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Isolation and identification of novel high strength phenol degrading bacterial strains from phenol-formaldehyde resin manufacturing industrial wastewater

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Abstract

Phenols are toxic to all types of organisms. Two bacterial strains capable of utilizing phenol as a sole carbon source were isolated from the phenol bearing industrial wastewater. Based on the biochemical test results the organisms were identified as *Pseudomonas cepacia* and *Bacillus brevis*. The organisms were very efficient in phenol degradation, the lag phase increased with increase in phenol concentration. The well-acclimatized cultures of *P. cepacia* and *B. brevis* degraded 2500 and 1750 mg l^{-1} of phenol in 144 h, respectively. The organisms degrade phenol even in the presence of toxicants like thiocyanate, sulphide and cyanide. The organisms can be effectively used for treating high strength phenol containing thiocyanate, sulphide and cyanide. The *P. cepacia* degrades phenol with a faster rate than *B. brevis*. *P. cepacia* can be used effectively for treating high strength phenolic wastewater.

Keywords: Phenol; Biodegradation; *Pseudomonas cepacia*; *Bacillus brevis*

1. Introduction

Phenol is an aromatic molecule containing hydroxyl group attached to the benzenoid ring structure. The origin of phenols in the environment is both anthropogenic as well as xenobiotic [1–3]. Phenol is a major environmental pollutant and phenol concentrations of upto 10,000 mg l^{-1} have been reported in many industrial wastes, being produced in several industries and operations such as petroleum refineries, gas and coke oven industries, pharmaceuticals, explosive manufacture, phenol-formaldehyde resin manufacture, plastic and varnish industries and related metallurgical operations, etc. [4–7]. Phenols are toxic to several biochemical functions [8] and to fish life [9]. It acts as a substrate inhibitor in the biotransformation [10]. WHO has prescribed a concentration of 1 μg as the guideline concentration for drinking water. Elimination of phenol, thus, is a necessity to preserve the environmental quality.

Phenolic wastes are treated by several physico-chemical methods like ozonisation, adsorption, reverse osmosis, electrolytic oxidation, H_2O_2, photo catalysis, etc. [11]. Phenol removal by biological methods is generally preferred to physico-chemical methods because of lower costs and the possibility of complete mineralization [12]. Bacteria, yeast and fungi are capable of utilizing phenolic compounds. Several bacterial strains belonging to the species of *Pseudomonas*, *Bacilli*, *Klebsiella*, *Ochrobactrum*, *Rhodococcus*, etc., were reported for phenol degradation [13–15]. Till date several works are in progress to isolate new and efficient microbial species to degrade high strength phenols. We report here the isolation and identification of novel high strength phenol degrading bacteria from

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the phenol-formaldehyde resin manufacturing industrial wastewater.

2. Materials and methods

2.1. Chemicals

All fine chemicals used were of Analar grade from Merck. The chemicals used for media preparations were of Bacteriological grade from Himedia.

2.2. Collection of samples

The phenolic waste samples both aqueous effluent and sludge were collected as per the standard procedure [16] from a phenol-formaldehyde resin manufacturing industry, Chennai, India and stored in plastic containers at 4 °C. The industry is discharging phenol concentration of about 4000 mg l^{-1} in the effluent [17].

2.3. Isolation of bacteria

The steam sterilized and cooled nutrient broth containing (per liter): glucose 5.0 g, peptone 5.0 g, NaCl 5.0 g, beef extract 3.0 g with 100 mg l^{-1} phenol and pH 8.01 was used for the enrichment studies. The effluent and sludge rich in phenol was used as a source of phenol-degrading bacteria. About 10 mL of aliquot from the enrichment flask was plated after suitable serial dilutions for obtaining individual colonies of different bacteria. The plates were incubated at 30 ± 0.1 °C and colonies developed were carefully observed for their uniformity and differences. The morphologically distinct colonies were selected and transferred to new plates. These isolates were screened by sub-culturing in a basal minimal medium containing (per liter): ammonium chloride 10 g, ammonium nitrate 4.0 g, K_2HPO_4 0.2 g, KH_2PO_4 0.8 g, $MgSO_4$ 0.2 g; phenol as the sole carbon and energy source. The pH was adjusted to 8.01 using buffers. The most efficient isolates were selected.

2.4. Identification of bacteria

The morphological, physiological, biochemical and carbon source utilization tests of the isolates are listed in Table 1 and identified as per Bergey's Manual of systematic bacteriology [18]. The organisms are further confirmed from MTCC & GB, IMTECH, Chandigarh, India.

2.5. Phenol degradation

In order to understand the growth pattern of the isolates, a growth study was performed in minimal medium with different initial concentrations of phenol in a shaker at 34 °C. The samples were analyzed regularly for phenol degradation and corresponding cell growth.

2.6. Analytical procedures

Growth of the organisms was recorded by monitoring the optical density (OD) of the culture in a Hitachi-U-2001 UV–vis Spectrophotometer at 600 nm. Phenol was estimated spectrometrically using 4-aminoantipyrene as per standard procedure [16].

3. Results and discussion

The effluent and sludge samples were collected from different sampling points in the phenol-formaldehyde resin manufacturing industry. The samples were inoculated in the medium containing phenol, initially about 55 isolates were grown out of which 16 were distinct and 2 out of 16 exhibit more growth in phenol.

The morphological, physiological, biochemical and carbon source utilization characteristics of the two isolates were given in Table 1. Based on the detailed biochemical test results, the first organism is identified as a Gram-negative bacterium having an effective growth between 25 and 42 °C, pH tolerance to a wide range of 5.0–11.0 and salt tolerance upto 5% on NaCl. The metabolic activity of this organism was vigorous both in aerobic and anaerobic conditions, showing a positive growth on MacConkey agar. The organism reduces nitrate and produces acid from the tested carbohydrates viz. Adonitol, Arabinose, Sorbitol, Raffinose, etc. Based on these characteristics the organism is identified as *Pseudomonas cepacia* as per Bergey's manual. Whereas, the second organism isolated from the same environment is a Gram-positive having an effective growth at the same ranges of temperature and pH, but the organism is having a less tolerance towards NaCl (2.5%). No metabolic activity was observed in the absence of oxygen indicating the strict aerobic nature. This organism is also nitrate-reducer. Compared to the first organism the second organism has a very poor response in acid formation from various carbohydrates and it also produces acid only from manosaccharides. The second organism has been identified as *Bacillus brevis*. Both organisms are non-pathogenic, fast multiplier and can grow aerobically.

The results of batch studies for phenol degradation in minimal media by *P. cepacia* and *B. brevis* are given in Figs. 1 and 2. The *P. cepacia* degrades about 97.7, 99.3, 98.7 and 98.6% of phenol with an initial concentration of phenol 1000, 1500, 2000 and 2500 mg l^{-1} in 84, 96, 108 and 144 h, respectively. The *B. brevis* degrades about 99.8, 99.7, 99.2 and 99.1% of phenol with an initial concentration of phenol 750, 1000, 1250, 1500 and 1750 mg l^{-1} in 72, 108, 132, 132 and 144 h, respectively.

The growth of both organisms increases with increase in phenol concentration is given in Figs. 3 and 4. But the lag phase was extended at higher concentrations of phenol. A

Table 1
Characteristics of the organisms

Morphological characteristics	I	II	Physiological characteristics	I	II	Biochemical characteristics	I	II	Acid production from carbohydrates characteristics	I	II
Configuration	Round	Round	Growth at temperature (°C)			Growth on MacConkey agar	+	–	Adonitol	+	–
Margin	Entire	Wavy	4	–	–	Indole test	–	–	Arabinose	+	–
Elevations	Convex	Convex	10	–	–	Methyl red test	–	–	Cellobiose	+	–
Surface	Smooth	Rough	15	–	–	Voges proskauer test	+	–	Dextrose	+	+
Density	Translucent	Translucent	25	+	+	Citrate utilization	+	–	Dulcitol	+	–
Pigments	–	–	30	+	+	Gas production from glucose	–	–	Fructose	+	+
Gram-reaction	Negative	Positive	37	+	+	Casein hydrolysis	±	–	Galactose	+	–
Shape	Rods	Rods	42	+	+	Starch hydrolysis	–	–	Inositol	+	–
Size	Short	Moderate	55	–	–	Urea hydrolysis	–	–	Lactose	+	–
Arrangement	Single	Single	65	–	–	Nitrate reduction	+	+	Maltose	+	–
Endospore	–	+	Growth at pH			Nitrite reduction	–	–	Mannitol	+	–
Position		Terminal	5.0	+	+	H_2S production	–	–	Melibiose	+	–
Shape		Oval	5.7	+	+	Cytochrome oxidase	+	–	Raffinose	+	–
Sporangia bulging		+	6.8	+	+	Catalase test	+	+	Rhamnose	+	–
Motility	+	+	8.0	+	+	Gelatin hydrolysis	±	–	Salicin	+	+
Fluorescence (UV)	–	–	9.0	+	+	Oxidation/fermentation	O	–	Sorbitol	+	–
			11.0	+	±	Arginine dihydrolase	+	+	Sucrose	+	–
			Growth on NaCl (%)			Lysine decarboxylase	–	–	Trehalose	+	–
			2.5	+	+	Ornithine decarboxylase	–	–	Xylose	+	–
			5.0	+	–						
			7.0	–	–						
			8.5	–	–						
			10.0	–	–						
			Growth under anaerobic condition	±	–						

I, *Pseudomonas cepacia*; II, *Bacillus brevis*.

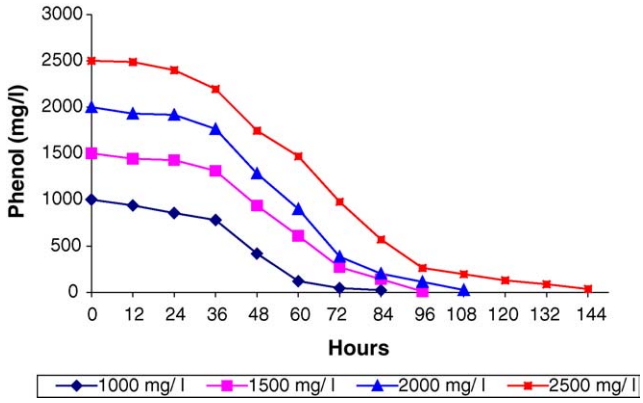

Fig. 1. Phenol degradation by *P. cepacia* in batch reactor [temperature = 34 ± 0.1 °C, pH 8.01].

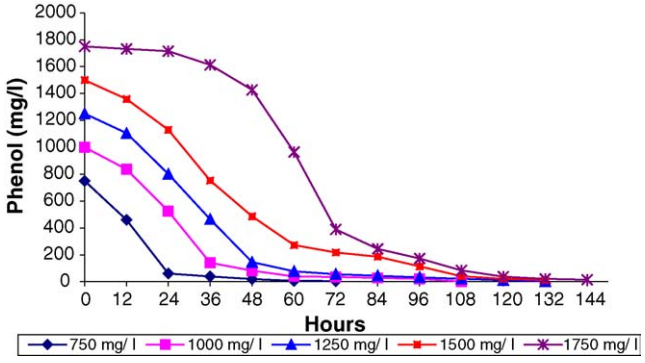

Fig. 2. Phenol degradation by *B. brevis* in batch reactor [temperature = 34 ± 0.1 °C, pH 8.01].

positive correlation between cell biomass and phenol degradation was observed. In the batch studies, the biomass concentration of *P. cepacia* was initially low, after that the growth increases exponentially. Whereas, the biomass concentration of *B. brevis* was initially low at 1750 than 1500 mg l^{-1} and the concentration becomes almost similar after the fourth day. During initial stages the biodegradation of *P. cepacia* for the corresponding biomass was less, subsequently in later stages

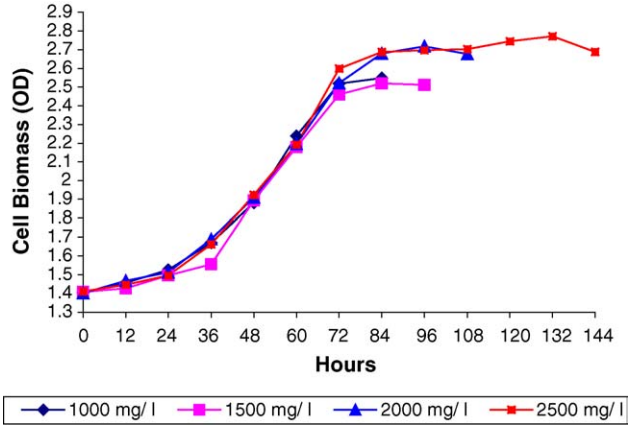

Fig. 3. Growth of *P. cepacia* in phenol [temperature = 34 ± 0.1 °C, pH 8.01].

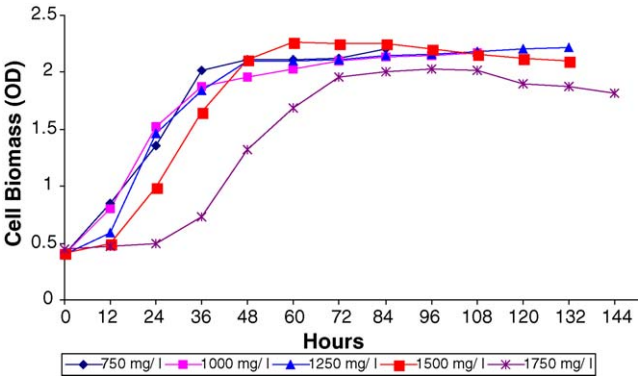

Fig. 4. Growth of *B. brevis* in phenol [temperature = 34 ± 0.1 °C, pH 8.01].

the biodegradation was much pronounced with less increase in biomass and this result was in conformity with the low conversion efficiency to biomass with high removal of phenol [19]. Out of the two isolates the order of phenol degradation was *P. cepacia* followed by *B. brevis*. The previous reports mainly on biodegradation of phenol in batch process were in the range of 0–1000 mg l^{-1} in maximum of 6.75 days [4]. In continuous process, the degradation of phenol was in the range of 1.8 kg phenol m^{-3} d^{-1} [12].

The phenol degrading capacity of the organisms were also tested in presence of thiocyanate, sulphide and cyanide, since these toxicants were present along with phenol in most of the industrial wastewaters. The effect of thiocyanate, sulphide and cyanide on phenol degradation was studied using *P. cepacia* and *B. brevis* with initial phenol concentration of 1500 mg l^{-1} in the batch process. The results of the degradation of phenol in presence of the toxicants are given in Figs. 5–10. From the observations it is clear that the organisms utilized phenol even in the presence of these toxicants.

Since, thiocyanate is a biodegradable compound [20] it was tried for three different higher concentrations of 500, 1000 and 1500 mg l^{-1}. Fig. 5 shows thiocyanate upto a concentration of 1000 mg l^{-1} not inhibited the phenol degradation by *P. cepacia*, whereas, for higher concentration of 1500 mg l^{-1} of thiocyanate the rate of degradation is reduced considerably. The *P. cepacia* degrades 1500 mg l^{-1} of phe-

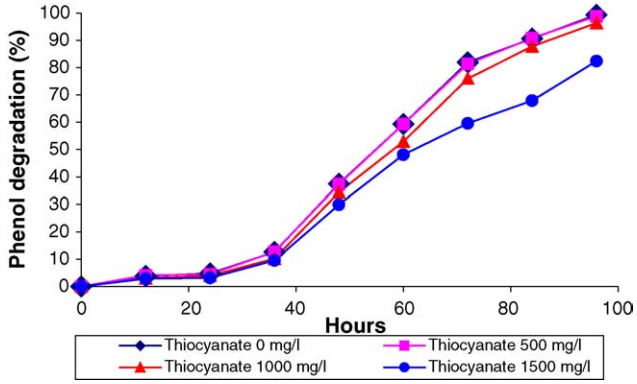

Fig. 5. Effect of thiocyanate on phenol degradation by *P. cepacia*.

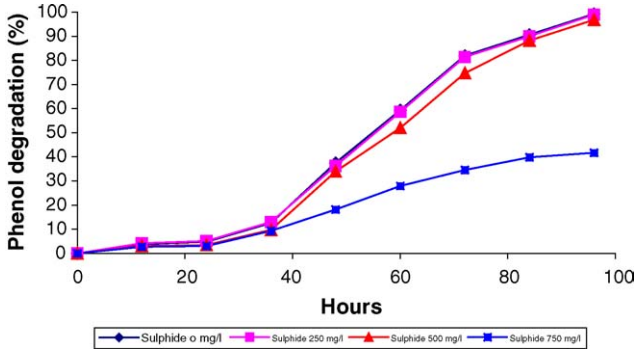

Fig. 6. Effect of sulphide on phenol degradation by *P. cepacia*.

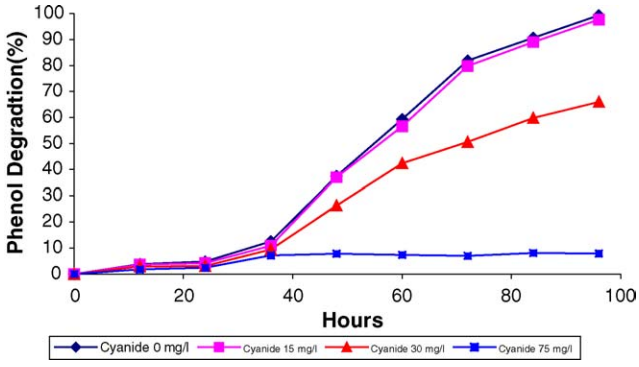

Fig. 7. Effect of cyanide on phenol degradation by *P. cepacia*.

nol by 98.9, 96.4 and 82.4% with initial concentration of 500, 1000 and 1500 mg l^{-1} of thiocyanate in 96 h, respectively.

Phenol degradation by *B. brevis* was not inhibited by thiocyanate upto 1000 mg l^{-1}, but at higher concentration of 1500 mg l^{-1} of thiocyanate the phenol removal efficiency was reduced even lesser than the strain *P. cepacia* shown in Fig. 8. *B. brevis* degrades 1500 mg l^{-1} of phenol by 98.5, 94.8 and 64.8% with initial concentration of 500, 1000 and 1500 mg l^{-1} of thiocyanate in 132 h, respectively. Whereas, when compare to the *P. cepacia*, strain *B. brevis* takes more time for degradation of 1500 mg l^{-1} of phenol.

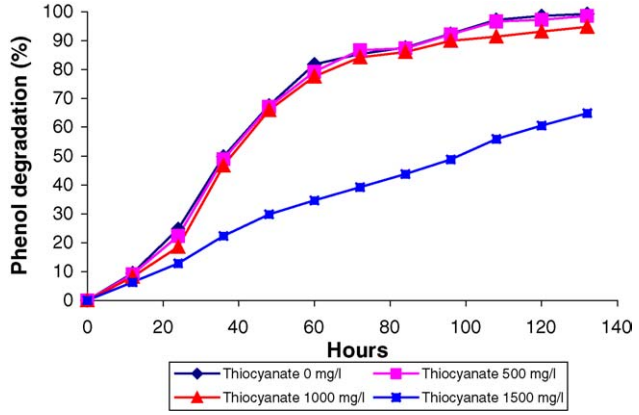

Fig. 8. Effect of thiocyanate on phenol degradation by *B. brevis*.

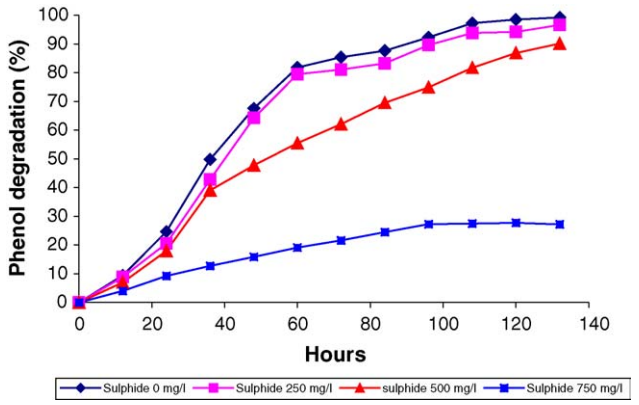

Fig. 9. Effect of sulphide on phenol degradation by *B. brevis*.

Biodegradation of phenol by the *P. cepacia* was tried in presence of sulphide of 250, 500 and 750 mg l^{-1} with initial concentration of 1500 mg l^{-1} of phenol. Fig. 6 shows the sulphide at lower concentration of 250 and 500 mg l^{-1} did not inhibit much the phenol degradation and achieved 98.9 and 96.8% degradation in 96 h, respectively. But the same sulphide at a higher concentration of 750 mg l^{-1} inhibits the phenol degradation by 41.7% in the same duration.

Fig. 9 shows that low concentration of sulphide did not inhibit the phenol degradation by *B. brevis* with same initial concentration. But at higher concentration of 750 mg l^{-1} of sulphide phenol degradation is inhibited. The phenol removal was in the order of 96.6, 90.1 and 27.2% with 250, 500 and 750 mg l^{-1} of sulphide in 132 h, respectively.

P. cepacia degraded 1500 mg l^{-1} of phenol in the presence of 15 mg l^{-1} cyanide, whereas, the degradation rate was reduced for cyanide concentration of 30 mg l^{-1} and there was a no growth or degradation of phenol at 75 mg l^{-1} of cyanide. The phenol degradation was 97.6, 66.0 and 7.9% in the presence of cyanide of 15, 30 and 75 mg l^{-1} in 96 h, respectively, as shown in Fig. 7.

B. brevis also degrade phenol in the presence of cyanide with initial concentration of 15, 30 and 75 mg l^{-1} and

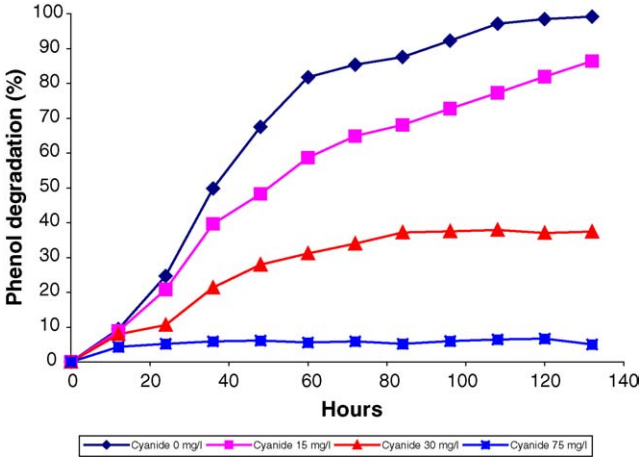

Fig. 10. Effect of cyanide on phenol degradation by *B. brevis*.

observed that in 15 mg l^{-1} of cyanide the organism achieved about 86.4% of phenol removal. Whereas, at higher concentrations of cyanide the degradation efficiency of the organism was totally reduced to 37.4 and 6.7% for 30 and 75 mg l^{-1} in the same duration of 132 h, respectively, is given in Fig. 10. In both organisms growth and degradation was not observed in presence of 75 mg l^{-1} cyanide.

4. Conclusion

Two bacterial strains capable of degrading phenol were isolated from phenol-formaldehyde resin manufacturing industry in Chennai, India. The organisms were identified as *P. cepacia* and *B. brevis*. The strain *P. cepacia* could be used as a more potential candidate for activated sludge process of phenolic waste because of its high degrading efficiency with less biomass. This experimental results shows that among the isolated organisms *P. cepacia* can be very effectively used than *B. brevis* for treating the phenolic wastewater containing cyanide, sulphide and thiocyanate viz. petroleum refinery and carbonization industrial wastewaters. Comparatively the *P. cepacia* degrades high strength phenol completely in the presence of toxicants within 96 h, whereas the *B. brevis* degrades high strength phenol only at lower concentrations of toxicants except thiocyanate, but takes a long duration of 132 h. Compare to the other organisms as well as the same genus reported by other researchers, the isolated strain *P. cepacia* was capable of degrading 1500 mg l^{-1} of phenol without the inhibition of thiocyanate at a concentration of 1500 mg l^{-1}. Sulphide inhibits the organism only at a concentration of 750 mg l^{-1}, whereas the cyanide exerted higher inhibitory effect on phenol degradation above 30 mg l^{-1}.

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