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Research Article

Ex situ Biodegradation of Phenol by Native Bacterial Flora Isolated from Industrial Effluent

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ABSTRACT

Phenol, a synthetically and naturally produced aromatic compound is one of the most widely used chemicals in the industrial processes. High solubility in water and the higher content in sewage make phenol a potential water pollutant. Due to its toxicity, phenol at higher levels reduces or even inhibits microbial population in effluents. A total of 28 bacterial forms were isolated from three industrial effluent samples of which, 3 isolates (A9, B5 and C3) showed considerable amount of growth and degradation potential at 200 mg/l concentration of phenol. Based on the 16S rDNA sequencing, A9, B5 and C3 were identified as Pseudomonas aeruginosa MTCC 1034, Pseudomonas fluorescens MTCC 2421 and Bacillus cereus ATCC 9634. The effects of parameters such as auxiliary carbon sources, nitrogen sources, pH and temperature on the growth and aerobic degradation potential of the isolates were evaluated. For all the isolates, the optimum conditions for growth and phenol degradation were 30°C, pH 7.0, and at glucose and peptone concentration of 0.25 g/l. Within 48 hrs, P. aeruginosa MTCC 1034, P. fluorescens MTCC 2421 and B. cereus ATCC 9634 degraded 76%, 83% and 74% of phenol, respectively. Thus, based on their potential to aerobically degrade phenol at a higher concentration these native microorganisms can be cost effectively used in the bioremediation of the polluted sites.

Keywords: Phenol, effluent, toxicity, degradation, sequencing.

INTRODUCTION

Wastewaters from industrial and municipal sources are characterized by presence of mixtures of chemicals. Co-contamination of natural environments with mixtures of organic and inorganic pollutants is an important problem. Phenol is the major group of organic compound characterized by a benzene ring with one or more hydroxyl groups. Phenol and chlorinated phenol like pentachlorophenol are some of the major industrial pollutants from the industries such as pesticides, paper and pulp, textile, timber, plastic,

dyes, pharmaceuticals, tanneries, paints, gas and coke, synthetic rubber, fertilizers, steel, oil refineries, etc[1].

The concentration of phenol in industrial effluents varies. Its concentration can be as high as 10,000 ppm in coal gasification effluent. The permissible limit of phenol in the discharged effluent is one milligram/liter[2].

Phenols are easily soluble in water and, hence, cause the carbolic odor in water. They cause toxic effects on aquatic flora and fauna and ultimately affect the ecological balance[1]. One of the major phenolic derivatives, o-phenyl phenol, which is a component of the disinfectant, binds to DNA of microorganisms. Similar to ethidium bromide, it intercalates between bases of nucleic acids, leading to lethal mutations[3]. Phenols inhibit respiration and bring about precipitation of proteins and are responsible for increased cases of testicular cancer, breast tumors and decreased sperm counts[4].

Phenol removal in water treatment and effluent treatment include super chlorination, ozonization and activated carbon adsorption[5]. But these methods are expensive as well as environmentally prohibitive. However, recently bioremediation is gaining more popularity in treating the industrial effluents over the physical and chemical methods because of its cost effectiveness and ecofriendly nature.

Microbes play an important role in degrading synthetic chemicals in soil[6]. They have the capacity to utilize virtually all naturally and synthetically occurring compounds as their sole carbon and energy source. Phenol biodegradation studies with the bacterial species have resulted in bringing out the possible mechanism and also the enzyme involved in the process. However, these microorganisms suffer from substrate inhibition at higher concentration of phenol, by which the growth is inhibited[7]. The efficiency of the phenol degradation under such a condition could be further enhanced by the process of media optimization and cell immobilization[8,9].

Biodegradation of phenol using different microorganisms is well documented. The potential of *Pseudomonas cepacia* PAA in bioremediation of aquatic wastes containing phenol, has been reported[1]. Microbial degradation of pentachlorophenol by the axenic bacterial cultures like *Flavobacterium sp., Rhodococcus sp., Arthrobacter sp., Pseudomonas sp.* and *Mycobacterium sp* had been reported[10]. Recently, white rot fungi like *Phanerochaete chrysosporium, Trametes sp., Pleurotus flabellatus* and *Agrocybe aegerita* have been well investigated for bioremediation of contaminated soil due to the activity of various lignolytic enzymes like lignin peroxidases and manganese peroxidases, which have major role in degradation of phenol and chlorinated phenols[4].

The present study aims at isolation of bacterial forms from different industrial effluents, screening for their phenol degrading potential and studying the effects of several parameters involved in the degradation.

EXPERIMENTAL

Collection of samples: Effluent samples were aseptically collected from three different locations of Jigani Industrial Area, Bangalore using pre-sterilized brown glass bottles (Schott Duran, Germany). The water samples were transported to the laboratory within one hour from the time of sample collection and stored under refrigeration condition until usage. Prior to use, the water samples were brought to room temperature, mixed well and processed on the same day.

Isolation of phenol degrading bacteria from the water samples: 1 ml of the water from each sample was serially diluted up to a dilution of 10^{-6}. Following serial dilution, 0.1 ml of each water sample was spread plated on mineral salt agar fortified with phenol at a concentration of 50 mg/ l. The compositions of mineral salt medium (MSM) in g /l were KH_2PO_4 0.42, K_2HPO_4 0.375, $(NH_4)_2SO_4$ 0.244, NaCl 0.015, $CaCl_2$. $2H_2O$ 0.015, $MgSO_4.7H_2O$ 0.05, and $FeCl_3.6H_2O$ 0.054, agar 15; pH 7 ± 0.1.The plates were incubated at 37 ºC for 24 hrs in a bacteriological incubator for the appearance of the bacterial colonies. The colonies obtained were inoculated in slants containing MSM fortified with 50 mg/l of phenol. All the slants were stored at 4ºC until used.

Utilization of phenol at higher concentration: In the next step, in order to test the ability of the isolates to utilize and degrade phenol at elevated concentration, 1 ml of the saline suspension of the isolates which showed growth on the Mineral Salt agar with a phenol concentration of 50 mg/l were then inoculated in the broth form of the Mineral Salt broth, similarly fortified with a phenol concentration of 200 mg/l. The ability of the isolates to degrade phenol was measured spectrophotometrically by following the 4-amino antipyrene method. Each of the tests in the determination of phenol degradation was conducted in triplicates.

The 4-amino antipyrene method: When phenol reacts with 4-amino antipyrene at pH 7.9±0.1 in the presence of potassium ferricyanide it forms a colored antipyrene dye. This dye is kept in aqueous solution and the absorbance is measured at 500 nm.

For degradation studies, 3 ml of the microbial growth were removed from each flask after every 24 hrs and the samples were clarified by centrifugation at 800 rpm for 5 minutes and supernatants were subjected to 4-amino antipyrene method for monitoring the phenol and toluene degradation.

70 µl of 0.5 N NH_4OH solution was added into each cell free supernatant (CFS) and pH was adjusted to 7.9±0.1 with phosphate buffer. 30 µl of 4 amino antipyrene solution was added and mixed well and 30 µl of $K_3Fe(CN)_6$ solution was added. After 15 minutes incubation in dark, the aliquots were transferred to quartz cuvettes and absorbance of sample was monitored against the blank at 500 nm. The controls were maintained by subjecting the uninoculated media with different concentrations of phenol to 4-amino antipyrene method and the optical density values were recorded. The efficiency of the isolates to degrade phenol was expressed as:

% Efficiency of degradation = [(Initial conc. of phenol – residual conc. of phenol)/ Initial conc. of phenol] x100

Characterization of the isolates: The screened bacterial isolates were identified based on their morphological and biochemical characteristics. The morphological characteristics were identified by culturing the isolates on nutrient agar plates and studying the shape, size, color, opacity, texture, elevation, spreading nature and margin of the colonies, followed by gram's staining and motility test. The biochemical characterization of the isolates was performed by indole, methyl red test, Vogesproskauer test, oxidase test, gelatin liquefaction test, lecithinase production test, starch hydrolysis test, nitrate test, testing growth at 4°C and 41°C and carbohydrate and amino acid utilization test. Bergey's Manual of Determinative Bacteriology (7^{th} Edition) was used as a reference to identify the isolates.

Selection of the carbon supplement: The MSM broth containing 200 mg/l concentrations of phenol was supplemented with different carbon sources like soluble starch, sucrose, lactose, maltose, fructose and glucose at a concentration of 0.1% (w/w). All the media were inoculated with 1 ml of the individual isolates and incubated for 72 hrs under dark condition. Following incubation, 4-amino antipyrene method of determining residual phenol was performed after every 24 hrs and the data obtained from the study were represented graphically.

Selection of the nitrogen supplement: The MSM broth containing 200 mg/l concentrations of phenol was supplemented with the 0.1 % of the selected carbon source and tested for the effect different nitrogen sources like peptone, tryptone, urea, casein, ammonium chloride, ammonium sulphate and sodium nitrate at a concentration of 0.1% (w/w). All the media were inoculated with 1 ml of the individual isolates and incubated for 72 hrs under dark condition. Following incubation, 4-amino antipyrene method of determining residual phenol was performed after every 24 hrs and the data obtained from the study were represented graphically.

Selection of the optimum pH: To optimize the growth condition for the best degradation of phenol by the bacterial isolates, pH is one of the important parameter that has to be optimized. So the MSM medium substituted respectively with the best carbon and nitrogen source was prepared in three Erlenmeyer flasks (each containing 100 ml of the media) and the pH of those semi synthetic medium were set from 5 to 9

with the increment of one unit and autoclaved. After autoclaving the media were cooled and 1 ml of the inoculum was added in a sterile condition. Following inoculation, the flasks were incubated under dark condition for 72 hrs. Decolourization was measured by 4-amino antipyrene method, every 24 hrs till 72 hrs using UV spectrophotometer at 500 nm.

Selection of the optimum temperature: Temperature plays an important role in the growth of the organisms, so as to optimize the temperature for the best degradation activity by the bacterial isolates; it has been checked with different temperatures such as 20ºC, 25ºC, 30ºC, 35ºC, 40ºC and 45ºC. The Mineral Salt broth medium substituted respectively with the best carbon and nitrogen source was prepared in three Erlenmeyer flasks (each containing 100 ml of the media) and the optimized pH was set to the medium and autoclaved. After autoclaving the media were cooled and 1 ml of the inoculum was added in a sterile condition. Following inoculation, the flasks were incubated under dark condition at 20ºC, 25ºC, 30ºC, 35ºC, 40ºC and 45ºC for 72 hrs. Decolourization was measured by 4-amino antipyrene method, every 24 hrs till 72 hrs using UV spectrophotometer at 500 nm.

Molecular characterization of the isolates: The selected isolates were grown in LB broth and incubated at 37°C for 24 hrs at 200 rpm. Extraction of the genomic DNA was done using Bacterial Genomic DNA Isolation Kit RKT09 (Chromous Biotech Pvt. Ltd., Bangalore, India) according to the manufacturer instructions and visualized using 0.8% (w/v) agarose gel electrophoresis. The 16S rRNA gene sequences were amplified using the forward primer (5´-AGAGTTTGATCCTGGCT CA -3') and reverse primer (5´-ACGGCTACCTTGTTACGACT-`3). The parameters for PCR-based amplification were as follows: an initial denaturing cycle at 95°C for 4 minutes, followed by 25 cycles of denaturation at 95°C for 1 minute, annealing at 60°C for 1 minute and extension at 72°C for 3 minutes. A final extension cycle was carried out at 72°C for 7 minutes. The size of the PCR-amplified product was verified by 1% (w/v) agarose gel electrophoresis.

The genomic DNA of each selected isolate was sequenced at Chromous Biotech Pvt. Ltd., Bangalore, India. The sequence obtained (about 1500 bp) was manually aligned using the Basic Local Alignment Search Tool (BLASTN) and compared with small-subunit (SSU) sequences deposited with NCBI GenBank.

RESULTS AND DISCUSSION

The massive mobilization of organic pollutants in natural resources or the introduction of xenobiotics into the biosphere leads to the persistence of a number of chemicals in the biosphere and thus constitutes a source of contamination. Often these organic pollutants comprise a potential group of chemicals which can be dreadfully hazardous to human health and are resistant to degradation. As they persist in the environment, they are capable of long range transportation, bioaccumulation in human and animal tissue and biomagnification in food chain.

Biodegradation is used to describe the complete mineralization of the starting compound to simpler ones like CO_2, H_2O, NO_3 and other inorganic compounds[11]. Microorganisms play an important role in the field of environmental science by degrading and transforming toxic compounds into non-toxic or less toxic forms. Phenol and its derivatives are major xenobiotics, which are derived from the industries such as paper and pulp, textiles, gas and coke, fertilizers, pesticides, steel and oil refineries etc.[1]. They cause many of the ill effects on human beings including cancer, besides causing a natural imbalance. Phenol and pentachlorphenol cause carboxylic odour in water. Microbes found in natural water and soil have broad ability to utilize virtually all naturally and some synthetically occurring compounds as their sole carbon and energy sources, thus, recycling the fixed organic carbon back into harmless biomass and carbon dioxide and resulting in clean up of environment. There are reports on many microorganisms capable of degrading phenol through the action of variety of enzymes. These enzymes may include oxygenases hydroxylases, peroxidases, tyrosinases and oxidase.

Aerobically, phenol is first converted to catechol, and subsequently, the catechol is degraded via ortho or meta fission to intermediates of central metabolism. The initial ring fission is catalyzed by an ortho cleaving enzyme, catechol 1, 2 dioxygenase or by a meta cleaving enzyme catechol 2, 3 dioxygenase, where the product of ring fission is a cis-muconic acid for the former and 2-hydro cis muconic semi aldehyde for the latter[12].

Isolation of phenol utilizing bacteria: When cultured on the MSM agar plates (supplemented with 50 mg/l of phenol), a total of 28 bacterial isolates were obtained from the three effluent samples, of which 11 (A1, A2, A3, A4, A5, A6, A7, A8, A9, A10 and A11) were from sample A, 7 from sample B (B1, B2, B3, B4, B5, B6 and B7) and 10 from sample C (C1, C2, C3, C4, C5, C6, C7, C8, C9 and C10) respectively. Out of the 28 isolates, 3 isolates (A9, B5 and C3) showed considerable amount of degradation potential on 200 mg/l concentration of phenol.

Morphological characterization of the isolates: In the present study, based on the selective enrichment methods using phenol as sole carbon source, A9 and B5 grew as translucent, non spreading, bluish green pigment diffused colonies on nutrient agar plates and were found to be gram negative rods in scattered form while C3 appeared as opaque, flat, dirty white colonies and was found to be gram positive rods in short curved chains **(Table 1)**. All the three isolates were motile.

Table 1: Morphological characterization of the selected isolates

Characteristics	**Bacterial isolates**		
	A9	B5	C3
Shape	Circular	Circular	Irregular
Color	Green	Green	Dirty white
Opacity	Translucent	Translucent	Opaque
Texture	Mucoid	Mucoid	Non mucoid
Spreading nature	Non spreading	Non spreading	Spreading
Elevation	Flat	Flat	Flat
Margin	Smooth	Smooth	Irregular
Gram Staining	Negative rods, scattered	Negative rods, scattered	Positive rods ,short chains
Motility Test	Motile	Motile	Motile

Biochemical characterization of the isolates: Isolates A9 and B5 tested positive for indole production and methyl red test while C3 showed negative result. C3 exhibited positive result for vogesproskauer test and starch hydrolysis. All the three isolates were positive for gelatin hydrolysis, lecithinase production, nitrate reduction and oxidase reaction. Only B5 produced a diffusible, fluorescent pigment. PHB accumulation was accounted in C3 alone. Among the isolates, A9 and C3 grew well at 41°C while only B5 demonstrated growth on nutrient agar plates at 4°C. In terms of the utilization of sugars, ribose and mannitol remained unutilized. Xylose was utilized only by B5 and maltose was fermented by C3. Among the amino acids, none of the isolates could utilize valine, but alanine was utilized by A9 and arginine by B5 **(Table 2)**.

Several studies on biological degradation of phenol have been conducted using various pure and mixed cultures of *Pseudomonas sp.* [13], in which, phenol is degraded via the meta-pathway[14]. However it has been found that these bacteria suffer from substrate inhibition, whereby growth and consequently phenol degradation is inhibited at high phenol concentrations[13]. Various methods have been proposed to overcome substrate inhibition in order to treat high strength phenolic wastewater. These include adapting the cells to higher phenol concentration[15], immobilization of the cells[16] and using genetically engineered microorganisms[17]. Another possible method increasing the tolerance of the cells to substrate inhibition is to supplement the growth medium with conventional carbon sources, such as yeast extract or glucose. It

has also been noted that the presence of yeast extract enhanced the affinity of *Pseudomonas putida* for phenol[18].

Table 2: Biochemical characterization of isolates A9, B5 and C3

Biochemical Tests	A9	B5	C3
Fluorescent, diffusible pigment	-	+	-
Diffusible, non- fluorescent pigment	+	-	-
PHB accumulation	-	-	+
Growth at 4°C	-	+	-
Growth at 41°C	+	-	+
Gelatin Liquefaction	+	+	+
Lecithinase (egg yolk reaction)	+	+	+
Nitrate as nitrogen source	+	+	+
Denitification	+	-	-
Indole	+	+	-
Methyl red test	+	+	-
Vogesproskauer test	-	-	+
Oxidase reaction	+	+	+
Starch Hydrolysis	-	-	+
Utilization of D- Xylose	-	+	-
Utilization of Maltose	-	-	+
Utilization of D-Ribose	-	-	-
Utilization of Mannitol	-	-	-
Utilization of L- Valine	-	-	-
Utilization of B- Alanine	+	-	-
Utilization of L- Arginine	-	+	-
Identified isolate	*P. aeruginosa*	*P. fluorescens*	*B. cereus*

Effect of carbon supplement on phenol degradation: Microbial activities allow the mineralization of some recalcitrant components into carbon dioxide and water, and hence microbial transformation is considered a major route for complete degradation of these components[19]. The potency of microbes as agents for degradation of several compounds thus indicates biological treatment can be a major promising alternative to attenuate environmental impact caused by pollutants[20]. Many scientific approaches have been used in the *in situ* and *ex situ* biodegradation of organic pollutants. However, the extent of biodegradation is critically dependent on temperature, pH, heavy metals, surfactants, nutrients and presence of readily assimilable carbon sources[21].

In our study, when the MSM was supplemented with auxiliary carbon sources the best results of phenol degradation of around 75.4 % was achieved with *P. fluorescens* in media supplemented with glucose (**Figure 1)**.

Earlier studies on the role of simpler carbon sources revealed that glucose supports growth and the addition of this conventional carbon source substantially increases cell density[22]. Similarly, addition of non-toxic compounds may stimulate the viability of cells and enhance degradation. It was proposed that the presence of a more metabolizable carbon source permitted more rapid growth and the activity of the phenol degradation pathway was suppressed in order to quicken biomass acclimatization to glucose as the alternate carbon source[23].

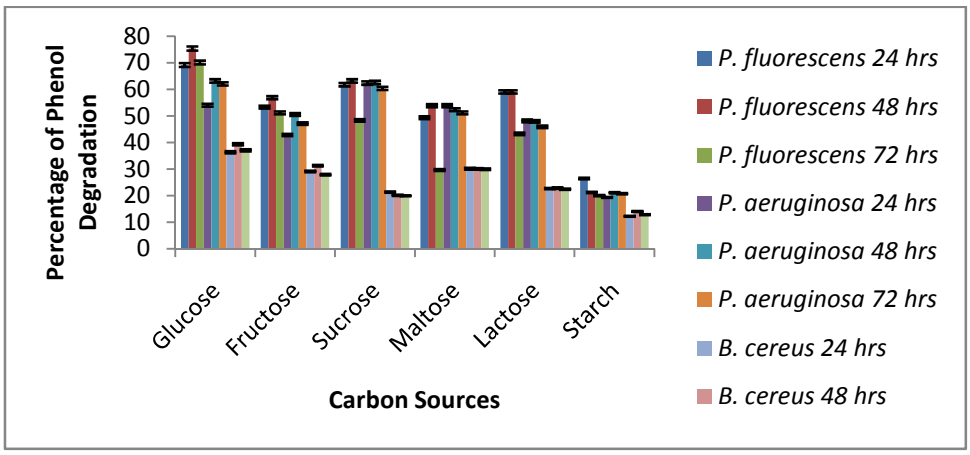

Figure 1: Effect of different carbon sources on percentage of phenol degradation

Effect of nitrogen supplement on phenol degradation: When the MSM was supplemented with different sources of nitrogen it was found that peptone supported the best degradation and a considerable amount of increase in the biomass occurred **(Figure 2)**. Our study is in accordance with an earlier study, where the presence of peptone attenuated the rate of phenol removal to the highest level by using *Pseudomonas sp.* in a medium with peptone and glucose as additional nutrients[24]. The rate of phenol degradation was improved when peptone was supplemented at the concentration between 0.25 and 1.0 g/L, with an optimum of 0.25 g/L. Peptone at low concentration influences the rate of degradation; however above 1.0 g/L peptone was inhibitory. A novel indigenous *Pseudomonas aeruginosa* strain MTCC 4996 was isolated from a pulp industrial effluent-contaminated site and was found to be capable of degrading phenol up to a concentration of 1,300 mg L^{-1} within 156 h using glucose and peptone at lower concentrations which enhanced phenol degradation[25].

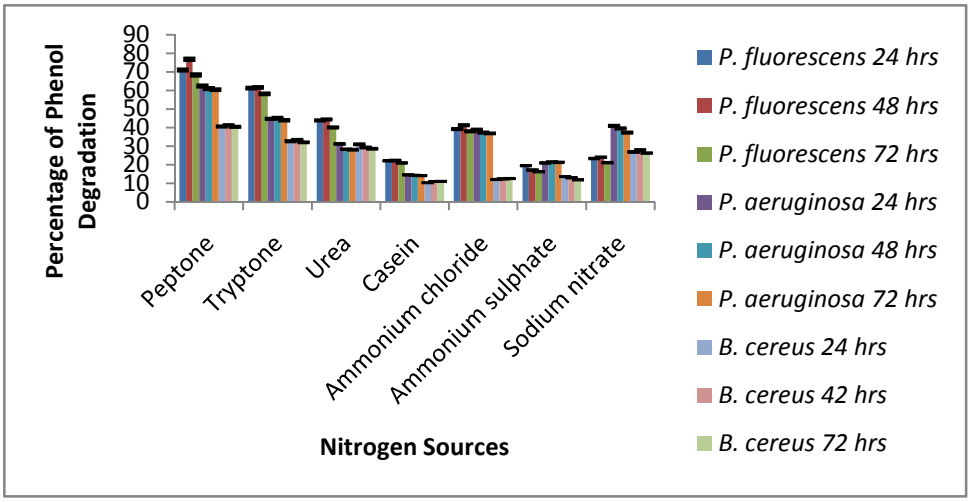

Figure 2: Effect of different nitrogen sources on percentage of phenol degradation

Effect of pH on biodegradation of phenol: For optimum microbial activity in the environment, the preferred range of pH is between pH 6 to 8[26]. Therefore, it is not surprising to find that most microorganisms have evolved with pH tolerances within this range[27]. **Figure 3** demonstrates the pattern of phenol degradation by the three isolates under the effect of different medium pH. All the three isolates were able to grow on a wide range of pH from 5.0 to 9.0 achieving the phenol degradation efficiency in a range of 29.3 % to 78.3 %.The maximum degradation efficiency of 78.3 % was achieved at 7.0 by *P.*

fluorescens. High degradation efficiency was recorded at pH 7.0 and 8.0. However, when the pH values were lower than 7.0 and higher than 8.0, biodegradation efficiency was affected significantly.

Following growth, the decrease in the medium pH can be an indicator of the phenol degradation and one of the factors significant in the success of the biological treatment. A slight reduction is observed as biomass grows and pH variation increases when the initial phenol concentration increases[28]. The decrease in pH suggests that biological degradation of phenol occurs and with a stable pH of about 7 (and a sufficient oxygen supply) phenol was successfully degraded. The pH significantly affects the biochemical reactions required for phenol degradation. Several studies indicate that the best pH range for the phenol degradation from effluents is 6.5- 7.5. Tests with pure *P. putida* could not efficiently resist pH change[29].

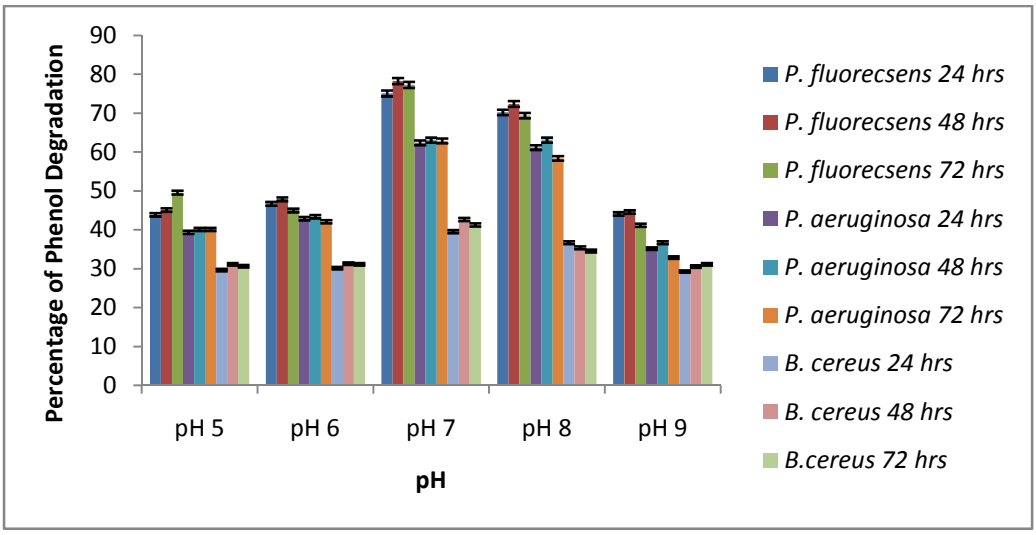

Figure 3: Effect of pH on percentage of phenol degradation

pH of the medium affects the substrate decomposition rate and phenol decomposition leads to a considerable decrease in pH. Consequently, phenol degradation is deteriorated as the medium pH deviates from neutral condition[30]. pH affects the surface charge of the cells of the activated sludge biomass. The surface charge of biomass is predominantly negative over the pH range of 3–10. Phenol could be expected to become negatively charged in phenoxide ion above a pH of 9. Below a pH of 3, the overall surface charge on cells becomes positive due to isoelectric point of activated sludge so the electrostatic attraction between phenol and activated sludge biomass will be insignificant[30].

Effect of incubation temperature on phenol degradation: Phenol degradation seems to be determined by some environmental factors such as temperature and pH[28, 29, 31, 32]. The rate and the extent of degradation are relatively sensitive to deviations outside the optimal range[28]. A variation of 5°C may cause a decrease in phenol degradation rate of at least 50% at the lower end and almost 100% at the higher end. Regarding the temperature effect, our results are almost unanimous with the findings of other workers, who found higher phenol removal efficiency near 30ºC **(Figure 4)**. Though significant level of degradation was observed both at 25°C and 35°C, it was less than that at 30°C. Degradation was affected both at low as well as high temperatures.

Such decrease in the degradation rates could be justified in terms of the effect of temperature on the bacterial growth. Growth rates, in general, roughly double for each 10°C rise in temperature within the usual mesophilic range and the growth rates generally do not change between 35°C to 40°C, but denaturation of proteins at higher temperatures slows growth rates of the mesophiles. However, different mixed cultures adapted at higher temperatures have optimum temperatures range of 40 to 45°C.

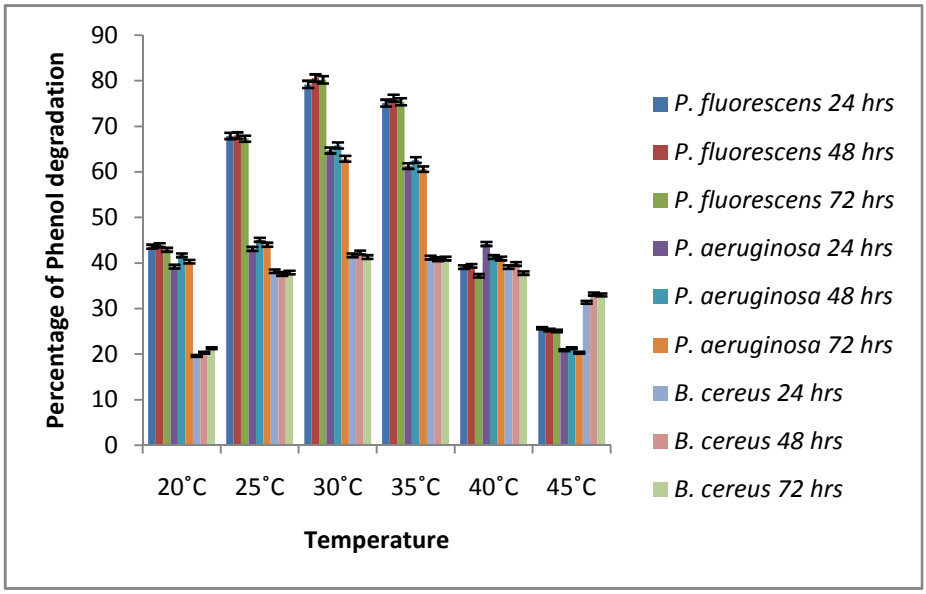

Figure 4: Effect of temperature on percentage of phenol degradation

The findings of our study are similar to that involving phenol degradation by soil pseudomonad and *Pseudomonas pictorum*[33, 34]. The difference between phenol removal efficiency at 30°C is probably due to the higher production of metabolites at this temperature[31]. However a study revealed that phenol degradation was significantly inhibited at 30ºC[35].

Molecular characterization of the isolates using partial 16S rDNA sequencing: The sequences of the isolates *P. aeruginosa, P. fluorescens* and *B. cereus* obtained after the partial 16S rDNA sequencing of the 1500 bp long PCR-amplified products were deposited in GenBank and subjected to the nucleotide BLAST. The query sequences revealed that the isolates of *P. aeruginosa, P. fluorescens* and *B. cereus* were MTCC 1034, MTCC 2421 and ATCC 9634 with the accession numbers AF094719.1, CP000076.1 and U02893.1, respectively. The homology value of BLASTN search showed 98%, 98% and 100% sequence homology, respectively, with the registered 16S rDNA gene sequence in NCBI data base.

CONCLUSION

Results from this study have demonstrated that the three isolates were capable to degrade phenol and the degradation was greatly influenced by the presence of auxiliary carbon and nitrogen sources and cultural conditions like pH and temperature. For all the isolates, the optimum conditions for growth and phenol degradation were 30°C, pH 7.0, 1 g/ l (0.1%) of glucose and peptone. A degradation of 76%, 83% and 74% was obtained for *P. aeruginosa* MTCC 1034, *P. fluorescens* MTCC 2421 and *B. cereus* ATCC 9634 respectively within a time period of 48 hrs. The success of the study lies in the fact that these were the natural isolates from the industrial effluents polluted with phenol and its derivatives and do not need much attenuation to bring about degradation. While most of the phenol degradation studies have been performed anaerobically, this study was an attempt to study the potential of the organisms to degrade phenol aerobically. This observation can be considered in future as an alternate method to aerobically treat phenol contaminated site .We recommend that the process can be studied further for it to be applied as a future technology.

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