Biodegradation of phenol using pure and mixed Culture Bacteria

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ABSTRACT

Present study is aimed at biodegradation of phenol using pure and mixed culture bacteria. The experiments are carried out at ambient temperature and near neutral pH for 100mg/liter and 200mg/liter phenol concentrations. The Biomass growth and the removal efficiency for both cultures have been established. The time required for complete removal of phenol using pure culture was found to be higher than that of mixed culture for both concentrations of phenol considered. The lag phases for biomass growth for mixed as well pure culture have been established. Considering the problems associated with pure culture, use of mixed culture in place of pure culture is proposed.

Key words: Phenol, Bacteria, pure and mixed culture, biomass growth.

1.0 INRODUCTION

Phenol is an organic, aromatic compound that occurs naturally in the environment and its origin is both anthropogenic as well as xenobiotic [1]. They are regular constituents of coke processing unit waste waters. Even at relatively low concentrations of 5–25 mg/L phenol affects aquatic life. This imparts medicinal taste and odour to municipal drinking water. The presence of the compounds in inland waters creates lot of havoc and stress on eco-systems. [2, 3].

According to the U.S. Environmental Protection Agency, phenols represent a group of organics frequently found in various industrial effluents and wastewaters [4]. As per Hazardous Wastes (Management and Handling) amendment rules, 2000, phenol and phenolic compounds are classified under category of Class B (B.19) of schedule-II in the hazardous wastes list [5]. Moreover according to the environmental protection rules of the Central Pollution Control Board (CPCB) and IS: 2490-1974 the discharge limit of phenols in inland water is 1 mg/L and in public sewers as per IS: 3306-1974 is 5.0mg/l.[6, 7].

Heterotrophic bacteria cultures are able to survive on polyaromatic hydrocarbons under proper environmental conditions, seed source and acclimation time. Phenol can be oxidized by a wide variety of oxygenase producing bacteria. Non specific mono oxygenase or di oxygenase enzymes produced mediate a reaction with oxygen and hydrogen. [8]

Aggary et al., [3] investigated the phenol utilization kinetics of a pure culture of an indigenous Pseudomonas fluorescence under steady state and non-steady state (washout) conditions. Lin et al., [9] have worked with bio degradation of 2,4,6-trichlorophenol by Pseudomonas fluorescens. Hank et al., [10] studied on a bacterium, Pseudomonas aeruginosa (ATTC27853), for its ability to grow and to degrade phenol as sole carbon source, in aerobic batch culture. Prasanna et al., [11] studied on biodegradation of phenol and toluene by Pseudomonas sp., Bacillus sp., Staphylococcus sp and mixed culture. Swaminathan et al., [12] researched on

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biodegradation of 2,4- dichloro phenol using mixed culture for different organic loading rates in modified 4- stage RBC. Effect of inhabitation was observed in the studies using phenol concentration greater than 500 ppm. [13, 14]

Literature was available for biodegradation of phenol using pure and mixed cultures. But very few reports were available for biological degradation of toxic phenolic waste wasters using acclimatized cultures. The advantages of acclimatized culture is that, easy to adapt for different toxic wastes, simplicity of operation and maintenance. Acclimatized mixed culture has better advantage over pure culture, where in the substrate has to be sterile and more factors influence/retards its growth and performance. [12, 15]. Hence, in the present study a comparison of performance of pure and mixed culture is done for their capacity to degrade phenol.

2.0 Materials and Methods

In the present study, Phenol and other chemicals are of reagent grade were used without purification. The desired concentration of phenol solutions was prepared by using distilled water.

2.1 Reactor for batch study

During start up operation, the sequential batch reactor was filled with sewage obtained from activated sludge unit from local industry. Culture was incubated for developing the biomass. Synthetic sewage having the constituents indicated in Table 1 was used for the growth of microorganisms.

Table 1: Composition of Synthetic wastewater [12]

Constituent	Quantity (mg/l)
Glucose	1000
Magnesium Sulphate	100
Di Potassium hydrogen phosphate	1070
Potassium di-hydrogen phosphate	527
Urea	227
Calcium chloride	0.7

Batch cultivation experiments were carried out using phenol as single limiting substrate for mixed and pure culture. Loop full of biomass is transferred into reactor with 100mg/l and 200mg/l phenol. The extent of phenol degradation using these different initial phenol concentrations was investigated for several batch residence times by intermittent sampling at every six hour interval. The concentration of the phenol was determined using the colorimetric 4-aminoantipyerene method. [11, 12, 16] using UV-Vis spectrophotometer at 500 nm Wave length.

Results and Discussions

3.1 Batch studies

Fig.1 shows the variation of removal efficiency and biomass growth for mixed culture bacteria. BG in the figure refers to Biomass growth. It can be seen from the figure that complete removal of phenol removal with pure culture in 96 hours for 100mg/l phenol concentration where as for 200mg/l phenol concentration, 156 hours time is required for complete removal. The lag phase for pure and mixed culture was found to be

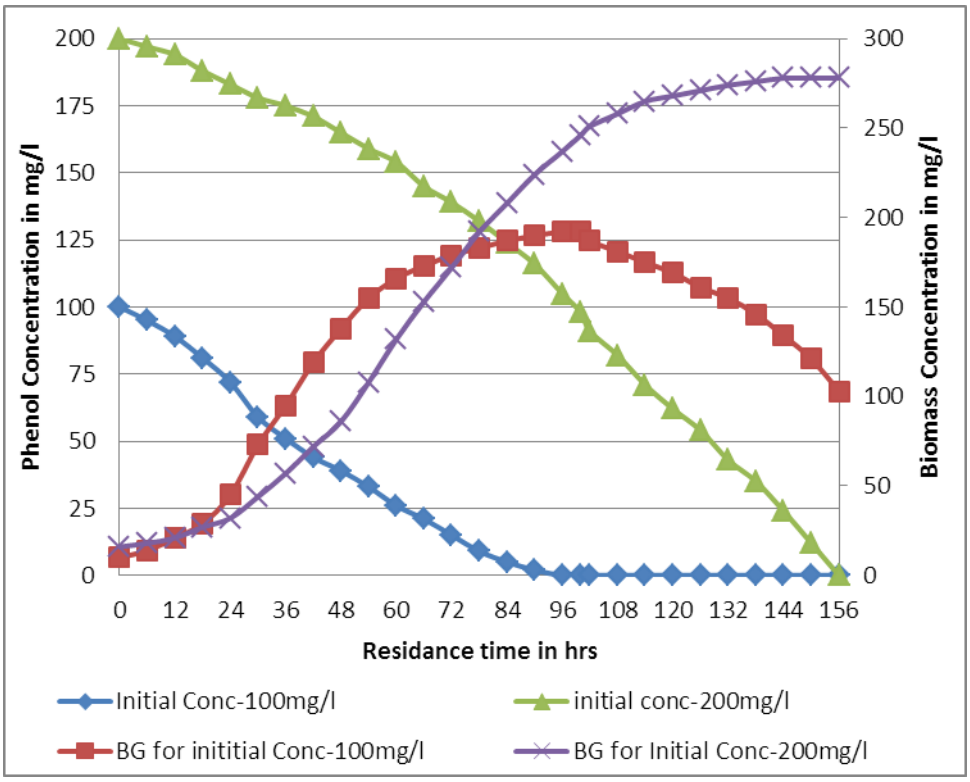

Figure 1: Substrate removal efficiency and Biomass growth with time for mixed culture bacteria

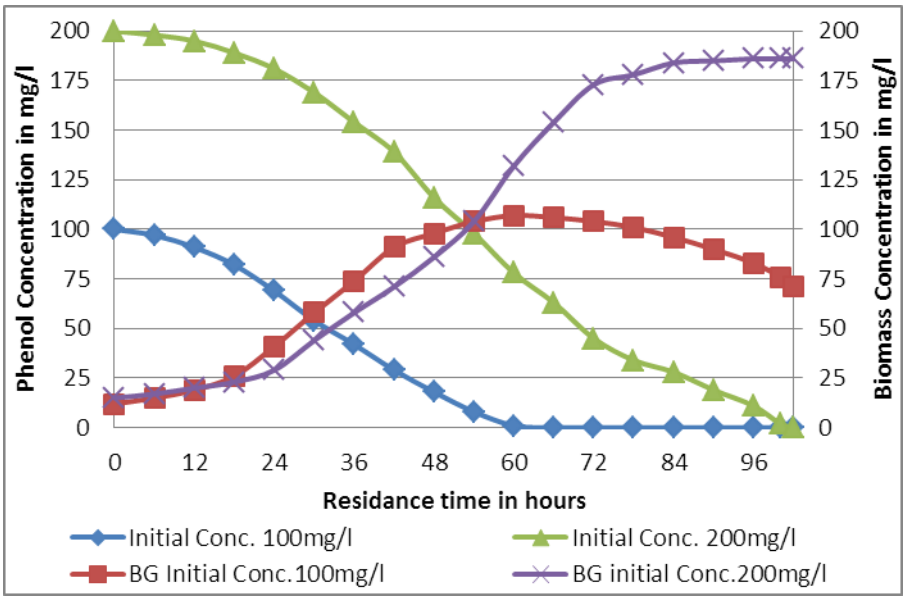

Figure 2: Substrate removal efficiency and Biomass growth with time for pure culture bacteria

The cell growth curve has typical exponential and stationary phases with increasing lag phase. The substrate with initial concentration 100mg/l was degraded in 96 hrs and 60 hrs by mixed and pure cultures with a lag phase of 12 hrs and18 hrs. System with substrate with 200 mg/l initial concentration was degraded in 156 hrs and 102 hours by mixed and pure cultures with lag phase of 18 and 24 hrs.

The culture was inoculated for 48 hours using glucose as substrate which had a strong effect on the length of lag phase. The batch reactor was operated with phenol as single

limiting substrate. Accumulation of the substrate induces toxicity by restructuring the cell. To minimize the duration of lag phase, cells should be adopted to the growth medium and conditions before inoculation. The medium contain phenol and glucose as substrates inducing multiple lag phases due to diauxic growth shifting metabolic pathways in the growth cycles leading to inhibitory effect. After lag phase cell mass density increased exponentially in which all components of cell grow at the same rate. Cellular metabolic control system showed maximum rates of degradation and reproduction during 30-66 hours for initial concentration of 200mg/l. The deceleration growth phase followed the exponential phase. In this phase, both the cultures growth decelerated due to depletion of nutrients exerting stresses on cell morphology increasing cellular survival in a hostile environment [17, 18].

Conclusions

1. Both the cultures even though takes a longer time for acclimatization, once acclimatized, accept phenol as sole source of carbon for their metabolic activities
2. The substrate with initial concentration 100mg/l was degraded in 96 hrs and 60 hrs by mixed and pure cultures with a lag phase of 12 hrs and18 hrs. System with substrate with 200 mg/l initial concentration was degraded in 156 hrs and 102 hours by mixed and pure cultures with lag phase of 18 and 24 hrs. This suggests that higher the concentration of substrate, higher the degradation time and higher the lag phase.
3. Since acclimatized mixed culture has better advantage over pure culture, where in the substrate has to be sterile and more factors influence/retards its growth and performance. [12, 15]. Hence use of mixed culture is proposed.

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