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Isolation and Identification of Phenol Degrading Bacteria from Effluent Treatment Plant of Textile Industry in Kerala

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ABSTRACT

Environmental pollution is considered as a side effect of modern industrial society. With the immense growth of industries major problem is encountered as contamination of the environment with hazardous and toxic chemicals. Phenolic, one of the major pollutants, are discharged in the waste water from the various industries such as phenol resin and pharmaceutical, oil refineries, petrochemical plants, ceramic plants, steel plants, and coal conversion processes. Phenol and its derivatives is the basic structural unit in a wide variety of synthetic organic compounds. The samples were collected from effluent treatment plants of Textile Industry in Kerala. Serially diluted samples were transferred to 10,20,30,40 and 50 ppm phenol having minimal media. Growth and total phenol was recorded according to its incubation periods. From the 50 ppm culture, organisms were selected and cultured in sorbitol agar medium with varying concentrations of phenol (200, 400, 600, 800 and 1000 ppm). Out of 13 strains isolated, 5 strains were selected for further analysis. Biochemical characterization was done to identify the selected five strains. The strains are also used to study the effects of temperature and pH.From this preliminary study, it could be concluded that the standard prescribed temperature and pH were suitable for better growth and phenol degradation. The comparative study between the consortium and the individual organisms revealed that use of individual organism had shown the maximum degradation of phenol rate than that of consortium of organism. Among the selected 5 strains, Micrococcus sp and Pseudomonas sp were the strains that could withstand all the selected concentrations of phenol.

Key words*; Textile effluent, Consortium, Total phenol, Phenol tolerance, Micrococcus sp.*

INTRODUCTION

In the development of the world today, human health and the environment have become the most pressing issues. Due to those concerns, biodegradation of aromatic compounds have received a great attention because of their toxicity and persistence in the environment. Among all the toxic compounds, phenol and its substituent phenolic compounds contribute a remarkable adverse impact to the environment. These are major xenobiotics, which are often found in the effluents discharged from the industries such as paper and pulp, textiles, gas and coke, fertilizers, pesticides, steel and oil refineries etc[10,13].

During the last two decades, phenolic compounds have become the subject of intense research in the preservation of our environment.

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Toxicity of phenolic compounds inhibits biological treatment or even eliminates sensitive micro-organisms from biological wastewater treatment process and significantly reduces the biodegradation of the other components[22]. Many aerobic bacteria have been confirmed to use aromatic compounds as the sole source of carbon and energy[17] which suggests using phenol as nutrient to the organism and thereby converts phenol to nontoxic component. Most of the efficient phenol degrading microorganisms are capable of using phenol as the sole source of carbon and energy for their growth and metabolism. Microorganisms capable of degrading phenol do so with the action of a variety of enzymes. The focus on the microbial degradation of phenols in recent years has resulted in the isolation, culture, adaptation and enrichment of a number of microorganisms that can grow on the compound as a sole carbon and energy source. The present study aimed at the isolation and identification of potent phenol degrading bacteria from the effluent treatment plant of textile industry in Kerala and also to study the effect of pH and temperature on their degradation potential.

MATERIALS AND METHODS

Samples from effluent treatment plant of Textile industry in Kerala were collected and serially diluted. Microbial Enrichment was done using nutrient broth with different phenol concentrations (10, 20, 30, 40, 50 ppm). From the 50 ppm culture, organisms were selected and cultured in sorbitol agar medium with varying concentrations of phenol (200, 400, 600, 800 and 1000 ppm).

Estimation of Phenol tolerance

Estimation of total phenol was carried out the method of Bray andThorpe W. V. T[7].

Observation of Total Growth

The growth rates of the microbes were observed by spectrophotomeric analysis.

Identification of Isolates

Biochemical Characterization was done based on Bergeys manual of Determinative Bacteriology[6] and Cappucino *et al*[8].

Effect of pH and temperature on total growth and phenol degradation

The effectof pH and temperature on total growth and phenol degradation of the selected strains were studied.

RESULTS AND DISCUSSION

One of the most alarming situations in today's world is the generation of a huge amount of waste water contaminated with the toxic organic substances like phenolics from the industrial sector. Phenol is highly water soluble, and its presence in the water imparts a carbolic odor to the receiving water bodies and can have baleful effects on aquatic as well as terrestrial flora and fauna including human beings[1]. Hence removal of phenol from the discharged sewage and effluent is highly necessary. Conventional methods for treatment of phenol have their own set of disadvantages hence biological method is the current choice. Reports on possibility of treatment of phenol bearing waste water using microorganisms are also available[14]. Till date, research communities have focused only on the isolation of microorganisms from the sites contaminated with phenol industrial effluents and use them for the treatment of the industrial wastewater.

In the present investigation, attempts were made to isolate phenol degrading microorganisms from the textile industrial effluent of Kerala. Serially diluted samples were transferred to 10,20,30,40 and 50 ppm phenol having minimal media. Neumann *et al*[15]., adapted *Pseudomonas* strains to high concentrations of phenol (1000 mg/L) and further biodegradation was carried out at a concentration of 500 mg/L. They opinioned that the cultures could grow by utilizing phenol as a source of carbon and energy. Growth and total phenol was recorded according to its incubation periods. The growth rate and the total phenol content for the microbes were given in the table 1. In the study, the least total phenol was shown in 40 ppm and the maximum in 20 ppm. In the sample all the strains were active upto 72 hrs then gradually decreased during 96 hrs of incubation. It has been suggested that several factors affect the growth of bacteria in a microbial community[20], some of these factors are nutrient availability, the presence of toxins, attachment of cells to matrices, and physical parameter. Out of 13 strains isolated, 5 strains were selected

for further analysis. They are *Micrococcus sp, Brucella sp, Pseudomonas sp, Aquaspirillum sp* and *Moraxella sp* (Plate 1 to 5). All the 5 strains were inoculated in sorbitol agar media with different concentrations of phenol (200 ppm, 400 ppm, 600 ppm, 800 ppm and 1000 ppm). Total phenol content and growth rates were observed for 24 to 96 hrs of incubation and the results are given in the tables 4 to 8.

Identification of microbial strains

The biochemical test of the selected 5 strains were done on the Bergeys manual of Determinative Bacteriology[6] and Cappucino*et al*[8]., (Plate 1 to 5). All the selected strains had shown +ve towards the oxidase test and –ve towards the citrate utlilzation, methyl red, vogesproskauer test and urea hydrolysis tests.

In medium with 200 ppm, the maximum degradation was shown by *Aquaspirillum sp* followed by *Pseudomonas sp, Moraxella sp, Micrococcus sp* and the least one was *Brucella sp.* The *Micrococcus sp* having the highest growth in 400 ppm concentration of phenol, followed by *Moraxella sp, Brucella sp, Aquaspirillum sp* and *Pseudomonas sp.* In 600 ppm, the maximum degradation potential shown by strain *Pseudomonas* and the second most was *Moraxella sp* followed by *Pseudomonas sp, Micrococcus sp* and *Aquaspirillum sp.*

In the medium with 800 ppm of phenol, the highest degradation potential was shown by *Micrococcus sp* followed by *Pseudomonas sp, Aquaspirillum sp, Moraxella sp* and the least one was *Brucella sp.* In 1000 ppm concentration of phenol, maximum growth and phenol degradation were shown by *Pseudomonas sp* followed by *Moraxella sp, Aquaspirillu sp, Micrococcus sp* and *Brucella sp.* Bandyopadhyay *et al*[5]., have studied the biodegradation of phenol by *Pseudomonas putida* immobilized in calcium alginate and reported that on increasing the concentration of the phenol above 750 ppm the reaction behavior deviates from michelean kinetics which may be due to the effect of intra-particle diffusion.

From the analysis it was clear that *Micrococcus sp* was having the potential to survive in 400, 600 ppm and 800ppm concentrations of phenol. *Brucella sp* was actively found in 400 ppm and 600 ppm concentrations of phenol. *Pseudomonas sp* thrive well in 600 ppm, 800 ppm and 1000 ppm concentrations of phenol and *Aquaspirillum sp* showed highest growth in 200 ppm concentration of phenol. *Moraxella sp* showed highest degradation potential in 400 ppm.

The total phenol was slightly increased after the 48 hrs of incubation and the same time growth was decreased. After that when growth rate increased, total phenol decreased. This was shown in all the concentrations of phenol (by 200-1000 ppm) with various strains. Among the selected 5 strains, *Micrococcus sp* and *Pseudomonas sp* were the strains that could withstand all the selected concentrations of phenol.

Effect of pH and Temperature on the selected strains

For the study of effect of pH on the degradation of phenol from the selected strains of the samples, an acidic (pH 5) and basic (pH 9) medium were selected (Table 11 and 12). When pH decreases, growth also slightly decreases and the degradation does not occur properly. In the case of increasing pH, growth was also increased but degradation rate was very less. In this study, only standard optimum pH had given the accurate phenol degradation correspondingly with their growth. The internal environment of all living cell is believed to be approximately neutral. Most organisms cannot tolerate pH values below 4.0 or above 9.0[11]. At low (4.0) or high (9.0) pH values acids or bases can penetrate into cells more easily, because they tend to exist in undissociated form under these conditions and electrostatic force cannot prevent them from entering cells[2,9,19]. The optimum pH for phenol degradation is 7.0 for *Pseudomonas putida* NICM 2174[3].

For the analysis of effect of temperature, two optimum temperatures were selected (27^0 C and 47^0 C) (Table 13 and 14). From this study, it was clear that all the selected strains had shown very negligible amount of phenol degradation in low and high temperature. So it can be concluded that the standard prescribed temperature is suitable for better growth and phenol degradation. Temperature plays an important role than nutrient availability in the degradation of organic pollutants. According to Pakula *et al*[16]., phenol biodegradation was significantly inhibited at 30°C. However, most laboratory studies on phenol degradation have been carried out at an optimum temperature of 30°C[4,18]. Annadurai *et al*[2]., and Chitra[9] described that when the temperature increased from 30°C to 34°C no phenol degradation was

observed due to cell decay, which shows that the phenol degradation is a temperature dependent process. Growth rates in general roughly double for each 10°C rise in temperature within the usual mesophilic operational range from 10 to 30°C. Growth rates generally do not change between 35°C and 40°C, but denaturation of proteins at higher temperatures slows growth rates for mesophiles. However, different mixed cultures adapted to thermophilic temperatures have optimum temperatures range of 55 to 65°C. Thermophiles do not function well at the intermediate temperature of 40 to 45°C as mesophilic organisms. Thus, onemust make the decision to operate at the lower mesophilic range with an optimum temperature of around 35°C or in the thermophilic range with a temperature optimum of 55 to 60°C.

Combination of microbes for biodegradation

In order to find out and compare the efficiency of consortium and individual organism'sin terms of degradation potential, the medium with 800 ppm was selected and used for total growth and total phenol studies. When consortium of the organism was introduced in nutrient broth of 800 ppm concentration, it showed a rapid degradation according to the growth. After 48 hrs of incubation they expressed less degradation rate (Table 3). This study it revealed that introducing of individual organism shown the maximum degradation of phenol rate than the rate of consortium of organism degradation rate. Evolution of efficient degradative pathways is also likely to be slow when degradation is accomplished by co-metabolism by a consortium of microbes[21]. For example, several microbes catalyze transformations of TNT via co-metabolic processes that reduce the nitro substituent, or less commonly, remove a nitro substituent. However, microbe carries out enough reactions to derive a benefit from its degradation. Evolution of an efficient degradative pathway may be more rapid when several consecutive reactions, or even the entire pathway, are found within a single microbe that can reap the advantages of accessing a novel source of carbon, nitrogen, or phosphorous.

With the immense growth of industries, major problem is encountered as contamination of the environment with hazardous and toxic chemicals. Phenolics, one of the major pollutants, are discharged in the waste water from the various industries such as phenolic resin and pharmaceuticals, oil refineries, petrochemical plants, textile industry, ceramic plants, steel plants, and coal conversion processes. Soils contaminated by oil are the most potential source to isolate high performance phenol degrading microorganisms. The degradation performance of selected strains was examined for different phenol concentrations. Serial exposure to increasing level of phenol concentration was used to determine the resistance of isolated strain. Acclimatization of the microorganisms overcomes the substrate inhibition problems that normally occurred in phenol biodegradation at high concentration[12]. The experiment aimed to find the highest tolerance level of phenol concentrations and found that it was able to survive and degrade up to 800 mg/L phenol. The growth of bacteria and phenol concentration in the media showed the inverse proportion with each other. The decrease in phenol concentration accompanied with increase in biomass.

Waste water from Textile industry contains a variety of substances including phenolic components. Their degradation mechanism was examined in a series of different phenol concentrations. Serial exposure to increasing level of phenol concentration can be used to determine acclimatizability of a particular isolate. Highly acclimatizable microbes are those which are able to degrade phenol at high concentration and at greatest rate will be the best phenol degrader candidates. In this textile effluent, *Micrococcus sp* and *Pseudomonas sp* were the best strains having the potential to withstand majority of the phenol concentrations given during the study. Future studies should be carried out to analyze the best phenol degrading bacteria from the textile effluent.

Table 1 - Growth and total phenol of strains from textile effluent

	Growth (600nm)				Total phenol (725nm)			
Con.in ppm	24 hrs	48 hrs	72 hrs	96 hrs	24 hrs	48 hrs	72 hrs	96 hrs
10	0.071	0.126	0.020	0.018	0.908	0.673	0.462	0.318
20	0.068	0.093	0.015	0.008	0.711	0.708	0.483	0.304
30	0.099	0.186	0.019	0.014	0.860	0.634	0.454	0.226
40	0.070	0.145	0.028	0.014	0.732	0.641	0.421	0.416
50	0.091	0.163	0.023	0.013	0.721	0.653	0.443	0.436

Table 2- Biochemical test results

Tests / Strains	Grams staining	Oxidase test	Catalase test	Citrate utilization	Methyl red	Vogesproskauer	Indole test	motality	Urea hydrolysis	Gelatin hydrolysis	Gas production
Micrococcus sp	+cocci	+	+	-	-	-	-	+	-	+	+
Brucella sp	-cocci	+	+	-	-	-	-	-	-	+	-
Pseudomonas sp	-rod	+	-	-	-	-	+	+	-	-	+
Aquaspirillum sp	-cocci	+	-	-	-	-	-	-	-	+	+
Moraxella sp	-cocci	+	+	-	-	-	-	-	-	-	-

Table 3 - Growth and Total phenol of Consortium of bacteria (800 ppm)

Time	24 hrs	48 hrs	72 hrs	96 hrs
Growth(600 nm)	0.141	0.157	0.025	0.017
Total phenol(720 nm)	0.039	0.400	0.307	0.209

Table 4- Growth and Total Phenol – 200 ppm

	Growth (600nm)				Total Phenol (725nm)			
Strains	24 hrs	48 hrs	72 hrs	96 hrs	24 hrs	48 hrs	72 hrs	96 hrs
Micrococcus sp	0.019	0.004	0.005	0.005	0.110	0.147	0.097	0.090
Brucella sp	0.001	0.002	0.004	0.003	0.104	0.129	0.100	0.100
Pseudomonas sp	0.021	0.005	0.007	0.004	0.134	0.072	0.060	0.060
Aquaspirillum sp	0.014	0.005	0.009	0.005	0.152	0.018	0.018	0.017
Moraxella sp	0.018	0.004	0.006	0.005	0.130	0.131	0.099	0.099

Table 5- Growth and Total Phenol – 400 ppm

	Growth (600 nm)				Total phenol (725 nm)			
Strains	24 hrs	48 hrs	72 hrs	96 hrs	24 hrs	48 hrs	72 hrs	96 hrs
Micrococcus sp	0.006	0.005	0.007	0.005	0.009	0.192	0.066	0.006
Brucella sp	0.006	0.005	0.005	0.004	0.047	0.198	0.104	0.100
Pseudomonas sp	0.007	0.003	0.004	0.001	0.086	0.214	0.198	0.161
Aquaspirillum sp	0.008	0.005	0.005	0.004	0.209	0.119	0.140	0.135
Moraxella sp	0.006	0.005	0.006	0.004	0.240	0.174	0.040	0.026

Table 6- Growth and Total Phenol – 600 ppm (725 nm)

	Growth (600 nm)				Total phenol (725 nm)			
Strains	24 hrs	48 hrs	72 hrs	96 hrs	24 hrs	48 hrs	72 hrs	96 hrs
Micrococcus sp	0.004	0.004	0.005	0.004	0.244	0.336	0.121	0.111
Brucella sp	0.004	0.004	0.009	0.004	0.283	0.371	0.099	0.006
Pseudomonas sp	0.004	0.004	0.006	0.004	0.388	0.381	0.204	0.105
Aquaspirillum sp	0.005	0.004	0.005	0.001	0.267	0.356	0.201	0.198
Moraxella sp	0.005	0.003	0.007	0.003	0.261	0.378	0.099	0.066

Table 7- Growth and Total Phenol - 800ppm (725 nm)

	Growth (600 nm)				Total phenol (725 nm)			
Strains	24 hrs	48 hrs	72 hrs	96 hrs	24 hrs	48 hrs	72 hrs	96 hrs
Micrococcus sp	0.005	0.004	0.009	0.005	0.153	0.291	0.129	0.108
Brucella sp	0.006	0.002	0.003	0.001	0.141	0.263	0.172	0.170
Pseudomonas sp	0.006	0.004	0.007	0.004	0.135	0.249	0.123	0.119
Aquaspirillum sp	0.006	0.004	0.006	0.004	0.111	0.278	0.139	0.121
Moraxella sp	0.007	0.004	0.005	0.004	0.126	0.281	0.146	0.121

Table-8- GROWTH and TOTAL PHENOL - 1000ppm (725 nm) (Textile effluent)

	Growth (600 nm)				Total phenol (725 nm)			
Strains	24 hrs	48 hrs	72 hrs	96 hrs	24 hrs	48 hrs	72 hrs	96 hrs
Micrococcus sp	0.001	0.002	0.003	0.002	0.239	0.375	0.037	0.357
Brucella sp	0.006	0.001	0.002	0.001	0.252	0.371	0.370	0.370
Pseudomonas sp	0.004	0.004	0.005	0.003	0.224	0.393	0.303	0.302
Aquaspirillum sp	0.007	0.002	0.003	0.001	0.214	0.369	0.360	0.351
Moraxella sp	0.004	0.004	0.004	0.003	0.396	0.400	0326	0.309

Plates showing the selected 5 strains

Plate 1 - *Micrococcus sp*

Plate 2 *–Brucella sp*

Plate 3-*Pseudomonas sp*

Plate 4- *Aquaspirillum sp*

Plate -5 *Moraxella sp*

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