pyrococcus horikoshii* are recommended to be used for the pretreatment of coconut retting waters before the discharge in to coastal ecosystems so as to enable to reduce the contamination and to reduce the mass mortality of the fishes due to anoxic condition created by the retting effluent.

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