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Biosorption of methyl violet, basic fuchsin and their mixture using dead fungal biomass

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The effect of different parameters on sorption of methyl violet, basic fuchsin and their mixture in an aqueous solution was studied. Dead biomass of *Aspergillus niger* was observed to be an efficient biosorbent. Maximum sorption was seen within 10 min. The sorption was independent of dye concentration and pH. The interaction of pH, dye concentration and biosorbent concentration showed variable results. The desorption profile showed that 0.1 M HCl was a better eluant than 50% ethanol.

CONTROL of pollution is one of the prime concerns of society today. With economic constraints on pollution control processes, affordable and effective methods have become a necessity. Untreated or partially treated wastewaters and industrial effluent discharges into natural ecosystems pose a serious problem to the ecosystem and the life forms. Among the many types of organics present, the most difficult to remove is colour. With increasing consciousness about pollution control, biosorption is

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achieving prime attention in effluent treatment processes, especially where wastewaters contain a mixture of dyes or compounds such as in effluents of textile industries, paper and pulp industries, etc.

Synthetic dyes are widely used in a number of industrial processes, such as textile industries, paper printing and photography[1]. Several strategies are currently available to remove colour from the industrial effluent. These include physical processes such as membrane technologies, chemical processes such as ozonation, physico-chemical methods, adsorption, chemical precipitation, flocculation, photolysis, and ion pair extraction and biological processes such as biodegradation and bioadsorption[2]. The available methods require considerable start-up costs and cannot meet increasingly stringent effluent colour standards. Also, the use of biodegradation processes can be unpredictable, cumbersome and sometimes difficult to operate at a large scale, because stringent conditions have to be maintained in order to support microbial growth. Also, such wastewaters may contain concentrations of toxic metals and widely fluctuating pH conditions that are not conducive for growth and maintenance of the active microbial population. This necessitates provision of a constant energy source in the form of organic substrates provided for the sustenance of life.

Although dye decolorization of industrial effluents has been achieved by degradation using bacterial[3–5] and fungal[6–9] isolates, increasing demands for effective and economical technologies for colour removal have led to research into a biosorption-based process that utilizes the sorption capacity of biological material for the removal of pollutants[10]. The biosorption techniques have been used effectively in the effluent treatment processes mainly for the heavy metals[11,12] and dyes[10,13,14].

Biosorption can be defined as a process in which solids of natural origin are employed for sequestration or separation and isolation of heavy metals from an aqueous environment[15]. The removal of organic colour using biosorption process stems from the work on the removal of metals in biological wastewater treatment systems[13]. Adsorption of solutes (adsorbate) in solution or suspension onto solid materials (adsorbent), occurs mainly through one of the following mechanisms: exchange of molecules from adsorbent to the solution, physical adsorption due to van der Waals forces and chemical adsorption (chemisorption)[16]. Most work has however been carried out on the biosorption of metals with little recent advances in the biosorption of dyes. In this study, we have investigated sorption of two frequently used textile dyes (methyl violet and basic fuchsin), and their mixture, using dead biomass of *Aspergillus niger*. The desorption profiles of the sorbed dyes from the biosorbent were also studied.

Dye concentration in aqueous solution was measured using spectrophotometry (Systronics 104) at specific λ_{max} values of methyl violet, basic fuchsin and their mixture. Standard dose response curves for dyes were established.

The λ_{max} of methyl violet, basic fuchsin and their mixture was determined on a spectrophotometer (Systronics 119). The observed λ_{max} of methyl violet was 585 nm, basic fuchsin 550 nm and that of the mixture, 550 nm. Though evident as a single peak in spectrum analysis, the mixture of both the dyes could be resolved by thin layer chromatography (Merck Silica Gel $60F_{254}$; solvent system – benzene : methanol :: 25 : 9; R_f values: basic fuchsin = 1.8 cm; methyl violet = 1.4 cm), indicating that there was no physical/chemical association between the two dyes when present in a mixture.

Aspergillus niger (#BU10) isolated in our laboratory was used as a biosorbent. The fungal culture was maintained on Czapex Dox Thom agar slants. Spore suspension (10^6 spores/ml) was inoculated in 1000 ml of yeast extract malt extract sucrose (YMS) liquid medium (yeast extract, 10 g; malt extract, 2 g; sucrose, 30 g; distilled water, 1000 ml; pH = 6.8–7.0). The flasks were incubated on a rotary shaker for 48–72 h (28–30°C). Fungal growth (pellets) was strained through a plastic sieve, wet biomass washed thrice with tap water and autoclaved in the final wash water, at 15 psi for 20 min. Such heat treatment has been reported to not only kill the organism but also to increase the sorption efficiency[15]. Water was drained-off and the wet biomass was dried at 37–40°C for 4–5 days. Flakes of dry fungal biomass were crushed in a mortar and pestle and the powdered biomass was sieved on an electrical sieve; particles of 140-mesh size were separated for use since they have been shown to give maximum sorption and consistent results[17].

Sorption profile was studied by adding 0.1 g of biosorbent in 50 ml of dye solution in a 150 ml Erlenmeyer flask. The contents were mixed on a rotary shaker (170 rpm) for 10 min and centrifuged at 4000 rpm to completely sediment the biosorbent; unsorbed dye was estimated in the supernatant. The biosorbent in the sediment (with the sorbed dye) was dried at 37°C for 2–3 days and preserved for further desorption studies.

The contact time for maximum sorption was determined in the initial sorption experiments, which were monitored at 10 min intervals for 100 min. Since maximum sorption was achieved within the first 10 min (Figure 1), this was used for all further experiments. The effect of different factors on the sorption process was investigated by applying a factorial design as this allows effects of the factor to be estimated at several levels of other factors, yielding conclusions that are valid over a range of experimental conditions[18].

The key parameters controlling sorption of the dye are expected to be dye concentration, biomass concentration and initial pH of the aqueous solution. Our previous studies revealed that dye biosorption exhibits saturation kinetics (unpublished data).

For further sorption, fresh biosorbent would have to be added to the dye solution. However, the presence of relatively high concentration of biosorbent in the solution re-
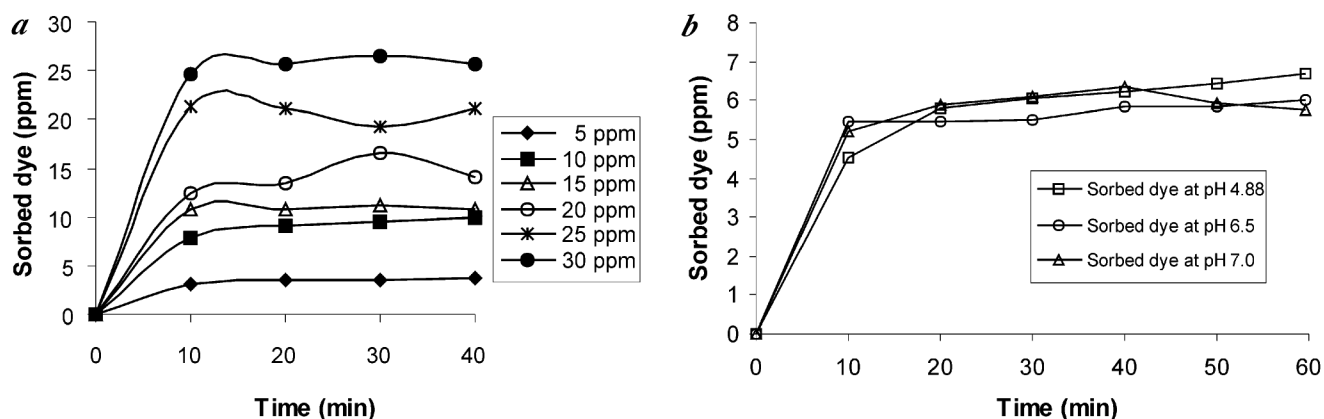
Figure 1. Sorption profile of dye. *a*, Sorption profile of methyl violet at varied initial dye concentration (mixture of methyl violet and basic fuchsin showed similar profile). *b*, Sorption profile of methyl violet at varied pH.

Table 1. Two-way and three-way interaction of pH, dye concentration and biosorbent concentration on sorption of methyl violet, basic fuchsin and their mixture. Significance of interaction was measured using analysis of variance

Dye	Interaction	*F* value	*P* value	Inference
Methyl violet	pH–dye	412.1	< 0.05	Marginal differences in pH
	pH–biomass	77.84	< 0.05	Sorption profile shows slight decrease at pH 6 and slight increase at pH 7
	Dye–biomass	249	< 0.05	With increase in dye concentration, there is increase in sorption at different concentrations of biomass
	Biomass–dye (pH 5)	36.56	< 0.05	Maximum sorption
	Biomass–dye (pH 6)	36.56	< 0.05	Least sorption
	Biomass–dye (pH 7)	36.56	< 0.05	Maximum sorption
Basic fuchsin	pH–dye	4.87	< 0.05	Sorption profile at 10 ppm shows maximum sorption at pH 6
	pH–biomass	1.07	> 0.05	–
	Dye–biomass	1.29	> 0.05	–
Mixture	pH–dye	7.8	< 0.05	Maximum sorption occurs at pH 5 when dye concentration is 20 ppm
	pH–biomass	0.27	> 0.05	–
	Dye–biomass	4.85	< 0.05	With increase in biomass concentration there is increase in sorption

sults in reduced distances between the biosorbent particles, thus making many binding sites unoccupied[13]. This would result in low dye adsorption per unit weight of biomass[10]. However, the H$^+$ ions either act as a bridging moiety between dye molecules and the surface of the biosorbent or may help produce repulsive forces between the dye molecules and the negatively charged cell surface[15].

To define the interrelationship between the parameters (initial pH, dye concentration and biosorbent concentration) controlling sorption kinetics, a series of sorption experiments were performed. Different levels of each variable were investigated, viz. pH 5–7; initial dye concentration 5, 10 and 20 ppm and biosorbent concentration 1–3 g/l. The effects of interaction of two or more variables with each other were determined. The significance of interactions between variables was determined by analysis of variance.

After determining the sorption profile, the possibility of recycling the biosorbent was investigated. Desorption of the dye from the biosorbent was achieved using two different eluants, 0.1 M HCl and 50% ethanol. The former

was chosen because of its usage in desorption of metals[18], and the latter because both the dyes are most soluble in ethanol. Next, 0.05 g of biosorbent (sedimented and dried from the sorption experiments) was added in 5 ml of 0.1 M HCl or 50% ethanol. The suspension was stirred for 10 min on a magnetic stirrer and the contents were then centrifuged. The biosorbent pellet was treated three more times with fresh 5 ml aliquots of the eluants, till maximum colour was eluted out. All aliquots were pooled and the amount of dye desorbed was estimated colorimetrically.

Independent of dye concentration and initial pH, maximum sorption occurred within the first 10 min (Figure 1 *a*). Change in pH between 5 and 7 does not affect the sorption profile (Figure 1 *b*).

The results of experiments to determine the interaction between pH, initial dye concentration and biosorbent concentration are shown in Table 1. Initial pH and dye concentration showed significant interaction for sorption of the dyes. For methyl violet, a marginal difference in sorption was observed with change in pH, while maximum

sorption for basic fuchsin was seen at pH 6. In case of the dye mixture, maximum sorption was seen at pH 5 and 20 ppm initial dye concentration. The two-way interaction of initial dye concentration and biosorbent concentration was significant only for sorption of methyl violet, but not for basic fuchsin (Figure 2). The three-way combined interactive effect of pH, initial dye concentration and biosorbent concentration was significant only for sorption of methyl violet. When examined for the interaction between biomass and dye, maximum sorption was seen at pH 5 and 7, while least sorption was observed at pH 6.0.
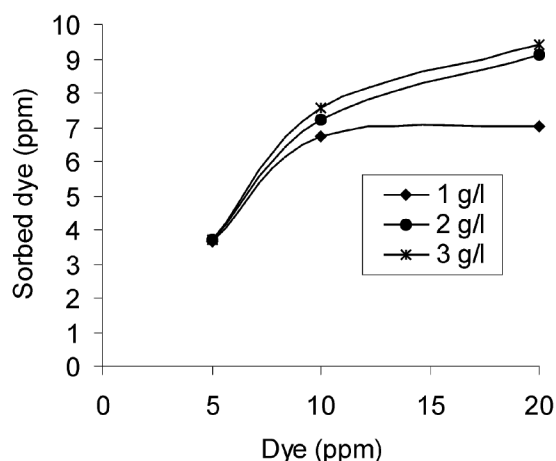

Figure 2. Two-way interaction of initial dye concentration and biosorbent concentration in sorption of dyes in a mixture.
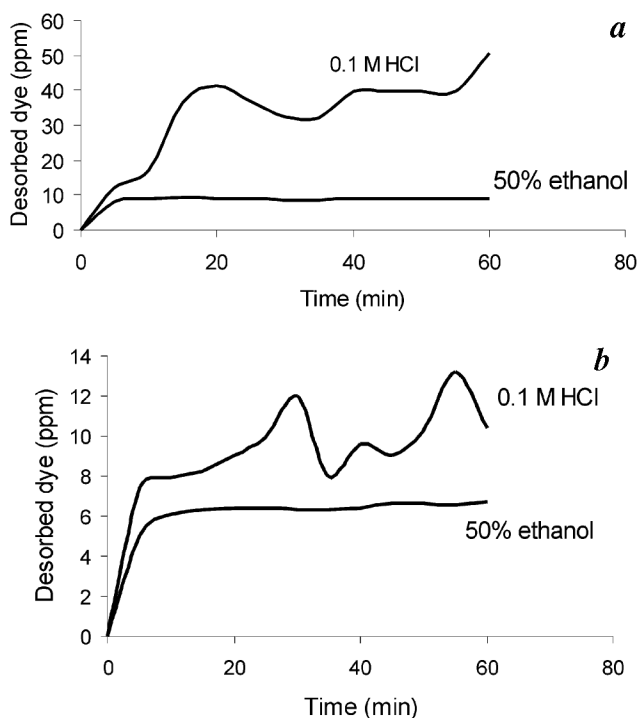

Figure 3. Desorption profile of (*a*) basic fuchsin and (*b*) mixture of methyl violet and basic fuchsin using 0.1 M HCl and 50% ethanol.

Desorption of the single dye or the mixture was better achieved using 0.1M HCl than 50% ethanol (Figure 3).

The studies show that the sorption pattern for dyes in aqueous solutions is similar to that shown for metals. The results show that biosorbent concentration and initial dye concentration affect sorption significantly. Sorption saturation curves observed suggest that sorption efficiency is directly proportional to surface area. The mixture of the dyes in solution showed a single absorption maximum, but was resolved by chromatographic separation. This suggests that the λ_{max} of the two dyes has shifted due to the presence of each other; or that absorption of one dye at one wavelength is affected by the presence of the second dye molecule.

Further application studies would be focused on 'preferential sorption' of a particular dye in a mixture, thus making the process of biosorption cost-efficient. Desorption of the dye from the biosorbent and its regeneration is an attractive proposal. The regenerated biomass thus can be recycled for sorption of the next batch of effluents and the recovered dye can find applications in secondary staining procedures such as plastic staining, paper colouring, etc.

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Species-specific proteins in closely-related seahorses

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Non-denatured polyacrylamide gel shows the respective species-specific characteristics on the muscle protein of *Hippocampus kuda* and *H. trimaculatus*. Two proteins of molecular weight 66.8 and 39.8 kDa were found exclusively in *H. kuda*. These constituted about 69.8 and 16.2% respectively of its protein. In *H. trimaculatus*, two other specific proteins with molecular weight of 674.3 and 50.5 kDa were recorded, which constituted 46.0 and 7.5% respectively of its protein. These species-specific proteins are important for species identification, which paves avenues for further characterization and upgrading of the available information on seahorse taxonomy.

MOST seahorse species were listed as 'vulnerable' in the *Red List of Threatened Animals* during 1996 at the IUCN[1].

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Among them, *Hippocampus kuda* has been used to denote almost all the non-spiny seahorses in the Indo-Pacific region. More than 15 names of apparent species could be regarded as merely synonyms of *H. kuda*[2], which suggests that it may incorporate cryptic (morphologically similar, but genetically different) species. Thus, *H. kuda* complex still warrants further research to clarify the relationship among the species it includes. The present study was undertaken with protein markers to identify the species of seahorses *H. kuda* and *H. trimaculatus*[3].

Relatively similar size group of *H. kuda* and *H. trimaculatus* were collected from peninsular Tamil Nadu (India) coast and from each of them 500 mg of tail muscle was dissected out and stored at –30°C. For preparing the extract, the stored samples were homogenized with 400 μl of extracting buffer [Tris base (0.05 M), EDTA (0.01 M), PMSF (0.1 M), β-mercaptoethanol (0.2%), Triton-X 100 (0.1%)] at pH 8 using a glass–glass homogenizer. The homogenates were centrifuged at 4000 g for 10 min. From the supernatant the total sarcoplasmic protein was quantified by the method of Lowry *et al.*[4].

The 10% separating and 6.5% stacking non-denatured polyacrylamide gel was prepared according to the standard protocols[5]. For this, 60 μg protein of each sample and 20 μl of molecular weight marker (PMW-H, Genei) with equal volume of sample buffer [1 ml glycerol, 0.1 ml 0.5% bromophenol blue, 1 ml 0.5 M Tris HCl (pH 6.8)] were loaded. The gel was run at constant current of 10 mA for 18 h at 15°C in the presence of tank buffer (0.3% Tris base, 1.4% glycine). The gel was then fixed in 7% acetic acid and stained in a staining solution (0.02% CBB-R 250, 40% methanol, 7% acetic acid). The gel was subsequently de-stained in 7% acetic acid and 40% methanol till clear bands appeared.

The correlation between molecular weight and relative mobility was obtained with the proteins in the molecular weight marker kit (PMW-H). The size of the muscle protein was analysed and documented by a gel documentation software system (Syngene, UK).

The sarcoplasmic proteins which account for 20 to 30% of the total protein in fishes, have a unique property that the separation profile obtained on electrophoresis can be used for unequivocal identification of fish species with reference of authentic sample profile[6]. The electrophoretic pattern obtained for the two seahorse species is shown in Figure 1. The distance travelled by each protein component (expressed as *rf* values) for the two species is given in Table 1. The mobility patterns expressed as densitometry scan graphs for *H. kuda* and *H. trimaculatus* are given in Figure 2 *a* and *b* respectively.

Two proteins with a molecular weight of about 66.8 and 39.8 kDa respectively became specifically apparent in the muscle protein of *H. kuda* (Table 1 and Figure 2). Their concentration was about 69.8 and 16.2% respectively, of the total protein. However, two entirely different proteins with molecular weight of about 674.3 kDa