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Comparative Biodegradation Analysis of Phenol from Paper & Pulp Industrial Effluent by Free and Immobilized Cells of *Aspergillus Niger*

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Abstract. Biodegradation of industrial phenol by a fungal isolate *Aspergillus niger* was studied in batch flask system with synthetic & industrial effluent. *Aspergillus niger*, was efficiently immobilized on sodium alginate beads. The immobilized cells were used in the batch culture flasks for paper & pulp industry as well as synthetic effluent phenol removal. All the flasks were operated at temperature 25°C at 125 rpm for five days in continuous mode. The immobilized cells showed over all better performance as compared to free cells. The average overall pH, Temp., Conductivity, B.O.D, C.O.D, T.S, T.D.S, T.S.S, Chlorides and phenol were upto 7.5, 34.75°C, 39.6 µmohs/cm, 139.5 mg/l, 430.5 mg/l, 1490 mg/l, 900 mg/l, 590 mg/l, 281.25 mg/l and 268 mg/l respectively. While culture with immobilized cell reached 110 mg/L whereas in free cell it is 119 mg/L in industry effluent whereas in synthetic effluent culture with immobilized cell reach 28 mg/L whereas in free cell it is 150 mg/L with the same conditions. Reduction in phenol level proved the biodegradation.

Keywords: Biodegradation, *Aspergillus niger*, Batch culture, Phenol, Immobilized cells.

1. Introduction

Phenol and its derivatives is the basic structural unit in a wide variety of synthetic organic compounds. (Annadurai et. al , 2000). Phenol and its higher homology are aromatic molecules containing hydroxyl group attached to the benzene ring structure. The origin of phenol in the environment is both industrial and natural. Phenol pollution is associated with pulp and paper mills, coal mines, refineries, wood preservation, plants & various chemicals industries as well as their wastewaters (Paula and young, 1998). Due to their high inhibitory and antibacterial activity, phenols may create problems in the operation of biological treatment plants (Rigo and Alegre, 2004). They also add odour to drinking and food processing water (Adav et.al, 2007) and have mutagenic and carcinogenic effects (Bolanos et.al, 2001). Phenol is also a priority pollutant and is included in the list of EPA (1979).

Biological processes using microbial systems provide an alternative to the existing physical/ chemical technologies (expensive and commercially unattractive) because they are more cost-effective, environment friendly and do not produce large quantities of sludge (Kariminiaae – Hamedaani et al., 2007; Asad et al., 2007). No. of microorganisms can utilize phenol under aerobic conditions as source of carbon and energy (Chen et al., 2002; Hidalgo et al. 2002; Riso and Alegre, 2004; Santos and linardi, 2004; yan et al.,2005 ; Adav et al., 2007; Juang and Wu,2007; Nair et al., 2007). Biodegradation is used to describe the complete mineralization of the starting compound to simpler ones like CO_2, H_2O, NO_3 and other inorganic compounds (Atlas and Bartha, 1998). Microbial degradation of phenol with different initial concentration ranging from 50-2000mg/L have been actively studied using shake flask, fluidized- bed reactor, continuous stirred tank bioreactor, multi stage bubble column reactor, air lift fermenter and two phase partitioning bioreactor methods (Bettmann and Rehm, 1984; Sokal, 1988; Annadurai et al., 2000; Reardon et al., 2000; Ruiz-ordaz et al., 2001; Oboirien et al., 2005; Saravanan et al., 2008) and these studies have shown that phenol can be aerobically degraded by wide variety of fungi and bacteria cultures such as *Candida tropicalis* (Chang et al., 1998) *Pseudomonas putida* (Hill and Robinson,1975).

The efficiency of the phenol degradation could be further enhanced by the process of cell immobilization (Annadurai et al., 2000). Various methods have been described for the immobilization (Klein and Wagner, 1983). Alginates represent however several advantages such as high porosity and chemical stability with a mild, fast, simple and low cost immobilization method (Fukui and tanaka, 1982). Under many conditions, immobilized cells have advantages over either free cells or immobilized cells. The biodegradation of phenol by immobilized cells had been investigated for several microorganisms but the use of immobilized *Aspergillus niger* cells for phenol biodegradation is scanty. This study has the results obtained from the biodegradation of phenol by free and immobilized cells of *Aspergillus sp*. Batch experiments were carried out in order to obtain the maximum phenol biodegradation rates by analyzing the influence of the immobilization in sodium- alginate gel beads on biodegradation performance.

2. Material & Methods

2.1. Chemicals and reagents

All the chemicals used were of analytical grade and the chemicals were supplied by Qualigens Fine chemicals (Mumbai, India).

2.2. Characterization of industrial effluent

The effluent was collected from Anand tissues ltd. (Meerut) which is an paper industry. The effluent collected from industry, was analyzed for different physico- chemical properties (Table-1) viz Temperature, pH, Conductivity, B.O.D, C.O.D, T.S, T.D.S, T.S.S, Chlorides, Phenol. The concentration of each of the component was determined as per the procedure outlined in APHA (2005).

Table 1: Physico- Chemical parameters of effluent

S.No	**Physico – chemical parameters of effluent samples for respective bioassay tests. Parameter**	**Test**	**Standard**
1	Temp	34.75ºC	-
2	pH	7.5	-
3	Conductivity	39.6 µmohs/cm	5 µmohs/cm
4	B.O.D	139.5 mg/L	30 mg/L
5	C.O.D	430.5 mg/L	250 mg/L
6	T.S	1490 mg/L	1200
7	T.D.S	900 mg/L	1000
8	TSS	590 mg/L	200 mg/L
9	Chlorides	281.25 mg/L	1000 mg/L
10	Phenol	268 mg/L	1 mg/L

2.3. Synthetic effluent

Sterile synthetic effluent composition (mg/l) was proposed by Passos et al. (2009): KH_2PO_4; 200 $MgSO_4.7H_2O$; 100 NaCl; 25 $Cacl_2.2H_2O$; $3MnSO_4.H_2O$; 500 $NH_4NO_3.H_2O$; 500 Glucose; Phenol (250 or 500).

2.4. Isolation, growth and processing of cells

Aspergillus niger previously isolated from paper industry effluent, and was capable of using phenol as carbon source (Santos et al., 2008). Strain was maintained on potato dextrose agar (PDA) at 4°C. For mass culturing, liquid broth was used as a culture medium which was having the following composition (gl^{-1}): 20 Dextrose; 10 Peptone; 0.2 NaCl; 0.1 $CaCl_2.2H_2O$; 0.1 KCl ; 0.5 K_2HPO_4; 0.05 $NaHCO_3$; 0.25 $MgSO_4$; and 0.005 $FeSO_4.7H_2O$. The liquid phase pH was adjusted to 4.5 by using the 0.1 M HCl and 0.1N NaOH. The liquid broth was inoculated with a loop of culture grown on PDA medium and incubated on an orbital shaker (Orbitek, sci, Genics Biotech Ltd) at 125 rpm and 25°C for 5 days in 500 ml conical flasks. The biomass produced was collected by filtration and washed twice with extra pure double distilled water.

2.5. Fungal spore immobilization

Spores were immobilized in calcium alginate according to Ellaiah et al. (2004). About 20 ml of sterile sodium alginate solution (3% W/V) and 5 ml of spore suspension (5×10^6 spores/ml) were mixed fully and the slurry was dripped into 0.2 M $CaCl_2.2H_2O$ solution at room temperature. The beads were maintained in $CaCl_2$ solution for 1 hour at 4°C, and then the beds were washed three times with sterile distilled water.

2.6. Inoculum

The free and immobilized spores were inoculated in 250 ml flasks with cotton plugs containing 100 ml of synthetic and industry effluent and were incubated at 25°C for 5 days to spores germination.

2.7. Phenol Biodegradation Experiment

Experiments in triplicate were carried out. Flasks containing synthetic and industry effluent were inoculated with free and immobilized cultures. Temperature and agitation were controlled at 25°C and 200 rpm on a rotary shaker. Control assay (without inoculum) were performed under the same experimental conditions in order to verify abiotic losses. Samples were withdrawn at regular intervals for phenol determination.

2.8. Phenol Determination

For Phenol determination, the Folin-Ciocalteau phenol reagent was used, involving the successive addition of 1ml sodium carbonate(200mg/l) and 0.5 ml Folin- Ciocalteau phenol reagent to 10 ml sample. After 60 minutes at 20°C, the absorbance was measured at 725 nm against a distilled water and regent blank (Garcia et al., 2000).

3. Result & Discussion

A batch cultivation experiment was carried out using phenol as limiting substrate for *Aspergillus niger.* Initial phenol concentration in synthetic effluent was taken as 250 mg/L.

Table 2: Comparative analysis of phenol biodegradation

Sample	**Synthetic wastewater**		**Industrial wastewater**	
Phenol degradation time (Days)	**Free cell (O.D at 725 nm)**	**Immobilized cell (O.D at 725 nm)**	**Free cell (O.D at 725 nm)**	**Immobilized cell (O.D at 725 nm)**
1	1.72	2.04	2.8	2.84
2	1.70	1.71	2.22	2.26
3	1.68	1.58	2.14	2.13
4	1.64	1.32	1.74	1.91
5	1.46	0.22	1.21	1.18

The extent of phenol concentration was investigated for several batch residence time by intermittent sampling showing the biodegradation potential of *Aspergillus niger* in degrading synthetic phenol waste. The initial phenol concentration observed in industrial effluent is 268 mg/L. After five days of incubation with *Aspergillus niger* tremendous reduction in phenol concentration is observed. The culture with immobilized cell reached 110 mg/L whereas in free cell it is 119 mg/L. As shown in Table-2 phenol concentration in synthetic effluent initially provided was 250 mg/L, after five days of incubation the culture with immobilized cell reach 28 mg/L whereas in free cell it is 150 mg/L. Immobilize cell of *Aspergillus niger* results in better performance than the free cell in batch process by reducing the adaptation and consequently the time for complete phenol biodegradation.

In encapsulated cell culture, the carrier material act as a protective cover against toxicity of phenol by forming networks of the beads, a diffusion barrier for phenol is build up which is not present in free cell culture. (Chen et al., 2002). The present finding will be useful to treat the waste containing phenol to convert the toxicant into nutrient, biomass and CO_2 via biodegradation through their intermediates. This technology will be useful to the Paper industry which generates the waste containing compounds such as phenol. The present technology will also be efficient and beneficial to treat the waste generated by paper industry.

Our work shows that *Aspergillus niger*. immobilized cells in calcium alginate is promising for application in bio-degradation schemes in order to degrade phenol and possibly other related aromatic compounds at high concentrations in industry generated wastewater which leads to a reduction in time for complete phenol removal in relation to free cells.

Better biodegradation rate of phenol was observed in immobilized cells due to absence of internal and external mass transfer resistance. An immobilized cell is one of the approaches for incorporating fungal biomass into an engineering process. The advantage of the process based on immobilized biomass include enhancing microbial cell stability, allowing continuous process operation and avoiding the biomass – liquid separation requirement.

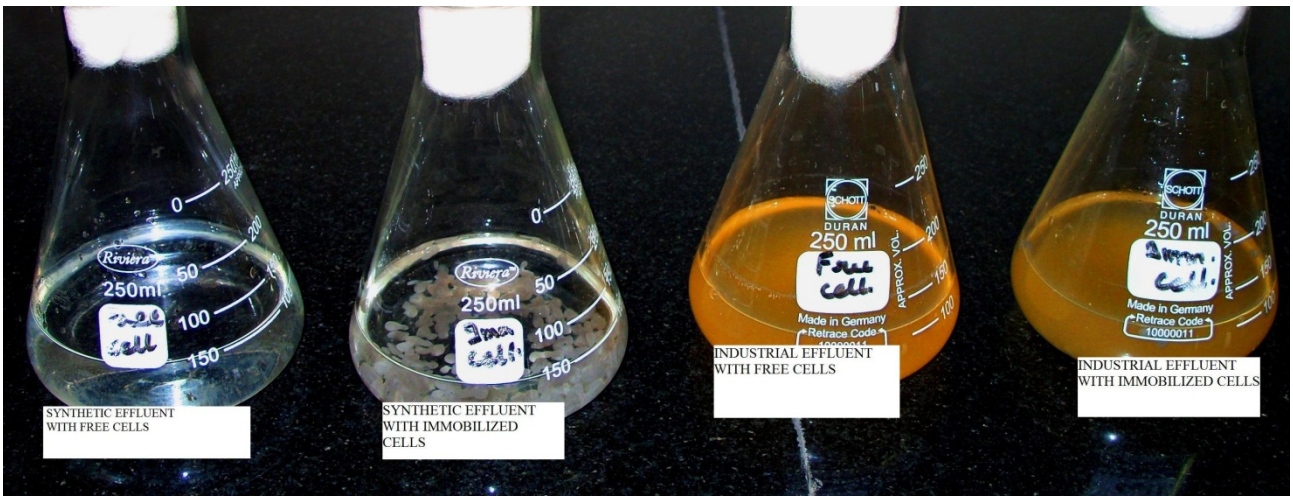

Fig. 1: Phenol biodegradation by free and immobilized cells in synthetic & industrial effluent.

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