A Preliminary Study on Phenol Degrading Bacteria from Effluent Treatment Plant of Paper Industry, Kerala, India.

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ABSTRACT: Phenol is used to make pharmaceuticals, synthetic resin, dyes, pesticides, synthetic tanning agents, lubricating oils and solvents. In the manufacture of industrial and agricultural products, phenols are used as common starting materials and often it is produced as waste products. The increasing presence of phenols represents a significant environmental toxicity hazard. Isolation of microbial strain capable of degrading chemical compounds can be achieved from polluted sources, such as soil and water. The increasing phenol and phenol wastes necessitates the screening of bacteria that are able to degrade phenol. The objective of the study was to isolate and identify the phenol degrading bacteria from effluent treatment plant of paper industry. Bacterial strains capable of degrading phenol were isolated from enrichments containing 50 mg/L of phenol. From this 50 ppm medium, the strains were transferred and grown in selective sorbitol media. 5 potential strains were isolated and were subjected to biochemical characterization and were identified as Vibrio sp, Moraxella sp, Brucella sp, Micrococcus sp and Neisseria sp. The cultures were subjected to phenol tolerance level of minimum medium containing different concentrations of phenol (200 ppm to 1000 ppm). The most potent phenol degrading bacteria among the selected strains was Micrococcus sp. This study will help to find out the phenol degraders from effluent treatment plant which can be isolated and cultured in large scale and also can be used in waste management.

KEYWORDS: Phenol, Micrococcus sp, Paper industry, Industrial effluent.

INTRODUCTION

Phenolic compounds are natural as well as manmade aromatic compounds. They are associated with pulp mills, coal mines, gasoline, petrochemicals, wood preservation plants, pesticides, insecticides, herbicides, detergents, solvents, polymeric resin production, plastic, rubber-proofing, disinfectants, pharmaceutical, metallurgical, explosive, textile, colour, coffee industries, domestic wastes, agricultural run-off and chemical spills (Aksu S, Yener J,1998; Gupta VK,*et al*., 1998; Loh KC *et al*.,2000).They also enter the environment as intermediates during the biodegradation of natural polymers containing aromatic rings, such as lignin and tannins and from aromatic amino acid precursors. They are also produced as intermediates during the biodegradation of xenobiotic compounds (Balfanz J and Rehm H J., 1991; Van Schie PM and Young LY, 1998). Sitting M (1975) has reported a very high phenol concentration in industrial wastewater e.g., plastic manufacturing (600-2000mg/l), petrochemical manufacturing (50-600mg/l) and stocking production (6000mg/l). The concentration of phenols in industrial wastewater varies from 10 mg/l to 3000 mg/l (Annadurai G, *et al*., 2000). Effluents discharged from petrochemical, textile, tannery and coal gasification units generally contain phenolic compounds ranging from 6-2000 mg/l. Their admissible limit is only 3mg/l in the receiving water bodies (Manivasakam N, 1984).

The need to remove the contaminations has led to the development of new technologies that emphasize the

detoxification and destruction of the pollutants rather than the conventional approach of disposal. Different methods of treatment are available for reduction of phenol content in wastewater with different initial concentration. Kostyeav, V. Ya (1973) had indicated the probable technologies for the treatment of wastewater containing phenol, which include chlorination, ozonation, adsorption, solvent extraction, membrane process, coagulation, flocculation and biological treatment. But physicochemical methods of the treatment of phenolic wastewater have the inherent drawbacks due to the tendency of the formation of secondary toxic materials. Moreover, the physico-chemical treatment processes have proven to be costly. Thus, biological method of treatment has turned out to be a favourable alternative for phenol degradation. Biodegradation means the biological transformation of an organic pollutant to another form (Klein JA and Lee DD, 1978). It is a cost-effective alternative to conventional disposal method, which emphasizes the detoxification and destruction of pollutants by acclimatized microorganisms.

Natural way of recycling wastes or breaking down organic matter into nutrients that can be used by the organisms is called biodegradation. There are two types of biodegradation- aerobic which takes place in presence of oxygen and anaerobic biodegradation which takes place in the absence of oxygen. There are basically two generalized category of biodegradation- mineralization and biotransformation. Mineralization involves total degradation of the organic matter. In case of biotransformation organic matter is not degraded totally, while a part of it is degraded; another part is converted into other smaller chain organic compounds. Because of widespread occurrence of phenol in the environment many microorganisms utilizes phenol as the sole carbon and energy source which includes both aerobic and anaerobic microorganisms.

In the present study, an attempt was made to investigate the growth of the phenol degrading bacterial strains and total phenol content in the medium with varying phenol concentrations. The sample was collected from effluent treatment plant of paper industry in Kerala and from this five potent bacterial strains were isolated and identified and used for further studies.

MATERIALS AND METHODS

Samples from effluent treatment plant of paper industry 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 medium, organisms were collected and added to sorbitol agar medium with varying concentrations of phenol (200, 400, 600, 800 and 1000 ppm).

Estimation of Total Phenol

Estimation of total phenol was carried out the method of Bray *et al* (1954).

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 (1994) and Cappuccino *et al* (1999, 2000).

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 odour to the receiving water bodies and can have baleful effects on aquatic as well as terrestrial flora and fauna including human beings. 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. Biodegradation is one of the cheapest methods with no production of hazardous by-products. This method is generally preferred due to lower costs and possibility of complete mineralization.

In the present investigation, attempts were made to isolate phenol degrading microorganisms from effluent treatment plant of paper industry. Serially diluted samples from paper industry were transferred to 10,20,30,40 and 50 ppm phenol having minimal media. Neumann *et al.,* (2004) 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.

Here, in all the selected concentrations of phenol (10ppm-50ppm) the strains showed a slight increase in growth during 24 hours of incubation and on consecutive days, the growth rate was decreasing(table1). Corresponding change in phenol concentration was observed in all the selected concentrations. In the medium with 10ppm phenol, the initial growth was observed as 0.0104. After 24 hours of incubation there was a slight increase in growth. After 48, 72, 96 hours of incubation, the growth rate was found to be decreasing. Similar pattern was observed in all remaining four concentrations. Maximum growth rate was recorded during 48 hours of incubation in medium with 40 ppm phenol. The medium with 20 ppm during 96 hours of incubation recorded the minimum growth. The total phenol content showed values in accordance with the changes in growth rates (table 1). It has been suggested that several factors affect the growth of bacteria in a microbial community (Roszak and Colwell, 1987). Some of these factors are nutrient availability, the presence of toxins, attachment of cells to matrices and physical parameters. 5 potent strains were selected for further analysis. These 5 strains were *Vibrio* sp, *Moraxella* sp, *Brucella* sp, *Micrococcus* sp, *Neisseria* sp (table 2)

All the 5 strains were inoculated in sorbitol agar media with different concentrations of phenol (200ppm, 400ppm, 600ppm, 800ppm and 1000ppm). Total phenol content and growth rate were observed for 24 to 96 hrs of incubation and the results are given in the (tables 3 to7).

In medium with 200 ppm, the highest phenol degradation was showed by *Vibrio* sp during 72 hrs of incubation and then followed by *Moraxella* sp, *Micrococcus* sp, *Neisseria* sp and *Brucella* sp. From this it was clear that *Brucella* sp was having the lowest degradation capacity in 200 ppm. The increase in the lag phase with increased initial concentration of phenol has been reported by Saravanan *et al*. (2008) and Bajaj *et al*. (2009).

In the medium with 400 ppm maximum growth was shown by *Micrococcus* sp followed by *Moraxella* sp, *Brucella* sp, *Neisseria* sp and *Vibrio* sp. In the medium with 600ppm, highest degradation potential was shown by *Micrococcus* sp, followed by *Moraxella* sp, *Vibrio* sp, *Brucella* sp and *Neisseria* sp. Bandyopadhyay *et al* (2001) have reported the inhibiting effect of phenol as substrate above the concentration of 600 ppm and 500 ppm respectively. Saravanan *et al*. (2008) have reported that microorganisms did not show any inhibitory effect and

almost no lag phase was observed during its growth between 100 mg/l and 500 mg/l. The most probable reason behind the extended lag phase is due to the increasing toxicity of phenol with increase in its concentration.

In the medium with 800 ppm the highest degradation potential was shown by *Micrococcus* sp and the second most was shown by *Neisseria* sp followed by *Moraxella* sp, *Vibrio* sp and *Brucella* sp. In medium with 1000 ppm maximum degradation and acclimatization was shown by *Micrococcus* sp followed by *Neisseria* sp, *Moraxella* sp, *Vibrio* sp and *Brucella* sp.

From this observation it was clear that among the selected strains, *Micrococcus* sp had highest phenol degrading potential. In 200ppm, *Vibrio* sp showed highest growth. Here all the bacterial strains were active till 72 hrs of incubation and after that a slight growth decline was observed. When the growth rate increased, the total phenol decreased. *Micrococcus* sp showed the most potential degradation in all the concentrations of phenol.

Combination of microbes for biodegradation

In order to find out the efficiency of consortium and individual organisms, the medium with 800ppm was selected (as this concentration had given maximum results for all the selected strains) 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 8). From this study it was revealed that with these selected strains, the use of individual organism had shown the maximum degradation of phenol than the consortium. Evolution of efficient degradative pathways is also likely to be slow when degradation is accomplished by co-metabolism by a consortium of microbes (Shelley D. Copley, 2009). For example, several microbes catalyze transformations of TNT (2, 4, 6 tri nitro toluene) 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.

SUMMARY AND CONCLUSION

Phenol is among the common toxic environmental pollutants that occur naturally and also originated from industrial effluents. The toxicity of phenol has been widely documented and there is a great concern over the disastrous effects upon both human being and the environment. 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.

Waste water from paper 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 paper effluent, *Micrococcus* sp was found to be the best strain among the selected strains having the potential to withstand majority of the phenol concentrations given during the study. The medium with 800ppm phenol had given better degradation for all the selected strains. Future studies should be carried out to isolate more bacterial strains and to analyze the best phenol degrading bacteria from various industrial effluents.

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

Growth (600nm)						Total Phenol (720nm)				
Con in ppm	**Initial**	**24 hrs**	**48 hrs**	**72 hrs**	**96 hrs**	**Initial**	**24 hrs**	**48 hrs**	**72 hrs**	**96 hrs**
10	0.0104	0.0147	0.0093	0.0064	0.0060	0.0064	0.0065	0.0160	0.0778	0.0129
20	0.0111	0.0121	0.0085	0.0061	0.0037	0.0086	0.0062	0.0261	0.0692	0.0135
30	0.0134	0.0141	0.0105	0.0055	0.0054	0.0082	0.0060	0.0267	0.0465	0.0137
40	0.0180	0.0198	0.0207	0.0052	0.0052	0.0196	0.0055	0.0313	0.0398	0.0142
50	0.0171	0.0182	0.0197	0.0057	0.0050	0.0231	0.0059	0.0259	0.0369	0.0152

Table 2- Biochemical test results

Tests	Gram staining	Oxidase test	Catalase test	Citrate utilization	Methyl red	Voges proskauer	Indole test	Motility	Urea hydrolysis	Gelatin hydrolysis
Strains										
***Vibrio* sp**	- rod	+	+	-	-	-	+		-	+
***Moraxella* sp**	- cocci	+	+	-	-	-	-	-	-	-
***Brucella* sp**	- cocci	+	+	-	-	-	-	-	-	+
***Micrococcus* sp**	+ cocci	+	+	-	-	-	-	+	-	+
***Neisseria* sp**	- cocci	+	+	-	-	+	+	-	-	+

Table 3- Growth and total phenol – 200 ppm

Growth (600nm)					Total Phenol (720nm)			
Strains	**24 hrs**	**48 hrs**	**72 hrs**	**96 hrs**	**24 hrs**	**48 hrs**	**72 hrs**	**96 hrs**
***Vibrio* sp**	0.0110	0.0080	0.0104	0.0098	0.0146	0.0357	0.0290	0.0275
***Moraxella* sp**	0.105	0.0074	0.0080	0.0072	0.0161	0.0407	0.0319	0.0133
***Brucella* sp**	0.0091	0.0086	0.0096	0.0065	0.0148	0.0272	0.0634	0.0415
***Micrococcus* sp**	0.0130	0.0065	0.0089	0.0068	0.0116	0.0326	0.0365	0.0310
***Neisseria* sp**	0.0089	0.0072	0.0090	0.0072	0.0141	0.0288	0.0475	0.0323

Table 4- Growth and total phenol – 400 ppm

Growth (600nm)					Total Phenol (720nm)			
Strains	**24 hrs**	**48 hrs**	**72 hrs**	**96 hrs**	**24 hrs**	**48 hrs**	**72 hrs**	**96 hrs**
***Vibrio* sp**	0.0171	0.0096	0.0103	0.0068	0.0133	0.0265	0.0758	0.0257
***Moraxella* sp**	0.0104	0.0072	0.0126	0.0062	0.0141	0.0224	0.0380	0.0234
***Brucella* sp**	0.0131	0.0082	0.0115	0.0066	0.0102	0.0252	0.0386	0.0194
***Micrococcus* sp**	0.0144	0.0074	0.0128	0.0074	0.0209	0.0225	0.0250	0.0174
***Neisseria* sp**	0.0111	0.0071	0.0111	0.0064	0.0170	0.0248	0.0415	0.0305

Table 5- Growth and total phenol – 600 ppm

Growth (600nm)					Total Phenol (720nm)			
Strains	**24 hrs**	**48 hrs**	**72 hrs**	**96 hrs**	**24 hrs**	**48 hrs**	**72 hrs**	**96 hrs**
***Vibrio* sp**	0.0101	0.0062	0.0082	0.0056	0.0110	0.0113	0.0375	0.0241
***Moraxella* sp**	0.0103	0.0052	0.0099	0.0063	0.0120	0.0245	0.0339	0.0116
***Brucella* sp**	0.0072	0.0066	0.0080	0.053	0.0070	0.0368	0.0385	0.0308
***Micrococcus* sp**	0.0079	0.0062	0.0104	0.0059	0.0122	0.0184	0.0259	0.0206
***Neisseria* sp**	0.0065	0.0055	0.0071	0.0060	0.0159	0.0375	0.0395	0.0220

Table 6- Growth and total phenol – 800 ppm

Growth (600nm)					Total Phenol (720nm)			
Strains	**24 hrs**	**48 hrs**	**72 hrs**	**96 hrs**	**24 hrs**	**48 hrs**	**72 hrs**	**96 hrs**
***Vibrio* sp**	0.0071	0.0058	0.0068	0.0053	0.0225	0.0250	0.0378	0.0372
***Moraxella* sp**	0.0070	0.0057	0.0071	0.0057	0.0169	0.0278	0.0359	0.0208
***Brucella* sp**	0.0070	0.0068	0.0063	0.0070	0.0157	0.0253	0.0417	0.0204
***Micrococcus* sp**	0.0061	0.0050	0.0093	0.0060	0.0189	0.0261	0.0284	0.0172
***Neisseria* sp**	0.0069	0.0065	0.0074	0.0056	0.0259	0.0306	0.0332	0.0234

Table 7- Growth and total phenol – 1000 ppm

Growth (600nm)					Total Phenol (720nm)			
Strains	**24 hrs**	**48 hrs**	**72 hrs**	**96 hrs**	**24 hrs**	**48 hrs**	**72 hrs**	**96 hrs**
***Vibrio* sp**	0.0079	0.0054	0.0058	0.0050	0.0369	0.0384	0.0539	0.0255
***Moraxella* sp**	0.0065	0.0056	0.0068	0.0052	0.0354	0.0375	0.0516	0.0146
***Brucella* sp**	0.0056	0.0052	0.0055	0.0049	0.0338	0.0360	0.0635	0.0163
***Micrococcus* sp**	0.0082	0.0054	0.0094	0.0050	0.0350	0.0375	0.0390	0.0176
***Neisseria* sp**	0.0077	0.0055	0.0069	0.0057	0.0250	0.0366	0.0425	0.0248

Table 8- Growth and Total phenol of consortium of bacteria (800 ppm)

Effluent	Growth (600 nm)	Total phenol (700 nm)
24 hrs	0.0210	0.0231
48 hrs	0.0255	0.0274
72 hrs	0.0246	0.0294
96 hrs	0.0134	0.0150