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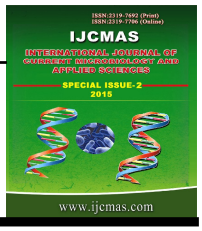

Original Research Article

Comparative Study of Biosorption of Textile Dyes Using Fungal Biosorbents

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Biosorption, Biosorbents, Azo dyes, *A. niger*, *A. oryzae*, *R. arrhizus*

A B S T R A C T

India produces ~200,000 tons of dyes such as direct, reactive, acid and basic dyes. During dyeing about 7×105 tons dyes per annum are lost in the effluent from dyeing processes. Most of these dyes represent acute ecological problems as they are toxic and carcinogenic. Removal of the color from the effluents is thus a major problem forcing waste creators to consider new options for the effluent treatment. Among all wastewater treatments, the biosorption of dyes using fungal biomass is an attractive option due to its cheap and constant supply from food and industrial fermentation processes. In the present study, three fungal biosorbents namely, dead biomasses of *A. niger*, *A. oryzae* and *R. arrhizus* were evaluated for their potential to remove 13 different dyes belonging to various classes. The dyes included Reactive dyes (Reactive orange 13, Reactive blue 256, Reactive blue 222, Reactive Black 5, Reactive Red 120, Reactive Green 8), Remazol dyes (Reactive orange 16, Reactive violet 5), Acid azo dyes (Acid Red 151, Acid orange 7), direct azo (Direct Blue 9, Direct Blue 199) and Basic azo dye (Basic blue 9). Effect of pH on the specific uptake capacities of the biomasses was studied which indicated significant contribution of pH on biosorption capacities. All the three fungal biosorbents tested, proved to be efficient in dye biosorption.

Introduction

Approximately 700,000 tons and 10,000 different types of disperse, reactive, direct dyes and pigments are produced annually across the world and are extensively used in many industries including textile, leather, pulp, paper, food and plastics (Kiran *et al.*, 2009; Aksakal and Ucun, 2010). Amongst the different dyes listed in the Color Index and available in the market, 60–70% belongs to the azo dyes compounds. These dyes are used in the textile industry for the colouring of polyester, nylon, cellulose diacetate and triacetate, acrylic fibres etc. The relative share of azo dyes among reactive, acid and direct dyes is even higher, it can be expected that they make up the vast majority of the dyes discharged by textile-processing industries. In 2010, India produced ~200,000 tons of dyes of which 50% were

reactive dyes which owing to their technical characteristics (Vijayaraghavan *et al.,* 2007). The dyes are designed to be chemically and photolytically stable and are unfavorable from the ecological point of view. The effluents produced are relatively heavily colored, contain high concentrations of salt and exhibit high BOD/COD values. Dyes, even at very low concentrations, reduce wastewater transparency and oxygen solubility and are often toxic, carcinogenic or mutagenic for various organisms and recalcitrant (Mathur and Bhatnagar, 2007). The release of the dyes therefore is both aesthetically unacceptable and presents an ecotoxic hazard, introducing the potential danger of bioaccumulation eventually affecting man by transport through the food chain (Asamudo *et al.,* 2005).

Currently, textile effluents are treated by physico-chemical methods which are of limited use due to the different constraints such as costs; general applicability and production of the solid wastes. Unfavorable conditions found in the textile dyeing effluents are known to inhibit the conventional biological wastewater treatment processes. Moreover, various azo dyes have been shown to be anaerobically decolorized by cleavage of the azo bond, resulting in the formation of potentially carcinogenic aromatic amines that persist and potentially accumulate in the environment (Aksu, 2007). Removal of the color from the effluents is thus a major problem and implementation of tighter constraints on the discharges is forcing waste creators and managers to consider new options for the effluent treatment and disposal (Zille, 2005).

Among all the wastewater treatments, the adsorption process has been recognized to be an effective and economical procedure for the removal of dyes from industrial effluents. Activated carbon is one of the most widely used adsorbents because of its excellent adsorption capacity for organic pollutants. However, its prohibitive cost and inability to regenerate it limit its commercial application (Russo *et al.,* 2010). Hence, low-cost biosorbent materials with high adsorption capacities have gained increasing attention (Chen and Chen, 2009).These include natural materials derived from waste materials from industry and agriculture, as well as biosorbents that are produced from microbial biomass like *Corynebacterium glutamicum, Escherichia coli, Pseudomonas luteola,* and *Rhizopus arrhizus* (Vijayraghavan, 2008).

The overall economics of the biosorption is influenced mainly by the cost of procuring/growing the biomass. Application of fungal biomass to remove textile dyes from industrial waste water is attractive for industry as it may decrease the overall effluent treatment cost. Food and industrial fermentation processes can provide a cheap and constant supply of fungal biomass or the biomass can be cultured using inexpensive growth media and unsophisticated fermentation techniques. Traditionally, the fungal biomass byproduct is incinerated for disposal or used as a fertilizer of a low economic value for agricultural use. This potential biosorbent can usually be obtained relatively free of charge in rather substantial quantities, from the respective producers since they already present disposal problems to them. The only costs incurred should be those of drying, if required and transport (Sağ, 2001; Wang and Chen, 2009).Thus, the use of fungal biomass as an adsorbent for dye pollution control can generate revenue for industries presently wasting the biomass and at the same time ease the burden of disposal costs associated with the waste biomass produced.

Compared to the live biomass, the use of dead fungal biomass offers various advantages such as reusability of biomaterial, easy storage, more efficiency, easy operation and hence cost effectiveness for the treatment of large volumes of wastewaters containing low dye concentrations, short operation time, and no production of secondary compounds which might be toxic (Donmez and Aksu, 2002; Amini *et al.,* 2009). The efficiency of the biosorption process of reactive azo dyes, under equilibrium conditions by some fungi has been shown to be more efficient than activated carbon. Generally, biosorptive processes can reduce capital costs by 20%, operational costs by 36% and total treatment costs by 28% when compared with conventional systems (Loukidou *et al.,* 2003). A major advantage of biosorption is that it can be used in situ, and with proper design may not need any industrial process operations and can be integrated with many systems in the most eco-friendly manner (Tewari *et al.,* 2005). Hence, the objective of this study was to compare the effect of varying pH on biosorptionof various azo dyes belonging to reactive, direct, acid and basic dyes by various fungi and compare them for their potential to be used as biosorbents.

Materials and Methods

Microorganism and Growth Condition

Aspergillus niger van Tieghem 595, *Aspergillus oryzae* NCIM 643 and *Rhizopus arrhizus* NCIM 997were obtained from National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory, Pune, and were routinely maintained at 4◦C on Potato Dextrose Agar (PDA) (g/L: Potato infusion from 200 g potatoes, Dextrose 20, Yeast extract 0.1,pH 5.0).

Preparation of the biosorbent

For experimental purposes, fungal biomasses were obtained by aseptically transferring mycelia from the PDA spread-plate cultures to 100 mL of Potato Dextrose Broth (PDB) containing 0.25% Tween 80 (to prevent sporulation) in 250 mL Erlenmeyer flasks. The flasks were incubated at 30 ± 1°C under static conditions with intermittent shaking. The biomass harvested after seven days were washed thoroughly with generous amounts of deionized distilled water and dried at 80◦C in an oven for 24 h. Care was taken to keep the particle size of the biomasses uniform, by grinding into powder and sieving through a 150-mesh sieve. The biomasses were stored in a desiccator until used for the biosorption experiments (Tunali *et al.,* 2005).

Preparation of the adsorbate

Reactive dyes (Reactive Orange 13, Reactive Green 8, Reactive Blue 256, Reactive Blue 222, Reactive Red 120, Reactive Black 5), Remazol dyes (Reactive Orange 16, Reactive Violet 5), Acid dyes (Acid Orange 7, Acid Red 151), direct dyes (Direct Blue 199, Direct Blue 9), and basic dye (Basic Blue 9) were obtained from Colourtex Dyes Pvt. Ltd., Mumbai and were used without further purification. All chemicals and reagents used for experiments were of analytical grade and supplied by HiMedia Laboratories.

Decolorization Assay

Stock solutions, 1000mg/L of the above mentioned dyes were prepared in double distilled water and were diluted as required according to the working concentration. The required pHwas adjusted by 0.1N HCl or 0.1N NaOH. Batch biosorption experiments

were carried out using dried biomasses (50 mg) of all the three fungal biosorbents in 250-mL conical flasks to elucidate their ability to sorb 50 mg/L of all the dyes separately over a pH range of 2.0-10.0. The flasks were kept under agitation at 120 rpm in a rotating orbital shaker for 120 min at 30oC.The residual dye concentration in the solution was determined after filtering the samples using Whatman No. 1 filter paper. Dye concentration was measured using UV–Vis Spectrophotometer (Shimadzu UV1800 UV/VIS) at wavelengths corresponding to the maximum absorbance of the respective dyes. A pre-determined calibration curve was used to convert the optical densities into concentrations. Blank without biosorbent was run simultaneously as a control. The concentration of the dye on the fungal biomasses at the corresponding equilibrium conditions was determined using a mass balance equation expressed as specific uptake capacity (SUC):

$$q = \frac{V(C0 - Ce)}{m}$$

Where,q is the amount of dye adsorbed per unit weight of the biosorbent (mg/g); C0theinitial concentration of the dye (ppm); Ce the concentration of dye in solution at equilibrium time(ppm); V the solution volume (L); m is the dosage of the biosorbent (g).

Result and Discussion

In the present investigation, three fungi were chosen based on their industrial use which in turn will determine their availability for commercial purposes. These included *Aspergillus* niger van Tieghem 595(production of citric acid, cellulase, β – glucanase, glucoamylase, glucose oxidase, etc.), *Aspergillus oryzae* NCIM 643(production of protease, amylase, glucoamylase, hemicellulose, lipase, koji), *Rhizopus* arrhizus NCIM 997 (production of lipase, lactic acid, fumaric acid, malic acid).Various types of dyes used in the study, their absorption maxima (λmax) and uses in the textile industry are shown in table 1.

The process of biosorption using non-living biomass is a rapid phenomenon and involves a solid phase (biosorbent) and a liquid phase (solvent, normally water) containing dissolved species to be sorbed (sorbate, dye). Biomass exhibits this property, similar to a chemical substance, of an ion exchanger of biological origin (Acheampong *et al.*, 2009). Studies on dye sorption have shown that pH plays a critical role in the biosorption processes. Change in the pH of the solution affects availability of dye in solution, speciation and the surface charge of the biosorbents, concentration of the counter ions on the functional groups of the biomass cell walls, the degree of ionization and the adsorptive process through dissociation of functional groups on the biosorbent surface active sites. The dye is taken up from water by ionization of negative functional molecular groups, which serve as the binding sites predominantly in exchange for counter ions present in the biomass (David and Volesky, 1998).

The effect of initial pH on various dye biosorption by the three fungi varied depending on the type of the dye as shown in Table 2. The adsorption of various anionic and cationic species on adsorbents has been explained, based on the competitive adsorption of H+ and dye ions with the adsorbates. It is a common observation that the surface adsorbs anions favorably at lower pH due to the presence of H^+ ions, whereas, the surface is active for the adsorption of cations at higher pH due to

the deposition of ions (Abdullah *et al.*, 2010). Similar results were obtained in the present study. Reactive dyes, Remazol dyes, direct dyes and Acid dyes, are anionic dyes which have negative electrical structure of the chromophore group. Upon dissolution, these ionic dyes release colored dye ions into the solution. As the pH of the solution decreases, more protons are available to protonate the amino groups of chitosan molecules on the fungal cell wall to form positively charged–NH3+ groups. This increases electrostatic attraction between the anionic groups of the dye and the protonated amino group ($–NH^{3+}$) of chitosan, causing an increase in dye adsorption. Under alkaline conditions, the decrease of biosorption capacity could be due to the increasing number of negative charges distributed on the fungal biomass surface, which would result in electrostatic repulsion between the adsorbent and dye molecules. At higher pH values, as the number of OH- ions increase, the competition for the adsorption sites between the chromophores and the ion increases, thereby decreasing the sorption (Chen and Chen, 2009; Xiong, 2009).

In the present investigation, the maximum sorption for all the anionic dyes was seen pH 2.0. The dyes were sorbed in the following order basic dye >reactive dyes > acid dyes >remazol dyes > direct dyes. The biosorption of all the reactive dyes decreased gradually from pH 2.0 to 10.0 by 65- 80% for all the biosorbents. Similar results were seen Acid Red 151, while there was a sudden decrease in biosorption at pH 3.0 for Reactive Orange 16.

Both the remazol dyes exhibited a drastic decrease in biosorption from pH 2.0 to 3.0 for all the three biosorbents followed by a gradual decrease in sorption with an increase in pH. Direct Blue 9, which is banned by the Government of India, but still being manufactured and used rampantly by the textile units was sorbed the least.However, the sorption of the other direct dye, Direct Blue 199 was equivalent to the reactive dyes employed in the present study.

Strong biosorbent behavior of microorganisms towards dye ions is a function of the chemical composition of the microbial cells. The cell wall is usually the first cellular structure that is exposed to the soluble dye species in the extracellular environment. A microbial cell wall is a well-defined polymeric matrix located just outside the plasma membrane of a cell (Donmez and Aksu, 1999).Cell walls of fungi present a multi-laminate architecture where up to 90% of their dry mass consists of amino or non-amino polysaccharides. The fungal cell walls can be considered as a two phase system consisting of chitin framework embedded on an amorphous polysaccharide matrix (Yan and Viraraghavan, 1999). The cell walls are rich in polysaccharides and glycoprotein's such as glycans (β- 1-6 and β-1-3 linked D-glucose residues), chitin (β - 1-4 linked N-acetyl-D- glucosamine), chitosan (β - 1-4 linked D-glucosamine), mannans (β-1-4 linked mannose) and phosphormannans (phosphorylated mannans). Various dye binding groups, viz. amine, imidazole, phosphate, sulphate, sulfhydryl and hydroxyl are present in the polymers (Alluri *et al.*, 2007).

The cell wall of *Aspergillus* sp., consists of neutral carbohydrate (73 to 83%) and hexosamine (9 to 13%), with smaller amounts of lipid (2 to 7%) and phosphorus (less than 0.1% of wall weight). The acetyl content is 3 to 3.4%, which corresponded to 1 mol/mol of hexosamine. This suggests the involvement of other components besides chitin in the dye sequestering (Sagˇ, 2001).

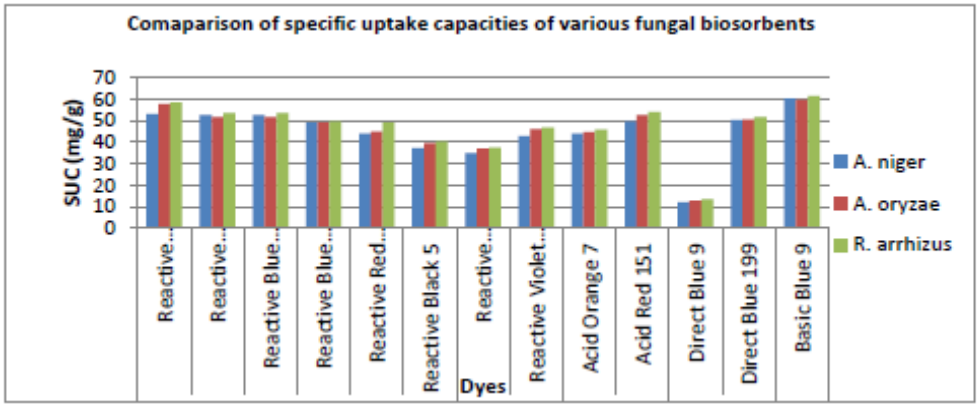
Table.1 List of dyes used in the study.

Name	Common name, Molecular weight (g/mol), Molecular formula	Class	Wavelength (λ max)	Application
Reactive Orange 13	Reactive Orange H-2R, 762.04 C24H15ClN7Na3O10S3	Monoazodye	489	Used for cotton viscose fabric or the wool, nylon and silk dyeing polyester/stick blended fabric dyeing and printing,
Reactive Green 8	Olive G-P, 1256.41 C40H23ClN11Na5O18S5	Monochloro triazinemonoazo dye	617	
Reactive Blue 256	Reactive Blue MEB, 1306.86 C38H22Cl2N15Na5O17S5	Diazo dye	574	
Reactive Blue 222	Reactive Blue BF, 1357.49 C37H23ClN10Na6O22S7	Diazo dye	609	
Reactive Red 120	Reactive Brilliant Red KE-4B, 1469.98, C44H24Cl2N14Na6O20S6	Polyaromaticdiazoorganosulphur dye	513	
Reactive Black 5	Cibacron Navy DP-B, 991.82 C26H21N5Na4O19S6	Diazovinylsulphone dye	597	
Reactive orange 16	Brilliant orange 3R, 617.54 C20H17N3Na2O11S3	Mono azo vinylsulphone remazol dye	594	Dyeing cellulosic fibers, such as cotton and wool.
Reactive violet 5	Brilliant violet 5R, 739.59 C20H16N3Na3O15S4	Copper complex monoazovinylsulphone dye	547	
Acid orange 7	Acid Orange 2, 350.32 C25H15ClN7O10S3.3Na	Monoazo acid dye	485	Applied to polyamide, silk, wool and wool fabric
Acid Red 151	Weak Acid Red BL, 454.43 C22H15N4NaO4S	Diazo acid dye	512	
Direct Blue 9	Sirius blue K- CFN, 860.73 C34H23N4Na3O13S3	Diazo dye	602.5	Used for cellulose fibre, silk and leather dyeing, paper color
Direct Blue 199	Direct Blue FBL, 921.57 C35H26N8Na4O17S4	Phthalocyaninedirect dye with a copper ion	610.5	
Basic Blue 9	Direct Blue BN, 870.63 C34H23N4Na3O13S3	Diazo dye	602.5	Applied to cotton, rayon, cellulosic fibre

Table 2: Effect of initial pH on biosorption of different classes of anionic dyes by various fungi

Dyes	pH																										
	2.0			3.0			4.0			5.0			6.0			7.0			8.0			9.0			10.0		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
	Reactive dyes																										
Reactive Orange 13	53.08	57.65	58.44	45.08	47.65	48.44	34.78	28.88	44.4	30.66	25.64	38.72	27.54	20.43	30.86	25.02	18.78	27.09	24.09	17.76	26.14	23.79	15.65	24.42	17.98	10.09	19.76
Reactive Green 8	52.51	51.6	53.48	42.51	41.6	43.48	35.93	39.53	40.12	23	27.02	35.75	20.53	23.81	28.02	17.09	18.32	25.07	15.9	16.98	24.03	10.09	11.03	18.05	9.03	10	15.01
Reactive Blue 256	52.51	51.6	53.48	35.93	39.53	43.12	23	27.02	30.75	20.53	15.52	28.02	17.87	13.89	25.69	15.08	12.76	16.32	13.23	10.98	14.43	12.32	10.09	13.97	10.69	8.69	11.65
Reactive Blue 222	49	49.01	49.73	38.28	38.28	45.45	24.87	24.87	26.9	11.72	11.72	12.08	11.43	11.23	11.79	11	11.21	11.43	10.02	10.12	10.89	9.79	9.87	10.32	9.01	8.49	9.65
Reactive Red 120	43.87	44.94	49.02	33.87	34.94	39.02	19.08	24.56	26.02	18.76	20.28	23.54	15.41	13.37	18.23	13.02	12.98	17.76	10.79	10.74	14.02	10.01	10.03	12.79	8.59	8.34	11.05
Reactive Black 5	37.05	39.53	40.36	23.96	20.92	22.84	14.82	13.28	17.6	13.28	10.94	16.2	13.02	10.23	15.67	12.76	10.01	15.23	11.09	9.87	13.79	10.57	9.43	12.89	9.45	9.02	11.21
	Remazol dyes																										
Reactive Orange 16	34.95	36.96	37.53	17.16	20.67	24.66	15.17	12.45	17.65	13.09	10.89	15.99	12.06	10.34	13.97	11.7	10.21	12.69	11.45	10	11.99	11	8.67	11.45	9.89	8.32	10.32
Reactive Violet 5	42.94	46.08	46.92	25.3	28.15	33.59	24.7	20.09	28.75	21.91	18.46	24.06	19.79	17.67	22.32	19.34	16.78	20.07	18.54	15.43	19.97	16.56	13.34	17.89	10.67	9.89	15.64
	Acid dyes																										
Acid Orange 7	43.97	44.71	45.93	22.89	26.47	29.03	19.58	23.88	24.01	11.8	11.43	16.96	10.98	10.89	13.98	9.97	9.76	11.43	9.89	9.73	10.87	9.54	9.23	10.04	8.67	8.54	9.02
Acid Red 151	49.58	52.63	53.99	45.35	43.85	47.45	43.23	43.27	45.35	41.56	42.87	43.88	41.23	41.19	42.09	40.98	40.87	41.69	39.98	39.78	40.88	39.54	39.32	40.17	38.17	38.09	39.78
	Direct dyes																										
Direct Blue 9	12.13	12.75	13.46	11.1	12.12	12.22	10.57	10.57	11.56	10.51	10.38	11.03	10.32	10.12	10.89	10.15	10.09	10.56	10.06	9.79	10.14	9.92	9.65	10.02	9.78	9.45	9.89
Direct Blue 199	50.23	50.58	51.58	40.88	42.27	42.66	23.61	25.55	27.47	23.18	23.22	26.68	20.09	20.13	21.05	15.09	15.08	17.89	14.98	14.76	15.78	13.09	13.11	14.76	12.87	12.78	13.09
	Basic dye																										
Basic Blue 9	26.35	27	26.9	33.36	35.37	35.36	39.8	39.65	39.56	45.9	46.98	47.12	50.12	49.89	51.01	54.98	53.75	54.09	57.76	56.98	57.99	59.09	58.34	59.21	60.2	59.67	61.43

Key: A: *A. niger*, B:*A. oryzae*, C: *R. arrhizus*



In *Rhizopus* sp. the native chitin exhibits some degree of de-acetylation of poly-N-acetyl glucosamine to chitosan (Araki and Ito, 1974). The hydroxyl, at position 2 in the D-glucose residue is replaced in chitosan by the amino group. In chitin, it is replaced by the acetamido (–NHCOCH3) group. The hexosamines- chitin and chitosan constituting approximately 24–40% of the cell dry weight of *Rhizopus* serve as a matrix of -COOH and -NH2 groups, which in turn take part in binding of various metals and dyes (Tsezos, 1989). Thus, the superior performance of *R. arrhizus* amongst the three biosorbents tested can be attributed to higher content of chitin and chitosan in its cell wall, making available more functional groups contributing to dye biosorption (Figure 1). Thus, all the three fungal biosorbents showed potential to be developed into biosorbents of commercial value after further optimization of other process parameters.

In countries, with the rush for rapid industrial development coupled with lack of awareness about dye toxicity there is an urgent need for developing an economical and eco-friendly technology which satisfies these demands when other conventional methods fail. The results of the present study showed that all the three fungi tested proved to be efficient in sorbing all the types of dyes used in this study. The process of biosorption was highly dependent on pH, where the maximum sorption for all the anionic dyes was at pH 2.0, while the maximum sorption of cationic dye was at pH 10.0. This is a step towards fulfilling the need for developing ecofriendly biosorbents which offers several advantages for industrial applications.

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