MICROBIAL DEGRADATION OF PHENOL

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ABSTRACT

Effluent from chemical industries contains many organic toxic pollutants which can be hazardous to aquatic life as well as human health. Phenol is one of the most hazardous pollutants present in industrial effluents. Even at very low concentration phenol is of considerable health concern. The increasing toxicity of phenol has become warning alarm for its removal. There are many conventional method such as chemical and physical for the treatment of phenol wastes from environment. Biological treatment using microorganism has proved to be most effective and successful process for the treatment of phenolic wastes. In order to find out phenol degrading bacteria, some soil samples were collected from industrial polluted sites. After proper enrichment of the samples, microorganisms were isolated on the solid media containing phenol. Isolated microorganisms were screened on the basis of their phenol removal capacity in the liquid broth. This process was carried out in shake flask batch culture at 120 rpm for 24 hrs at 30°C using a specific concentration of phenol in ppm. Removal of phenol was estimated using 4-Aminoantipyrine method.

Key words*: *4- Amino Antipyrine, Biodegradation, Phenol

I. INTRODUCTION

In last few decades, humans have faced many serious environmental pollution problems, increasing tremendously every day[1]. All the types of environmental pollutions are hazardous, among which water pollution is more dangerous and is the major concern today. Many types of pollutants like organic, inorganic, suspended solids and radioactive materials are majorly responsible for water pollution[2].

Wastewater from industrial effluent contains many pollutants like phenol, which are frequently disposed without any treatment. Phenol is highly toxic, corrosive and mutagenic; it is also teratogenic agent affecting both environment and living organisms[3]. Phenol is a hazardous for humans, plants and animals, when present in low concentration. High and acute concentration of phenol can cause a central nervous system disorders and myocardial depression. It also causes irritation of eyes, swelling, corneal whitening and also blindness. When present in low concentration, it can be toxic for some aquatic species. It cause taste and odor problems in water and this water cause serious skin damage, cardiovascular diseases, gastrointestinal damage and also death[4].It is most commonly used in perfumes, lubricating oils, dyes and in the manufacture of industrial and agricultural product[5]. It enters into the natural water bodies through the effluent of many industries like high temperature coal conversation, petroleum refining, resin and plastic manufacturing, wood and dye industries. It is also found in the contaminated drinking water[6].

Due to such toxic and life threatening effects of phenol on living organisms, it is essential to remove or degrade it completely from the ecosystem[7]. Currently many different physical, chemical and biological methods are used for the removal of phenol[8,9]. Current chemical procedures for phenol removal involve many expensive methods like distillation, liquid-liquid extraction with the use of different solvents, adsorption, and membrane

pervaporation and membrane solvent extraction. However, such treatments are very complex and expensive which triggers the development of new technologies and methods for phenol removal. Biological treatments have shown to be economically viable, practical and the most promising one[10].

Despite of being toxic, phenol can be effectively utilized by microbes as carbon and energy sources. A number of studies on phenol biodegradation with the help of microorganisms have been carried out in past years. Both fungi and bacteria are known for the degradation of phenol and its derivatives whereas only a few members of yeast genera are capable of phenol degradation[11]. The aim of the present study was to isolate and characterize indigenous microorganisms, capable of degrading phenol, form industrial effluents and to establish optimal physiological parameters for phenol degradation.

II. MATERIALS AND METHOD

2.1 Sample Collection and Enrichment:

Soil and effluent samples were collected from industrial chemically contaminated sites surrounding Surat City, Gujarat, India. Samples were collected in sterile plastic container and immediately transferred to the laboratory and stored in refrigerator (4°C) till use.

Enrichment of the samples was carried out at laboratory condition by inoculating samples in nutrient broth medium with addition of 50 ppm of phenol. The flasks were kept on rotator shaker at 100 rpm. 10 ml of enriched samples were transferred in 100 ml fresh medium containing phenol. Such five successive transfers were carried out.

2.2 Isolation and Partial Characterization of Phenol Degrading Microorganism

From the enriched samples, a loop full of suspension was streaked on nutrient agar plate containing 100 ppm phenol. The plates were incubated at 37°C for 24-48 hr. After incubation the plates were observed for phenol degrading microorganisms. Partial characterization of the isolated organism was carried out by studying the morphological, cultural and biochemical characteristics.

2.3 Phenol Degradation Experiments

The isolated organism was subjected to study phenol degradation in 250 ml Erlenmeyer flasks containing 100 ml of Bushnell Hass medium containing 100 ppm phenol and 1% glucose as additional carbon source. The flasks were incubated on rotary shaker at 120 rpm. Samples were removed at regular interval aseptically and were phenol content was measured spectrophotometrically using 4-aminoantipyrene method (mentioned below).

2.4 Phenol Estimation Assay (4-Aminoantipyrine Method) [12]

Removed sample was taken in micro-centrifuge tube and centrifuge at 10,000 rpm for 20 min to remove biomass.1 ml of supernatant was added to 9 ml of Distilled water. To this mixture 0.5 ml 2N NH_4OH solution, 0.25 ml of 2% 4-aminoantipyrine solution and 0.25 ml 8% K_3FeCN_6 is added. Absorbance of red color is measured in spectrophotometer at 510 nm and compared with standard phenol solution curve.

2.5 Optimization of Various Physicochemical Parameters

2.5.1 Optimization of Ph: Effect of initial pH of the media on degradation of phenol was checked. Here, initial

pH of the BH media was adjusted to 5, 6, 7, 8, 9, 10 and 11. Inoculum (1.5 ml) was inoculated in 100 ml BH medium containing phenol. At regular interval samples were removed and checked for phenol degradation.

2.5.2 Optimization of Temperature: Effect of incubation temperature on degradation of phenol was studied. 1.5 ml of inoculums was inoculated in 100 ml BH medium containing phenol. Inoculated BH media was incubated at various temperature i.e. 25ºC, 27ºC, 29ºC, 31ºC, 33ºC, 35ºC, 37ºC, 39ºC, 41ºC.

2.5.3 Optimization of Additional Carbon Source and Nitrogen Sources: Three additional carbon sources i.e. glucose, lactose, sucrose and two additional nitrogen sources i.e. organic nitrogen source(urea), inorganic nitrogen source(ammonium chloride) were tested for the degradation of phenol at various concentration i.e. 0.2, 0.5 and 1.0%(w/v). 1.5 ml of inoculums was inoculated in 100 ml BH medium containing phenol and different concentration of additional carbon and nitrogen source. All flasks were incubated at 37ºC on shaker. Sample was removed for the estimation of degraded phenol at different time interval.

III. RESULTS AND DISCUSSION

3.1 Sample Collection and Enrichment

Seven different effluent and soil sediment samples were collected from three different chemically contaminated sites, namely Bamroli Khadi, Pandesara GIDC, and Sachin GIDC, Surat. Enrichment was done in nutrient broth media supplemented with 50 ppm phenol. The essence of enrichment technique is to provide growth conditions that are very favorable for the organism of interest, and unfavorable for competing organisms, and also increase relative population size [13,14].

3.2 Isolation and Partial Characterization of Phenol Degrading Microorganism

Phenol degrading organism was isolated, on the bases of its growth on the media containing phenol. As a result of enrichment technique, a typical colony of cream color was isolated. On the basis of morphological characteristics it may be tentatively identified as yeast. Based on cultural and biochemical conditions, the isolated organism was identified as *Candida Spp.*(Data not shown) [15].

3.3 Phenol Degradation Experiments

The isolated microorganism was tested for its potential to degrade phenol. The assay was carried out in BH medium supplemented with 100 ppm phenol. The phenol removal efficiency was observed 60% in 96 h (Fig.-1).

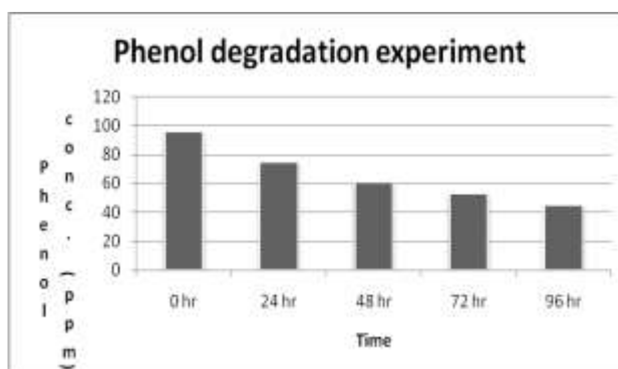

Fig.-1: Phenol Biodegradation Experiment.

3.4 Optimization of Physiological Parameters

Optimization of pH was done by adjusting the initial pH of media, result suggested that in maximum phenol degradation was observed at pH 7 (Fig.-2). Aysha OS [16] and Mumtaj K (2014) and Awan et al., (2013) [17] reported similar kind of results showing maximum phenol degradation at pH 7. Our results were supported by the work of Alexander and Robertson. They experimented that the majority of organisms could not survive in pH range below from 5.0 or above from 9.0. At high or low pH values acid or base could affect the enzyme activity of the cell [18].

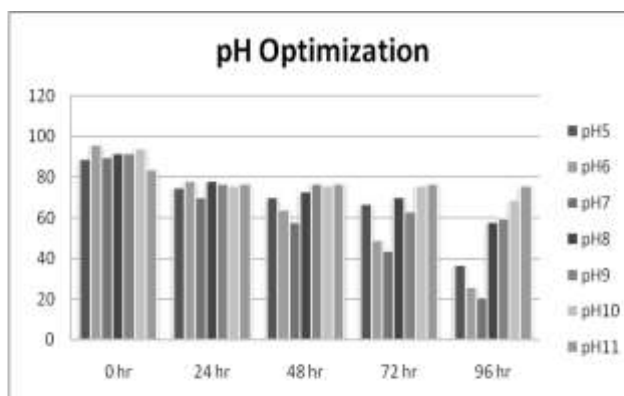

Fig.-2: ph OPTIMIZATION FOR PHENOL BIODEGRADATION.

Each life form has a minimum, optimum and maximum temperature for growth. For temperature optimization flasks were incubated at different temperature. These resulted in the maximum phenol degradation at 31 ºC temperature (Fig.-3). Chakraborty et al., (2010) also reported the same results for optimization of temperature for phenol degradation [19].

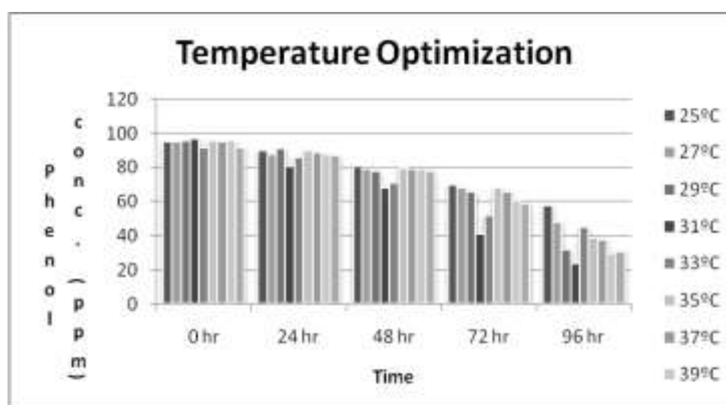

Fig.-3: Temperature Optimization for Phenol Biodegradation.

Three different carbon sources glucose, lactose and sucrose were used at the concentration of 0.2%, 0.5% and 1.0% for the optimization of additional carbon source. The maximum phenol degradation was observed in flask containing 1% glucose (Fig.-4) whereas there was no increase in the rate of phenol degradation when lactose (Fig.-5) and sucrose (Fig.-6) were added. In the previous study by the author, it was observed that 0.5% glucose was optimum for maximum biodegradation of Phenol by *Staphylococcus aureus* [20].

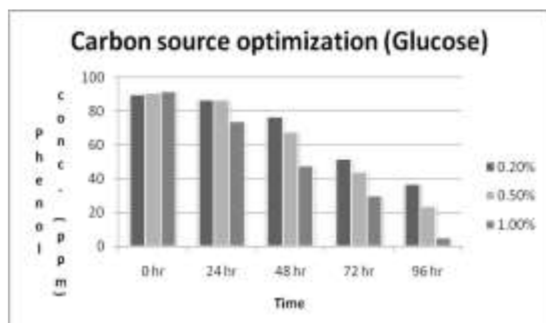

Fig.-4: Carbon source (Glucose) optimization for phenol biodegradation.

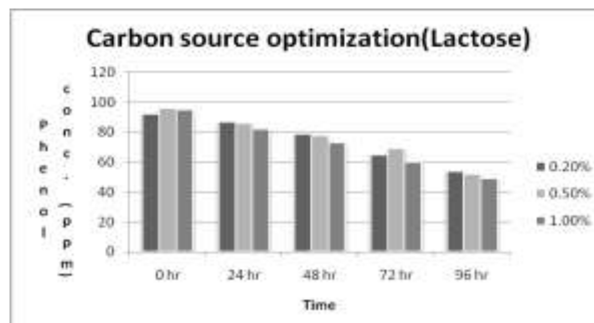

Fig.-5: Carbon source (Lactose) optimization for phenol biodegradation.

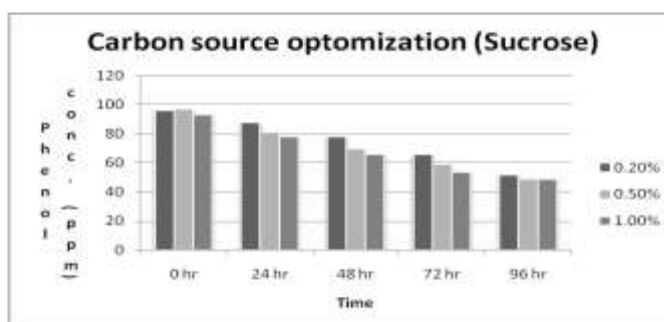

Fig.-6: Carbon Source (Sucrose) Optimization for Phenol Biodegradation.

Two different nitrogen sources (urea and ammonium chloride) were tested for the maximum phenol degradation by the isolated organism. The maximum phenol removal was observed at 1.0 % of urea (Fig.-7) and 1.0% of ammonium chloride (Fig.-8). The result suggest that ammonium chloride has impact on degradation of Phenol [20].

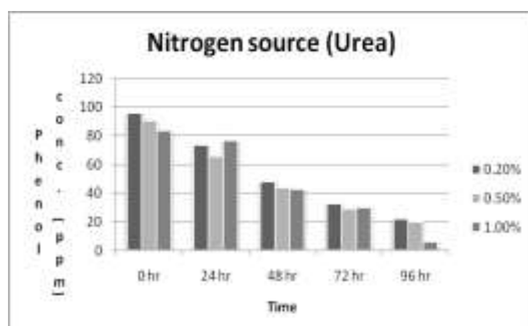

Fig.-7: Nitrogen Source (Urea) Optimization For Phenol Biodegradation.

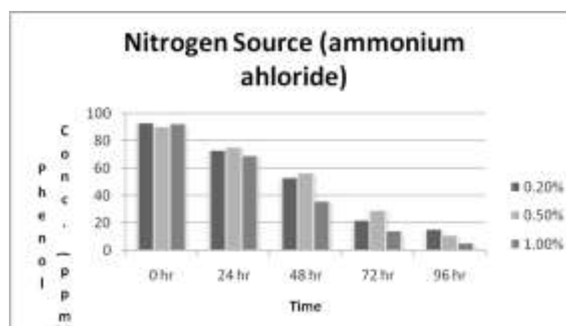

Fig.-8: Nitrogen Source (Ammonium Chloride) Optimization For Phenol Biodegradation.

IV. CONCLUSION

From this research report it can be concluded, isolated *Candida Spp*. had potential to degrade phenol. Results of optimization suggest that it can survive under harsh condition of effluent. It can be exploited as a potential degrader of phenol present in industrial effluent. Isolated strain can be used in bioremediation treatment of the industrial effluent.

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