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Research Article

BIOLOGICAL REMOVAL OF PHENOL FROM WASTE WATER USING A BACTERIAL BIOFILM

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ABSTRACT

In the present study, the uptake of Phenol was carried out by *B.cereus* DZ4. Screening of phenol tolerance was done by adding the culture to different concentrations of phenol in nutrient broth. Biodegradation of phenol was quantities using the standard method of Phenol analysis. Biological treatment for phenolic compounds using biofilms indicated that the uptake was highest at 50 µg/ml concentration. The dry weight of cells decreased by only 15% on increasing the concentration of phenol. Phenol uptake was in the 96% range, more uptakes were noted at higher concentrations of Phenol.

INTRODUCTION

Phenol is used to make pharmaceuticals, synthetic resin, dyes, perfumes, pharmaceuticals, 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. Especially phenolic compounds are often found in wastewaters from coke-oven batteries, coal gasification refinery and petrochemical plants and other industries, such as herbicides, synthetic chemicals, pesticides, pulp-and-paper, photo developing chemicals, antioxidants, tannery and foundries (washing of the gas effluents) etc. Thus, the presence of phenols in water generally comes from this industrial pollution. The increasing presence of phenols represents a significant environmental toxicity hazard (McCarty *et al*., 2008). On account of its toxicity it is thus recommended to fully remove the phenol from industrial effluents before it enters the water stream. Biological strategies are part of green technology that is cost effective and eco friendly (Ganguly and Tripathi, 2002).

The occurrence of Phenol was widespread in waste water as well as stored water samples procured for the study. Out of a number of methods available for detoxification of Phenol, biofilm based uptake is also a promising new process. It has been reported by Christenson (1989) that biofilms comprise the normal environment for most microbial cells in many natural and artificial habitats, and as such are complex associations of cells, extracellular products and detritus either trapped within the biofilm or released from cells which have lysed as the biofilm ages. They make best use of the concentration difference, which is known to be the driving force for sorption, and allow more efficient utilization of the sorbent capacity, resulting in better effluent quality (Aksu and Gonen, 2004).

Microbial degradation is a useful strategy to eliminate organic compounds and detoxify wastewaters and polluted environments (Gallego *et al.,* 2003). Phenol is degraded by diverse microorganisms including yeasts, fungi and bacteria (Table 1). Because of widespread occurrence of phenol in the environment, many microorganisms utilize phenol as the sole carbon and energy source which includes both aerobic and anaerobic microorganisms (Basha *et al.,* 2010).

From the literature review it was found that biodegradation is one of the most efficient methods for removal of toxic organic material like phenol from industrial effluent. Phenol and its derivatives are toxic and classified as hazardous materials. The phenolic compounds possess various degrees of toxicity. Even when it is present in waste water in low concentrations can be toxic to some aquatic species and causes taste and odor

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problems in drinking water. That's why; the removal of such chemicals from industrial effluents is necessary. It was found that the degradation mainly depends on bacterial growth and the growth of bacteria highly dependent on concentration of carbon source, pH and incubation temperature. The present study was attempted for removal phenol from waste water using biofilm of *B. cereus*

MATERIAL AND METHODS

Sample collection: Collection of waste water samples were done from different region of Aurangabad, Maharashtra State India. All the samples were collected in polythene bags and processed on the same day for the isolation of phenol degrading bacteria.

Isolation and screening of phenol degrading bacteria: Waste water samples were suspended in Basal salt medium (composition) and kept for growth at 30^0C for four days for enrichment. This suspension was further inoculated in Nutrient agar medium and Hichrome Bacillus agar medium for isolation of bacteria.

Characterization and identification of the strain

The strain was characterized as per biochemical and colony characteristics and also by 16s rDNA sequencing at NCIIM, Pune, Maharashtra, (India) and can be accessed at GenBank KF906248, NCBI). (Bhattacharya ,2014).

Screening for phenol tolerance

Screening of phenol tolerance was done by adding the culture to different concentrations of phenol in nutrient broth. After incubation for 24 to 48 hrs, the optical density at 600 nm was measured along with the wet weight by centrifuging one ml of the sample at 10,000 rpm for 10 minutes.

Development of biofilms

Biofilms were developed on previously sterilized small stones placed in glass separating funnels. Nutrient broth (Deshmukh, 1997) was used for buildup of the culture to be used for further biofilm development. A culture of *Bacillus cereus* DZ4 (Bhattacharya, 2014) was used for this study. The media was autoclaved separately along with stock solutions of phenol (10, 30, 50, and 70) ppm. The biofilm development was carried out at 10 µg/ml in fresh nutrient broth that was added to the separating funnel containing the presterilised stones. Biofilm development was allowed till the supernatant showed an optical density of 2.0 at 600nm. The residual media was drained out and biofilm was washed with distilled water without dislodging the cells. The previously calculated phenolic solutions (10 to 70 µg/ml) was added one by one to the developed biofilm and initial and final uptake were noted after 24h to 48h at room temperature. Samples removed from the generator were centrifuged a 10,000 rpm using a high speed centrifuge (REMI R24) to settle the cell debris and the supernatant was quantitated using the 4-amino antipyrine method.

(a) **(b)** **(c)**

Figure 1 a,b,c Biofilm developed on gravel for the treatment of Phenol, 2, 6 –Dichlorophenol and 4-Nitrophenol

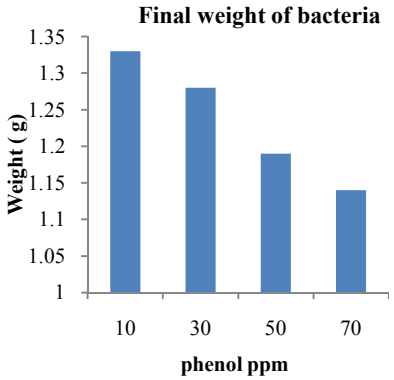

Figure 2 Dry weight of bacterial cells in presence of Phenol

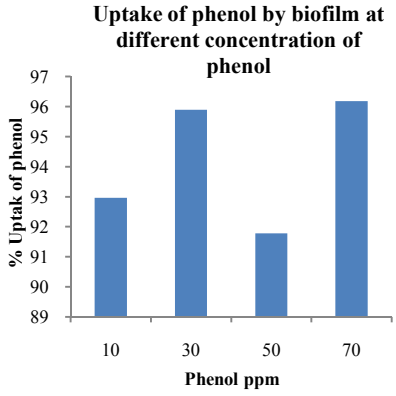

Figure 3 Uptake of different concentrations of Phenol in a bacterial biofilm

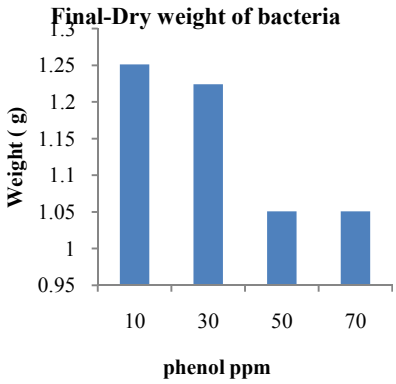

Figure 4 Bacterial dry weight in presence of different concentrations of Phenol

Biodegradation of phenol

Phenol analysis

An aliquote of the solution was removed immediately after addition of phenol to record the initial phenol present and similarly after 48h to note the residual phenol present in the sample. Phenol was estimated following 4- Aminoantipyrine method **(**Sinha *et al.,*2014**).** The concentrations were calculated from the standard curve prepared by using gradient concentrations of phenol. The color that develops after adding regents was measured at 510 nm using an UV –Vis Spectrophotometer (ElicoSL-244).

RESULTS AND DISCUSSION

A luxurious biofilm developed on small pebbles in glass separating funnels using nutrient broth as the growth medium. (Fig a,b,c).

It may be noted as per fig 2, that the dry weight of bacteria ranged from 1.33 to 1.14 at concentrations 10 and 70 µg/ml. A seven fold increase in Phenol concentration only reduced the dry weight by less than 15% .This shows that indicating that there is no marked toxicity exhibited by Phenol on the bacterial cells in the biofilm. Ayati *et al.,* (2009) observed that biomass can be used as a biosorbent as it mostly consists of bacteria with cell walls that fundamentally contain a variety of organic compounds for example acidic polysaccharides, lipids, amino acids, chitins, and other cellular components.

It may be noted as per fig 3 that uptake of Phenol ranged from 93% to 96% thereby showing a higher uptake at higher concentrations of Phenol. At 30 and 70 µg/ml, the uptake was found to be higher. The lower uptake at 10 and 50 µg/ml may be because of low culture density in the biofilm. It may be noted as per Fig 3 that the There are differences in dry weight observed due to unequal dislodgement of the bacteria from the biofilm, thus in some cases a lower dry weight may be noted at lower concentrations of Phenol (Figure 4).

It has been reported by several researchers that among the different column configurations, packed bed columns have been established as an effective, economical and most convenient for biosorption processes (Zhao *et al*., 1993; Saeed and Iqbal, 2003). It has been reported by several researchers including Burghate and Ingole, (2013) that the media on which microorganisms grow is in fluidized state and therefore the surface of the media available for the development of microorganisms is quite large which leads to high concentration of microorganisms and thus high flow rate can be achieved in fluidized bed bioreactors. Ayati (2009) has also reported that the major biosorption mechanisms appear to be extracellular interactions, complexion and subsequent accumulation, inactive sorption at binding sites on the envelopes of cells, and intercellular accumulation.

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The strain used in the study has been isolated and characterized by the Dr.Aditi Bhattacharya as per biochemical and colony characteristics and also by 16s rDNA sequencing at NCIIM, Pune, Maharashtra. (India) and can be accessed at GenBank KF906248, NCBI).

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