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Degradation of bisphenol a by *Aspergillus* Sp. isolated from tannery industry effluent

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Abstract

Bisphenol A (BPA) is a chemical produced in large quantities for use primarily in the production of polycarbonate plastics and epoxy resins. It is an endocrine disruptor, which can mimic the body's own hormones such as estrogen and may lead to negative health effects. The tannery effluent was used as a source for isolation of *Aspergillus* sp. Physiochemical analysis of the effluent sample was done. In this study batch experiments was used for the degradation of BPA at different concentrations from 20-100ppm. The highest removal of 77% was found in 20ppm concentration. The effect of pH 5.0 to 11.0 on the growth of fungi strain and BPA removal was examined and optimum was found at pH 9.0.

Key-Words: Bisphenol A, tannery effluent, isolation, *Aspergillus* sp, degradation

Introduction

Environmental pollution is a constant threat faced by humanity. Industrial effluents entering into the surface water are one of the most important sources of toxic contamination in the environment. Although industrialization is inevitable, various devastating ecological and human disasters which have continuously occurred over the last four decades, implicate industries as major contributors to environmental degradation and pollution problems of various magnitude[1].

Bisphenol A (2, 2-bis (4-hydroxyphenyl) propane, BPA) is widely used as the starting material for the industrial production of polycarbonates, epoxy resins, and other specialty chemicals. Owing to its mass production and widespread use, the probability of environmental contamination with BPA has increased. Environmental releases are possible via permitted outfalls of industrial wastewater treatment systems or sewage treatment plants that receive BPA. Other possible sources of BPA are found in the environment, such as waste plastics in waste landfills and sewage sludge from wastewater treatment facilities. There is a considerable amount of monitoring data on BPA in Europe, the United States, and Japan, and certain levels of BPA have been detected in many samples [2,3].

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Microbial degradation is expected to play a major role in the removal of BPA from the environment. Several BPA-degrading bacteria, including *Sphingomonas sp.* AO1[4], the unidentified gram-negative bacteria strain MV1[5,6] and strain WH1[7],*Sphingomonas paucimobilis* strain FJ-4[8,9], and many other studies[10-20], have been reported. Some examples of the biologic degradation of BPA by bacteria and basidiomycetes have recently been reported, but such knowledge on fungi is limited. However, these biologic methods to eliminate the pollutant in an aqueous solution are a successive process. The objective of this study was to isolate the fungi from the tannery industry effluent and evaluate them for use in biodegradation of Bisphenol A at different concentrations.

Material and methods

Chemicals

BPA (GC grade >99%) was purchased from Sigma-Aldrich (India). Czapek yeast-extract (CY) liquid medium or Czapek yeast-extract agar (CYA) medium was used for growth of the fungi. The CY medium contained per liter of distilled water: 3.0 g of $NaNO_3$, 1.0 g of K_2HPO_4,0.5 g of KCl, 0.5 g of $MgSO_4 \cdot 7H_2O$, 0.01 g of $FeSO_4 \cdot 7H_2O$, 0.01 g of $ZnSO_4 \cdot 7H_2O$, 0.005 g of $CuSO_4 \cdot 5H_2O$, 5.0 g of yeast extract, and 30.0 g of sucrose. For CYA medium, agar was added to CY medium at a concentration of 1.5% and the media were sterilized at 125°C for 15 min.

Sample collection

Effluent samples were collected from Namakkal district, Tamilnadu, India. These samples were

collected at low tide with a 2 L plastic hydrobios water sampler and transferred to clean 2 L polyethylene containers and 250 ml capacity borosilicate glass bottles. All samples were transported in ice chests and analyzed for physicochemical quality within 12 hrs of collection[21]. The results are expressed in mg/L for effluents and receiving water.

Isolation, Screening and Growth characteristics

Isolation of fungi was carried out on Czapek yeast-extract agar following serial dilution technique. The agar plates were incubated at 21^0C for 7 days. Morphologically distinct colonies were subjected to purification following sub culturing. The pure cultures were maintained on CYA slants at 4 ^{0}C in a refrigerator. The isolated fungal strains were tested for their independent tolerance of BPA in Czapek yeast – extract medium both in broth and plates. Filter sterilized BPA at different concentrations were added to medium after autoclaving. Strains were inoculated and observed for growth after 5 days at 28ºC. Out of tolerant strains one strain was chosen for the study.

Fourier Transform Infrared Analysis

The functional groups present in the fungi used for this study was examined using Fourier transform infrared (FTIR) spectroscopy. The spectra of the sample were recorded between 4000 and 400 cm^{-1} using thermo Nicolet, Avatar 370 spectrometer (Model: IR Affinity brand, Shimadzu) at Karpagam University, Coimbatore.

Biodegradation of BPA by batch experiment

The tolerant strain was aseptically inoculated (2 mL. of fresh spore suspension of inoculums strength: 5x 101 c.f.u./mL) in 100 ml of sterile distilled water supplemented with BPA of varied (20,40,60,80 and100ppm) concentrations in 500 ml glass flasks. Control sets without fungus were also run. The experimental set up was carried out in orbital shaker at room temperature to study ability of the strain in assimilating BPA as sole carbon source. The organism growth rate and BPA removal efficiency were calculated by optical density at 270nm for each day interval up to 5th day. BPA estimation was done with filtrate after harvesting fungal biomass by centrifugation at 10,000 rpm for 15 miNs. Effect of pH on the degradation process was tested by addition of fungal strain into 20ppm of BPA solution prepared at different pH (3.0, 5.0, 7.0, 9.0 and 11.0) levels.

Statistical analysis

Mean triplicate readings obtained in the study were subjected to analysis of variance (ANOVA) and Ducan's multiple range tests using statistical package for Social Scientist (SPSS 10.0) computer software[22].

Results and Discussion

In the present study the Tannery industry effluents were used as source for fungal strain isolation for the biodegradation of BPA. The physicochemical quality of the collected effluent was tested and reported (Table 1). Out of the few tolerant fungal strains one of the most frequently occurring genus was observed throughout the isolation procedure and it was identified as *Aspergillus* based on colony morphology and microscopic observations [23,24] Many studies have found that BPA is degraded in the environment, as in activated sludge[25,5], river water[26,27] and soil [28,4] and that some BPA degrading microorganisms were isolated from such places, but not from Tannery industry effluent.

FTIR can provide very useful information about functional groups. The technique can be used to analyze organic materials and some of inorganic materials. It also provide spatially resolved biochemical information on hyphal growth and spore development under normal and stressful conditions, complementing information gleaned from molecular genetics investigations[29]. In earlier study the elemental functional group and infra red spectroscopic analysis of actinomycete melanin form Brazilian samples was estimated by FTIR analysis [30]. In this work FTIR utilized to detect the functional group present in *Aspergillus* sp. In FTIR major peaks were observed at 3373.5 cm^{-1} which may be polymeric hydroxyl compounds due to O-H Stretching (bonded) functional group, 2926.01 cm^{-1} may be Cyclobutane due to C-H stretching and 1741.72 cm^{-1} which may be acid anhydrides and amides due to coupled C=O. The peaks were observed at 1548.84 cm^{-1} may be Aromatic nitro compound due to NO_2 asymmetric stretching, 1076.28 cm^{-1} which may be alkyl amine due to C-N stretching and 1033.85 cm^{-1} may be acid and RSO_3 ,ionic Sulphonates due to the presence of SO_3 symmetric stretching (Figure 1).

The growth rate of *Aspergillus* sp in different concentration ranges from 20ppm to 100ppm was measured for 5 days and are reported (Figure 2). The growth was observed in all working solutions, which shows that the fungi utilize the dissolved BPA as a carbon source for their growth. The growth percentage of fungi in BPA solution are in log phase during the time intervals of 1 day, then it reached lag phase during the 2 and 3rd day and finally it remain in stationary phase from 4-5 days. The results also revealed that the BPA was degraded in appropriate manner from 20ppm to 100ppm (Figure 3). Utilization of BPA for their few metabolic processes depends on the fungal biomass, so BPA declined in solution gradually.

The CZ medium (50 mL) spiked with BPA (2 mg, 40 ppm) was prepared to test the degradation of BPA by 26 strains of soil fungi. Among the 26 strains tested, 22 showed good growth for this initial concentration of BPA. After 14 days of reaction, these strains were grown to about 60 mg (dry wt) and 11 strains degraded BPA at 50% or higher[31]. In this present study distilled water supplemented with BPA was used as a medium instead any mineral supplement. The growth of fungi attained in this mixture indicated the ability of BPA utilization. In earlier studies highest degradation (>99%) was reported by *Aspergillus*, with *A. terreus* MT-13; Fusarium, with *F. moniliforme* 2-2 and *F. sporotrichioides* NFRI-1012; and Ascomycetes, with *Emericella nidulans* MT-78[31].In this experiment only *Aspergillus* sp was used and it showed growth at all concentration up to 100ppm. Nearly 77% and 50% degradation was obtained in minimum and maximum concentration respectively. The effect of pH for different fungal strains varies based on the materials which undergo degradation. In cyanide degradation the optimum pH observed was at 9.0[32]. pH range of 4.0 to 7.0 was used to determine the effect of changing pH on the adsorption of Zn by non-living biomass of both *Aspergillus fumigatus RH05* and *Aspergillus flavus* RH07, at 28°C and 100 mg/L Zn [33]. Biodegradation of BPA by various fungal sources has been reported in earlier but effect of pH in their growth and degradation were not reported (Chai *et al*., 2005). In our present study effect of pH on *Aspergillus* sp growth in synthetic BPA solutions at 20ppm with various pH (3.0 to 11.0) was studied. The data stated that the highest cell density and the highest degradation were obtained when pH was adjusted to 9.0, indicating pH preference of the fungi (Figure 4). In this study the efficiency of BPA degradation by *Aspergillus* sp was studied upto 100ppm range. Furthermore, the BPA removal depends on the *Aspergillus* sp growth in solutions because they may be utilize the BPA as carbon source. The fungal load, contact time and pH are also responsible for the degradation process. The degradability of Bisphenol A by *Aspergillus* sp might result from the following causes: resistance of cells to the toxicity of the chemicals, broad substrate specificities of initial enzymes in the metabolism of the chemicals, and inner-membrane permeability of the chemicals.

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Table 1: The physicochemical quality of Tannery effluents (mg/L)

Parameters	Effluent
pH	8.3±0.2
BOD	218±0.93
COD	234±1.81
conductivity (µS cm-1)	1139±12
Salinity	17.59±0.96
Phenol	71.12±2.2
ammonia (N)	25.79±0.9
nitrate (N)	1.88±0.33
Phosphate	14.9±0.92
Cyanide	<0.01
Sulphate	29.31±1.3
Sulphide	<0.01
TDS	337.6±2.8
lead (Pb)	<0.01
nickel (Ni)	<0.001
iron (Fe)	0.279±0.02
zinc (Zn)	0.179±0.02
copper (Cu)	<0.001
chromium (Cr)	0

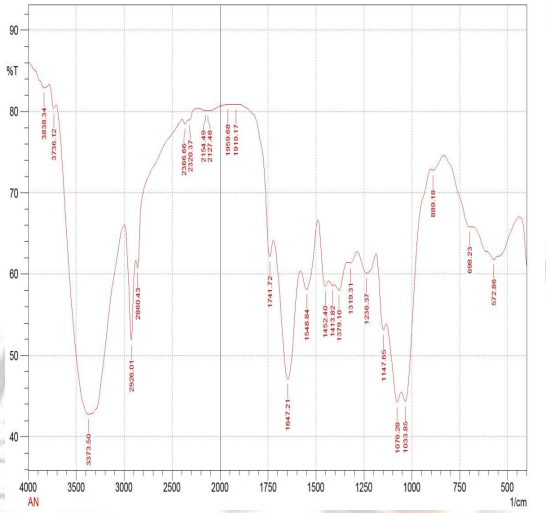

Fig. 1: FTIR analysis of selected *Aspergillus* sp

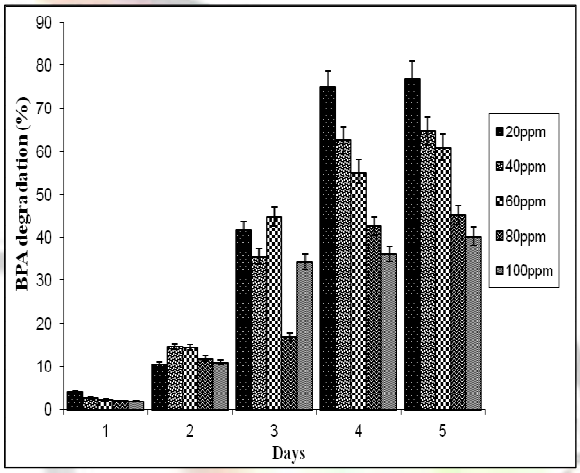

Fig. 2: Growth rate of *Penicillium* sp in different concentrations of BP

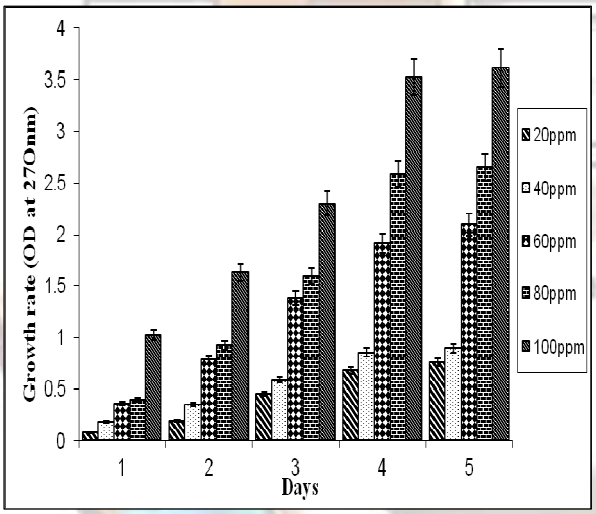

Fig. 3: BPA degradation efficiency by *Aspergillus* sp

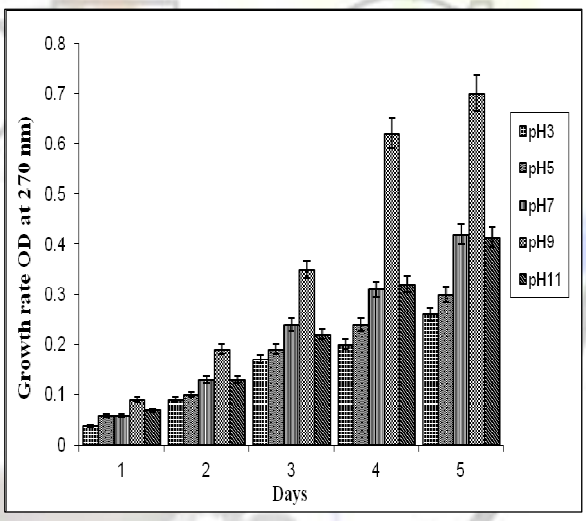

Fig. 4: Effect of pH on *Aspergillus* sp growth in 20ppm BPA solution