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Studies on removal of Phenol from contaminated water source by microbial route using *Bacillus cereus*

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A B S T R A C T

Phenolic compounds, a priority chemical, are discharged in waste water from many industries and need to be removed. Among various techniques available for removing phenol, biological treatment has been proved to be very effective. The present investigation was undertaken to assess the potentiality of *B. cereus* strain in removing phenol from synthetic solution, which was previously isolated for Chlorophenol (CP) degradation studies. The organism was acclimatized in phenol environment and growth rate was evaluated. The acclimatized culture was able to remove 98 % of phenol in 60 hours from a solution containing 500ppm phenol. Thus the bacterium *B. cereus* could be used for simultaneous removal of phenol and CP.

Introduction

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 pharmaceutical, oil refineries, petrochemical plants, ceramic plants, steel plants, and coal conversion processes (Santos and Linardi, 2004). Due to the toxic properties, including permeabilisation of cellular membranes and cytoplasm coagulation, phenolic contaminants can damage sensitive cells and cause profound health and environmental problems (Tziotzios et. al., 2005). The World Health Organization has limited phenol concentration in the water to 1 mg/L (WHO, 1994). 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 (Yan et al., 2006). The presence of phenol

in water imparts carbolic odor to receiving water bodies and can cause toxic effects on aquatic flora and fauna (Ghadhi and Sangodkar, 1995). Hence, it is necessary to remove phenol from industrial effluents before discharging them into the environment. Many technologies exist for the treatment of phenols, but their use is limited due to the fear of creating toxic intermediates particularly from non-biological process. Many aerobic bacteria have been confirmed to use aromatic compounds as the sole source of carbon and energy (Paller et al., 1995) which suggests to use phenol as nutrient to the organism and thereby converts phenol to nontoxic component.

Biological methods for the removal of phenolic compounds are possible because some organisms have the capacity to degrade phenol utilizing it as their nutrients (Kanekar et. al, 1999, Catia et.al, 2010). *Bacillus cereus* is very effective in removing pollutant from waste water. *B. cereus* (JUBT1) isolated from sludge of chlor-alkali industry has been successfully used to remove Mercury from simulated waste water stream both in free and attached bio-film form (S. Ghosal et al., 2011).

A systematic study was undertaken to isolate and screen microorganisms, capable of degrading phenol and 4CP separately in our laboratory. *B. cereus PSMD5* is one of such organisms which has been found to be a potent strain for removal of chlorinated phenol.

The waste water contaminated with chlorinated phenol would also usually contain phenol. So it is utmost necessary to test the effect of phenol on the behavior of isolated *B. cereus.* Accordingly it is needed to know the potentiality of this strain whether it is inhibited by phenol or as to what far it can remove. Reliability of this organism in phenol vis-a-vis probability of degrading phenol by it has been compared in this study. In this work, biological removal of phenol by *Bacillus cereus* has been studied

Materials and Methods

Acclimatization in phenol

The isolated and identified strain *B. cereus* (soil sample from Dankuni coal complex, West Bengal) was grown on mineral salt medium (MSM) having the following compositions(gm/l): Lactose-15, Beef extract-3, KH_2PO_4-1, KCl-0.5, $NaNO_3$-2, $MgSO_47H_2O$-0.5, $FeSO_4$-0.01 and pH 7.0 at 30^oC.

B. cereus, a 4-CP reducing bacteria is required to be adapted to the phenol environment. During acclimatization process certain enzymes in the bacteria are induced so that they are available for taking part in the metabolism reaction.
The isolated strain was grown in MSM enriched by weekly addition of the synthetic solutions of different concentrations of phenol (100-1000 ppm) for 6 weeks. After enrichment, the representative organisms growing on the agar plates as colony were separated and grown independently on agar medium as well as broth medium containing phenol (100-1000 ppm). The strains capable of growing at these concentrations were selected and sub-cultured for our studies.

Growth in phenol

The experimental studies were carried out in 250 ml Erlenmeyer flask as batch reactor under pertinent process variables. 100 mL of batch volume was taken along with

phenol in which 5 % of overnight cultured cells were inoculated and kept in temperature controlled orbital shaker with 130 rpm at 30°C for different time intervals. Each experiment was repeated twice to get the best result.

Biodegradation experiment

The biodegradation potential of the selected strain *B. cereus* was evaluated by monitoring growth of biomass and analyzing residual phenol at regular interval of time in a bioreactor (New Brunswick, BIOSTAT 410) having 2 liters working volume with auto sterilization option.

The optimum conditions like pH, temperature, agitation, dissolved oxygen concentration as 7, 30^0C, 200 rpm and 100 ppm respectively were automatically maintained throughout the study in the bioreactor into which 5% of overnight cultured cells *of Bacillus cereus* were added. Sampling was done at regular interval of time to study the degradation of phenol and the biomass formation.

Analytical methods

The biomass concentration was observed in a spectrophotometer by using O. D. at 600 nm and expressed as dry cell weight unit (DCW, mg/l) from a standard curve earlier prepared by measuring the OD value and corresponding DCW measured by oven drying method.

Phenol was estimated following 4-Aminoantipyrine method (Martin, 1949, Emerson, 1943). The concentrations were calculated from the standard curve prepared by using gradient concentrations of phenol developing color after adding regents and measuring absorbance at 510 nm.

Result and Discussion

Acclimatization

B. cereus was first acclimatized in phenol. Growth medium was supplemented by 100, 200, 300, 500, 700 and1000 ppm phenol. The biomass was separated from the broth and cultured in petri-plate. Sufficient growth was observed for the organism supplemented till 500 ppm phenol and the culture that tolerates 500ppm phenol was taken for further studies.

Growth of organism

The adjoining figures (1and 2) show a distinct exponential growth phase followed by a stationary phase. However no lag phase is identified. Qualitatively such cell growth behavior is an indication of the situation whose cell growth kinetics follow classical substrate uninhibited Monod equation. The nature of the cell growth is almost identical for both presence and absence of phenol limited systems.

It is evident that the exponential region extends up to 60 hrs of incubation time at 30°C when the cells are very much active. Accordingly this period is considered for the next phase of study. The inoculum is taken from this growth region and to make consistency of all the experiments overnight culture was used.

The growth characteristics of the organism has been compared in figure 3. It is observed that the growth of the organism in presence of phenol has been affected due to the toxicity of phenol. Since the organism is resistant to CP and acclimatized with phenol it can survive. So this organism can be used for the removal of phenol. In the presence of phenol the growth rate becomes asymptotic after 24 hrs of incubation and

Fig.1 Cell growth in MSM

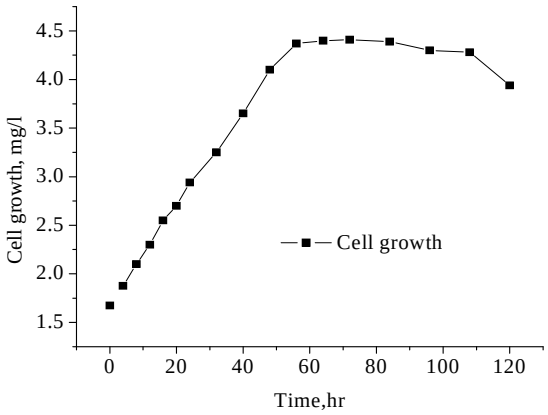


Fig.2 Cell growth in presence of phenol

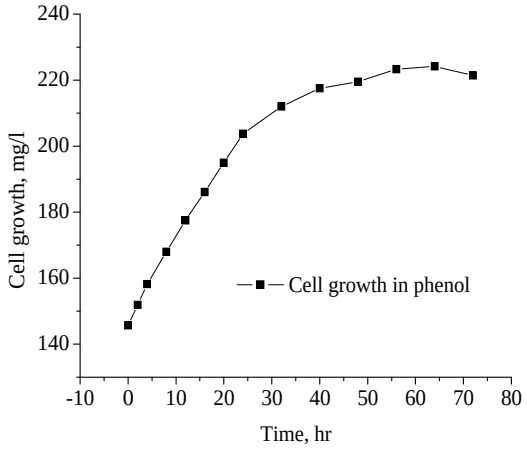


Fig.3 Comparison of growth of *B. cereus* in presence and absence

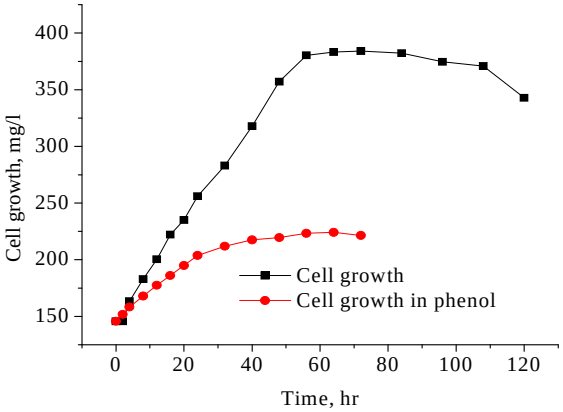


Fig.4 Effect of time on removal of phenol

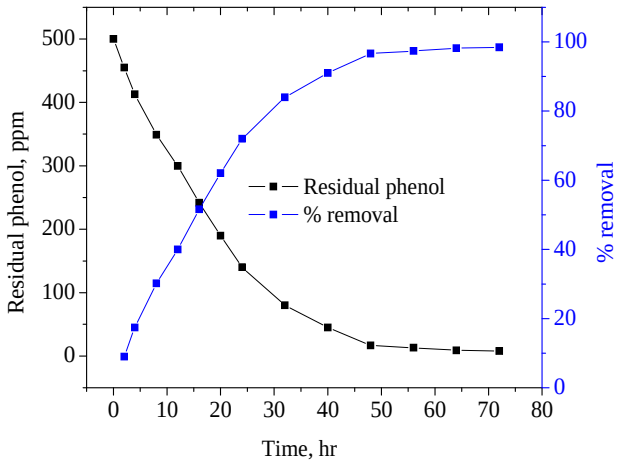
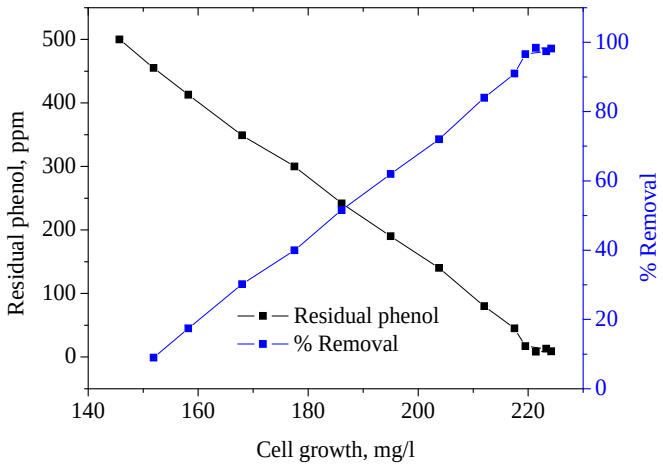


Fig.5 Removal characteristics of phenol



no substantial growth occurs after this period where as it is of the order of 60 hrs in absence of phenol. Phenol removal is associated with the growth of microorganism, hence the growth time is restricted to 24 hrs in further course of studies. One interesting observation is that the growth curve has two distinct specific growth rate one up to 24 hrs and the other from 24 hrs to 60 hrs for both the situations. The residual phenol concentration has been presented in Fig.4. It is found that the phenol is removed actively up to 50 hrs and at that time residual amount is 16 ppm. Rate of removal after this is not significant. Up to 24 hrs the rate is linear there after it is decreasing. 98% phenol removal has been achieved within 60 hrs.

The curve shows that the removal is linearly proportional up to 24 hrs and there after it decreases and becomes insignificant after 50 hrs. The growth curve (Fig. 2 or Fig. 3) signifies that the removal is closely related with the growth of the organism. If we look after the growth curve we found

that the growth rate is linear up to 25 hrs and decreases up to 50 hrs after which stationary phase starts. This signifies that the organism is consuming phenol as its substrate.

Removal efficiency of phenol with respect to cell growth has been presented in Fig.5. As the amount of cells in the medium is increased more number of cells are available to consume phenol and the phenol level in the solution depleted. But after the exponential phase the activity of the cells decreases and they no longer able to act on the remaining phenol in the solution with the same pace.

Bacillus cereus primarily isolated for the treatment of Chlorophenols can also be used effectively for the removal of phenol and it can grow in phenol solution having 500ppm initial concentration and can degrade phenol by 98.4 % after 60 hrs of treatment. Hence this strain has a remarkable potential for application in bioremediation of phenol and waste water treatment, especially in detoxification of phenol as wastes.

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