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Study On Effect Of Operating Parameters On Biodegradation Of Phenol By Aspergillus Fumigatus

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Abstract

Biodegradation of phenol by Aspergillus fumigatus was carried out in a batch stirred reactor. In the batch system studies, the effect of initial phenol concentration, pH, temperature and inoculums size on biodegradation rate was investigated .The maximum phenol removal yield of 94% was obtained at 100 mg/l initial phenol concentration. The phenol degradation rate was found to be increased with increasing concentration of phenol. Maximum dried organism concentration of 98g/l was obtained in the absence of phenol. Maximum degradation rate was achieved at pH 7.0,Temperature 30 ^{0}c and an inoculums size of 10%(v/v).In the range of phenol concentration used in the study, the degradation rate was observed to follow substrate inhibition kinetics .The specific growth rates of the culture at various initial phenol concentrations were fitted to Monod and Haldane models. Between the two models, the Haldane model was found to be a better fit with the experimental data. The inhibition was found to be a competitive inhibition for the degradation of phenol since the μ_{max} was same for other experiment in presence of phenol.

Keywords - phenol degradation.aspergillus fumigatus,kinetics

Introduction

Since world war II, depending on the increase of the world population and development of the industrial application, environmental pollution and other environmental problems became important. There has been a huge growth in the manufacture and uses of synthetic chemicals since the beginning of the 20 th century. There still are many possible sources of chemical contamination. These include wastes from industrial chemicals production, metal plating operations, and pesticide run off from agricultural lands, and the other industrial application and production. The number of organic compounds that have been synthesized since the turn of the century now exceeds half a million, and 10000 new compounds are added each year. As a result many of these compounds are now found in the wastewaters from most municipalities and communities.Currently,the release of volatile organic compounds (VOCs),non-volatile or semi-volatile organic compounds and volatile toxic organic compounds (VTOC) found in wastewater is of great concern in the operation of both collection systems and treatment plants. All industries use specific chemicals or the other raw material to produce their last products. Production has long

steps which is the total of many reactions. So, each process can produce hazardous wastes. A waste is considered a hazardous if it is reactive, ignitable, corrosive or toxic.

Ninety five chemicals have been defined as toxic including phenol on the basis of production volume, exposure, and biological effects. Organic compounds in water derive from the natural decomposition of plant and animal material from industrial, urban, or agricultural pollutants and from the reaction of halides with natural organics during water treatment. Concentrations range from none in protected ground waters to 10 – 30 mg/l in naturally productive or contaminated surface water. Hydrocarbons in these wastewaters are in many forms such as chlorinated hydrocarbons, halogenated hydrocarbon, organophosphates and non volatile or semi volatile or semi volatile aromatic hydrocarbons. Phenol ,as an aromatics semi volatile hydrocarbon, presents in wastewaters of most industries such as high temperature coal conversion, petroleum refining, resin and plastics, leather and textile manufacturing ,oil refineries, chemical plants, coke ovens, aircraft maintenance, foundry operation, paper-processing plant, paint manufacturing, rubber reclamation plants, nitrogen works and fiberglass manufacturing in different ranges from 1mg/l to 7000 mg/l.Phenolic

constitutes are 11th of the 126 chemicals which have been pointed as priority pollutants according to united states Environmental Protection Agency .

Materials And Methods

2.1 Microorganism.

Aspergillus fumigatus (MTCC No: 343) was obtained from Institute of Microbial Technology, Chandigarh, based on its ability to degrade phenol.

2.2 Preparation of growth medium

Czapek Yeast Extract Agar (CYA) medium was used as the growth medium for *Aspergillus fumigatus.*

Composition of Czapek Yeast extract Agar medium is made by Czapek concentrate 10ml, K_2HPO_4 1.0g, Yeast extract 5.0g, Glucose 30.0g, Agar 15.0g, Distilled water 1000ml.

Composition of Czapek concentrate is prepared by $NaNO_3$ 30.0g, KCl 5.0g, $MgSO_4.7H_2O$ 5.0g, $FeSO_4.7H_2O$ 0.1g, Distilled water 100ml.Czapek concentrate can be stored without sterilization. The precipitate of Fe $(OH)_3$ can be resuspended by shaking well before use.

2.3 Procedure for maintenance of cells

The culture *Aspergillus fumigatus* was maintained in the agar media. Agar-Agar was used for the preparation of slants. For growing the media on a large scale, fresh culture was transferred to 100 ml of liquid media containing the Czapek Yeast Extract medium without agar. The media was left for growth of 7 days. The 100ml media was transferred in to a 500 ml media and these cultures was used for further studies.In all the cases, the media was autoclaved under 121°c at 1.1Kgf/cm^2 guage pressure for 15 minutes and strict asceptic conditions should be maintained. Throughout the experiment precautions were taken while inoculating and transferring the culture.

2.4 Preparation of phenol solution

The test solution containing phenol was prepared by diluting the stock solution to the desired concentrations. The phenol concentrations were varied in the range of 50 to 500 mg/l. Stock solution of aqueous phenol was prepared by dissolving the exact quantity (1g) of phenol in double distilled water.

Experimental Procedures

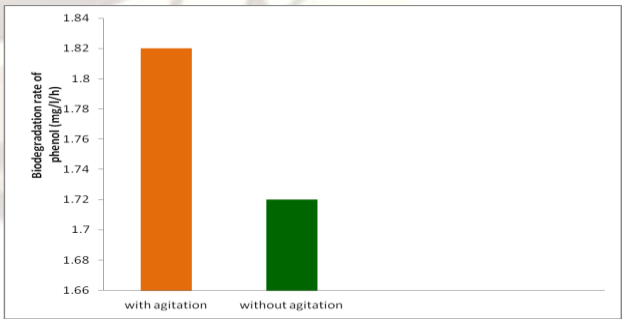
The factors affecting the growth and phenol degradation rate of growing *Aspergillus fumigatus* were examined in 250ml Erlenmeyer flask with 50ml accumulation medium. The accumulation medium was prepared by mixing 25 ml of aqueous phenol solution with 25ml of Czapek Yeast Extract medium. The pH of the Czapek Yeast Extract medium was adjusted to the desired value by adding acid or alkali solutions. CYA medium was autoclaved separately at 1.1Kgf/cm^{2}guage pressure for 15 min. A known amount of microorganism suspension (10%(v/v)) was added to the accumulation medium and the cultures were grown at 30°c for 7 days on a rotary shaker at 100rpm constant shaking rate. This shaking frequency supplied the culture with enough oxygen to attain logarithmic growth. For each phenol concentration a non-inoculated media was served as blank .The dry samples were drawn at predetermined time intervals and analyzed for residual phenol concentration and biomass concentration. The residual phenol concentration in the medium was determined by 4-amino antipyrene method at 510nm using UV-spectrophotometer. The dry weight of *A.fumigatus* was determined by drying the organism in an oven at 40°C for 2hrs.

Results And Discussion

4.1.Effect of agitation

To study the effect of agitation, experiments were conducted under shaking and static conditions by keeping the inoculated cultures on a rotary shaker at 100rpm shaking speed and resting conditions. The initial phenol concentration of 400mg/l, pH of 7.3 and at a temperature of 30°C was used for this study.

The phenol degrading ability of *Aspergillus fumigatus* was investigated by testing the shaking speed compared to static conditions. Table 4.1.1, Table 4.1.2, and Fig 4.1.1 shows the phenol degradation rate by *Aspergillus fumigatus* under shaking and static conditions. 1.82mg/l-h degradation rate of phenol was obtained for agitation condition as compared to 1.72mg/l-h degradation rate under static conditions. Shaking speed of 100rpm was used for further studies.



4.1.1 shows the phenol degradation rate by Aspergillus fumigates

4.2.Effect of initial concentration

Initial phenol concentration plays an important role in the biodegradation process, since some hydrocarbon contaminants, including phenol are known to have inhibitory effect on the activity of the biomass. Experiments were carried out at different initial phenol concentrations ranging from 100 mg/l to 500mg/l. The temperature was fixed at 30°c and the inoculum size was 10% (v/v). Table 4.2.1 and 4.2.2 shows the values of biomass concentration and phenol degradation due to the effect of initial phenol concentration by *A.fumigatus*. Fig 4.2.1 shows the effect of initial phenol concentration on biodegradation of phenol .Fig4.2.2 and 4.2.3 shows the effect of initial phenol concentration on phenol removal yield and biodegradation rate of phenol. The maximum phenol removal yield was determined as 94% at 100 mg/l initial phenol concentration. Phenol degradation rate increases from .55mg/l/hr to 2.14 mg /l/hr with increasing initial phenol concentration from 100 mg/l to 500mg/l and is given in fig 4.2.3. Lower biodegradation rate at low phenol concentration is believed to be due to mass transfer control, where less phenol is accessible for the biomass.

The initial phenol concentration in the feed medium varied in the range of 100mg/l to 500mg/l remarkably influenced the microbial growth rates and maximum dried biomass concentrations as shown in Table 4.2.1. Fig 4.2.4 indicates the biomass profile of the *A.fumigatus* at various initial phenol concentrations. Maximum dried microorganism concentrations of 98g/l was found in the absence of phenol. Raising the level of phenol concentration in the culture medium caused a reduction in the final biomass production. Phenol degradation was also highest at the end of growth. The presence and increasing of phenol concentration in the growth medium caused inhibition on the growth of microorganism.

Table 4.2.5 shows the effect of initial phenol concentration on the specific growth rate of *A.fumigatus.* It could be seen in Fig 4.2.5 that the maximum specific growth rate occurred at very low phenol concentration of 100mg/l. Generally a low specific growth rate indicates very intense substrate inhibition to the strain of *A.fumigatus*. But with the increase of initial phenol concentrations in the medium, the specific growth rate gradually decreased from 0.31 h^{-1} to 0.21 h^{-1} because of the lack of carbon source in the medium. The lower the phenol concentration in the medium, the weaker the substrate inhibition exhibited. More energy was required for *A.fumigatus* to overcome the effect of substrate inhibition at high phenol concentrations.

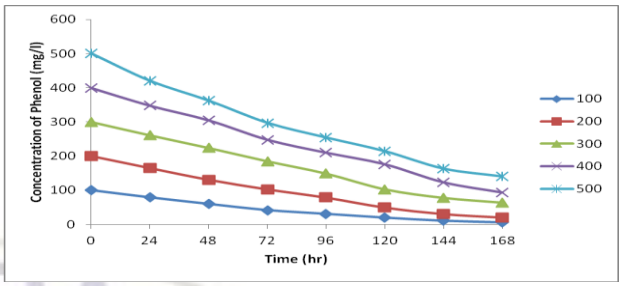


Fig.4.2.1 Effect of initial phenol concentration on biodegradation of phenol

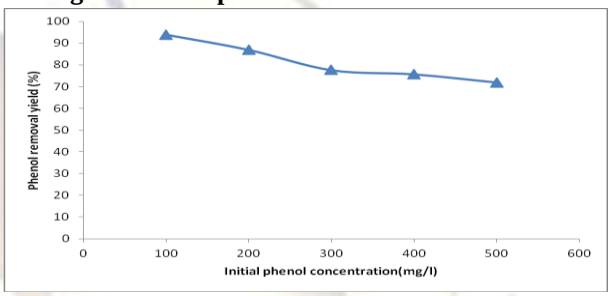


Fig: 4.2.2 Effect of initial phenol concentration on phenol removal yield

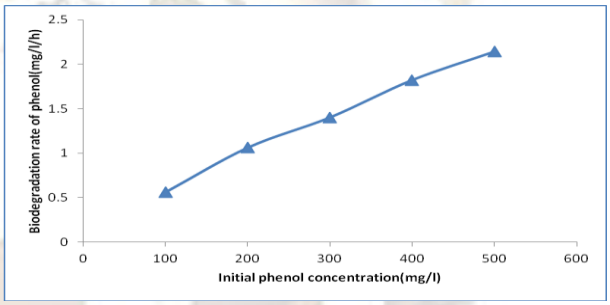


Fig: 4.2.3. Effect of initial phenol concentration on biodegradation rate of phenol

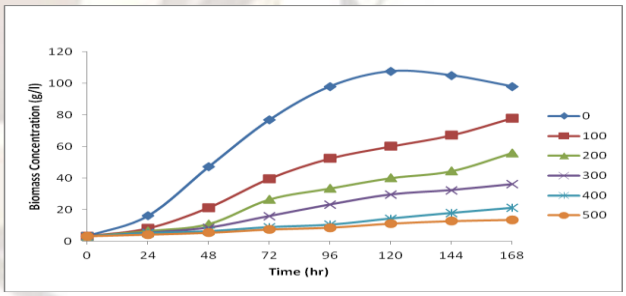


Fig.4.2.4: Biomass profile of the culture at various initial phenol concentrations.

4.3 Effect of temperature on biodegradation of phenol

Experiments were carried out to assess the effect of temperature on the biodegradation of phenol. Temperature might play an equivalent or larger role

than nutrient availability in the degradation of organic pollutants. All other parameters were kept constant, while varying the temperature from 30°C to 45°C. Table 4.3.1, 4.3.2 and 4.3.3 indicates the effect of temperature on degradation of phenol by *A.fumigatus*. A plot of the degradation rate of phenol versus temperature is shown in fig 4.3.1. From fig, 1.83 mg/l/h biodegradation rate was obtained at temperature 30°C and a slight increase in degradation rate of 1.84 mg/l/h was obtained at temperature of 35°C. Only slight variation of degradation rate was observed as the temperature was increased from 30°C to 35°C. Higher temperatures seen to negatively affect the activity of the fungal culture and hence hindered its biodegradation capabilities. It is believed that sudden exposure to temperatures higher than 35°C may have detrimental effect on the fungal enzymes, when the temperature increased beyond 35°C, mild phenol degradation was observed due to cell decay, which is a temperature –dependent parameter. However, in the current study, *A.fumigatus* was able to degrade phenol up to 45°C.

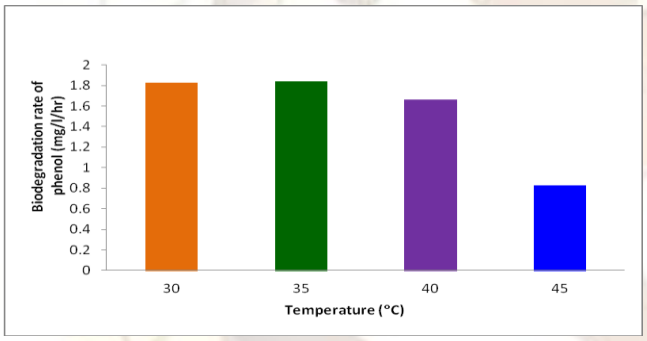


Fig4.3.1.Effect of temperature on biodegradation rate of phenol by *A.fumigatus*

4.4 Effect of pH on phenol degradation by *A.fumigatus*

pH plays an important role in the biodegradation of phenol by *A.fumigatus*. A batch test of phenol degradation with an initial phenol concentration of 400mg/l was conducted with *A.fumigatus* at pH 3-9.The experimental results for the effect of initial solution pH on the growth, concentration and the biodegradation rate of phenol are shown in table 4.4.1, 4.4.2 & 4.4.3 and in fig 4.4.1.These results reveal that the biodegradation rate increases with solution pH reaching a maximum value of 1.82 mg/l/h at a pH of 7.0. These behaviours are consistent with those reported in the literature or the biodegradation of phenol. At very low or high pH values, acids or bases can penetrate in to cells more easily, because they tend to exist in undissociated form under these conditions and electrostatic force cannot prevent them from entering cells.

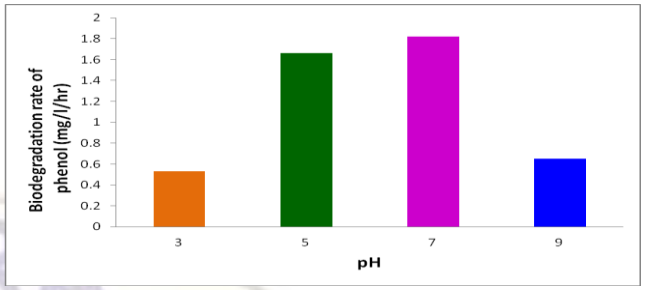
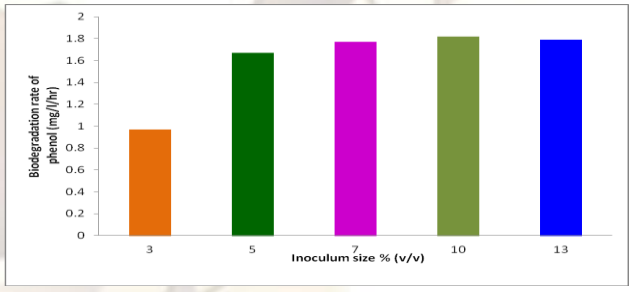


Fig.4.4.1.Effect of pH on biodegradation rate of phenol by *A.fumigatus*.

4.5. Effect of inoculum size on the growth of *A.fumigatus* and biodegradation rate of phenol.

A sufficient quantity of inoculums ensures rapid proliferation and biomass synthesis in cultivation. Phenol degradation at an initial concentration of 400 mg/l was achieved at an inoculum concentration of 3-13% (v/v) at pH 7.5 and 30°C. At low inoculums concentrations (3-5%), microbial growth had a prolonged lag phase when the inoculums concentration was increased to 7-10 %(v/v), the lag phase was largely eliminated. A sufficient quantity of inoculums was required to minimize the duration of the lag phase, increase the degradation rate, and induce the exponential growth phase after seeding. In this study optimum inoculums size was found to be 10 % (v/v) with the biodegradation rate of 1.83mg/l/h of phenol.



Conclusion

Biodegradation of pollutants by *Aspergillus fumigatus* in batch and packed bed reactor is a technically efficient and economically feasible technology for removal of organic pollutants. In the present research, the biodegradation of phenol from aqueous solution by suspended and immobilized *Aspergillus fumigatus* was performed in a batch stirred and in a packed bed reactor.

- From the batch studies, 94% phenol removal yield was obtained at 100 mg/l initial phenol concentration.

- Maximum amount of 98g/l dried biomass concentrations was obtained in the absence of phenol.

- The phenol degradation rate was found to be increased with increasing concentrations of phenol.

The optimum value of pH, temperature and inoculums size was found to be 7.0, 30^{o}C and 10% (v/v) for 400mg/l initial phenol concentration

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