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Microbial Bioremediation of Heavy Metal from Textile industry Effluent using Isolated Fungal Strain

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Abstract
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. Among pollution causing industries, textile industry achieve a major attention by environmentalists due to expenditure of large volume of water. The textile effluent consists of heavy metals such as Cadmium, Lead and Zinc etc either in free ionic metals or complex metals.
Recent advances towards effective treatment methods for the removal of heavy metals in aqueous media involve the use of microorganisms. It is a cost-effective process and the end products are non-hazardous. In this study heavy metal resistant fungal strain *Aspergillus niger* isolated from the textile effluent of South Gujarat Region and it was used for Bioremediation of heavy metal Zinc. The fungal strain *Aspergillus niger* effectively removes the heavy metal Zinc from the effluent.

Keywords: Effluent, Heavy Metal, Pollution, Microorganism

Introduction
The Textile printing and dyeing industry is a water-intensive industry requiring a large volume of fresh water at various steps of printing and therefore, the volume of wastewater produced is equally large. The discharge of textile waste water into aquatic habitats is of great concern since the discharges are mostly made untreated or partially treated due to poor enforcement of existing laws in the developing world including India. (17)Textile Industry plays an important role in Indian economy, as well. It is an employment oriented industry, occupying the second position i.e. after Agriculture. Clusters of processing units are located at Surat, Ahmedabad and Jetpur. Untreated or incompletely treated textile effluent is notoriously known to contain large amount of total suspended solids which increases the turbidity in water and prevents the light from reaching aquatic plants and animals, large amount of total dissolved solids limiting the industrial and agricultural use of water, high levels of chemical oxygen demand which is indicating high degree of pollution and biological oxygen demand which leads to lowering of the level of dissolved oxygen thereby inhibiting aquatic habitats and elevated temperatures which lower the rate of dissolution of atmospheric oxygen in the

water and affects the sustainability of the aquatic habitats due to reduction in the level of the dissolved oxygen.(9)

The problem of water pollution has become still worse due to toxic heavy metals (11, 13). The increasing trend in concentration of heavy metals in the environment has attracted considerable attention amongst ecologists globally during the last decades (15). 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. The textile effluent consists of heavy metals such as Cadmium, Lead and Zinc etc either in free ionic metals or complex metals, (19).This textile effluents cause serious environmental problems by absorbing light in receiving water bodies like streams, rivers and lakes etc and ultimately interfering with aquatic biological processes (6)

Recent advances towards effective treatment methods for the removal of heavy metals in aqueous media involve the use of microorganisms (5). Bioremediation is the use of natural biota and their processes for pollution reduction. It is a cost-effective process and the end products are non-hazardous. Microbial communities are of primary importance in bioremediation of metal contaminated water because microbes alter metal chemistry and mobility through reduction, accumulation, mobilization and immobilization.(19)Attention has been focused lately on the application of fungal species for metal removal from aqueous media (7).

The use of conventional technologies, such as ion exchange, Chemical precipitation, reverse osmosis, and evaporative recovery, for this purpose is often inefficient and/or very expensive.(2, 5, 7, 14&16).Recent developments in the field of environmental biotechnology include the search for microorganisms as sorbents for heavy metals. Bacteria, fungi, yeast and algae can remove heavy metals and radio nuclides from aqueous solutions insubstantial quantities (1, 10&18). The uptake of heavy metals by biomass can take place by an active mode (dependent on the metabolic activity) known as bioaccumulation or by a passive mode (sorption and/or complexation) termed as biosorption.

Materials and methods

Collection of Effluent

The Textile Effluent was collected from the Textile Mill of Udhana and Kadodara located in South Gujarat region, Gujarat, India.

Isolation and identification of Fungi

Sabouraud Dextrose Agar (SDA) was used for culturing fungi from Textile effluent. 100 µl of the effluent added in to the plate and poured autoclaved SDA medium temp 40 to 45^{o}C. The inoculated plates were incubated at 25˚C for 5 days. The fungi were identified using lactophenol cotton blue.

The technique of James and Natalie (2001) was adopted for identification of the unknown isolated fungi using cotton blue in lactophenol stain. The identification was achieved by placing a drop of the stain on clean slide with the aid of a mounting needle, where a small portion of the mycelium from the fungal cultures was removed and placed in a drop of lactophenol. The mycelium was spread very well on the slide with the aid of the needle. A cover slip was gently applied with little pressure to eliminate air bubbles. The slide was then mounted and observed with x10 and x40 objective lenses respectively.

Optimization of Growth conditions for Aspergillus niger

Fungi Aspergillus niger isolated from Textile effluent was selected for studies on Zn removal in Shake flask experimentation. Prior to analysis of adsorption ability of this fungus, growth condition was optimized. For subsequent experimentation this strain was grown in SDA plate 28^{o} C for 5 days in the incubator. From this plate, spore suspension was prepared by 5-7 loop fulls culture of Aspergillus niger, to inoculate 100 ml Sabouraud dextrose broth (SDB) in conical flask. All experiments were performed in duplicate to minimize errors in analysis.

Optimization of pH

Isolated fungi was grown in SDB on orbital shaker (100 rpm)at 25^o C for 5 days at different pH(4.0,5.0,6.0,7.0 & 8.0). Samples were drawn at 0,3,4 & 5 days of incubation, growth was monitored by filtering the biomass through Whatman filter paper No.1,followed by drying at
55^o C.

Optimization of Temperature

For the growth of Aspergillus niger, three temperatures, 25^o C, 28^o C and 31^o C were selected. Inoculums was added as a spore suspension to SDB having pH 5.0 and incubated on orbital shaker 100 rpm for 5 days. Samples were drawn at 0, 3, 4& 5 days of incubation. Filtered and dried biomass was weighed and recorded.

Preparation of Fungal Biomass

The pure culture of Aspergillus niger was isolated from textile effluent. Mycelial biomass of fungus was grown in pre autoclaved Sabouraud Dextrose Broth (SDB) and incubated at 25 C temperature in orbital shaker at 100 rpm for 5 days.

Preparation of biosorbent

The fungal cell mass was determined by filtering the culture medium through weighed Whatman filter paper no. (44). Mycelium was thoroughly washed with generous amounts of deionized water, re suspended and washed again. The biomass thus obtained was suspended in 0.5N Sodium Hydroxide solution for 15 min. The pretreated biomass was washed with deionized water until thepH of the solution was in a near neutral range (pH 6.8-7.2). This was autoclaved for15min at 121°C the biosorbent is dried in an oven at 80ºC for 24 hours. When the biomass is dry it is powdered and sieved to size 0.125mm.

Effect of Zn concentration on Adsorption

Zn concentration on adsorption to dead biomass was studied by ZnSo4 solutions. Prepared a different concentration of ZnSo4 covering a range from 100-300 mg/L at pH 5.0. Solutions were prepared in distilled deionized water in 100 ml flask, 0.4 gm dried biomass was added and kept at on orbital shaker (100 rpm) at 28^o C. Samples were drawn at time intervals of 30 minutes and samples drawn at 0 (zero) to 120 minutes.

Results and discussion

Isolation and Identification of Fungi from Textile effluent

Fungi isolated from the samples of different sites were having same morphological characteristics and it is identified as an Aspergillus niger. This fungus was common in each effluent samples. Hence it was selected as an adsorbent for Zn removal.

Optimization of pH

A pH range of 4.0 to 8.0 was selected to find a maximum growth of fungi. From the study, isolated fungi showed maximum growth at pH 5.0. Aspergillus niger produced a biomass 0.59 g/100 ml in Sabouraud dextrose broth (SDB), after5 days incubation. Trend analysis of the strain at different pH levels in the range of 4.0 to 8.0, are shown in Figure-1.

Optimization of Temperature

Three different temperatures 25^o C, 28^o C and 31^o C were selected for the optimum temperature of growth of Aspergillus niger. This fungal strain yielded maximum biomass 0.60 g/100 ml at 28^o C and pH 5.0, which is shown in Figure-2.

Table 1: Optimization of pH.

Optimization of pH				
	Dried Biomass (gm/100 ml)			
DAY	**0**	**3**	**4**	**5**
pH 4	0	0.12	0.2	0.34
pH 5	0	0.39	0.52	0.59
pH 6	0	0.21	0.27	0.22
pH 7	0	0.32	0.3	0.24
pH 8	0	0.28	0.23	0.24

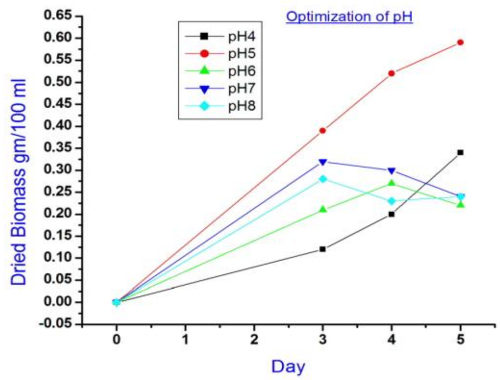

Fig. 1: Optimization of pH.

Table 2: Optimization of Temperature.

Optimization of Temperature				
Temp.	Dried Biomass (gm/100 ml)			
	0	**3**	**4**	**5**
25 C	0	0.26	0.32	0.45
28 C	0	0.35	0.38	0.6
31 C	0	0.29	0.36	0.49

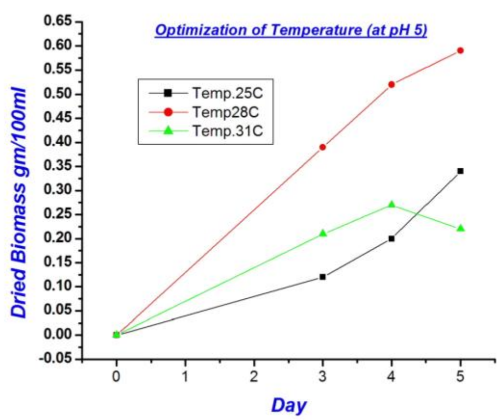

Fig. 2: Optimization of Temperature.

Effect of Zn concentration on Adsorption

Analysed the samples withdrawn at different time interval (0 – 120 minute), the results found that maximum adsorption of Zn was 64.67 % at 100 mg/l. Figure 3 is indicating the adsorption of Zn at 100,200 & 300 mg/l concentration.

Table 3: %Adsorption of Zn by Non-living biomass of A.niger at various Conc. (pH 5,28°C).

% Adsorption of Zn by Non-living biomass			
Concentration mg/l			
Time (min)	**100**	**200**	**300**
0	4.98	2.6	1.46
30	36.98	41.45	35.36
60	39.69	47.4	43.01
90	55.88	48.53	49.09
120	64.67	51.25	53.81

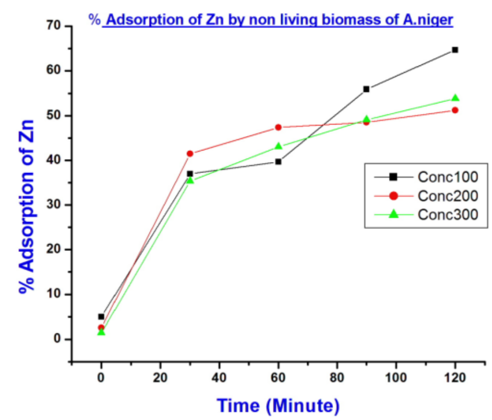

Fig. 3: %Adsorption of Zn at various concentrations.

Conclusion

The present study conclude that pretreated biomass of Aspergillus niger isolated from effluent of textile industries could be effectively utilized to remove heavy metal Zinc(Zn).It was concluded that the biosorption activity shows good performance for the removal of heavy metal

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