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Studies on biosorption of pollutants by fungal dead biomass

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Abstract
We studied the ability of dead biomass of *Aspergillus Niger* and *Rhizopus nigricans* to sorb dye released in the effluents, effect of different concentrations of fungal biomass on their efficiency to sorb the dye and effects of different contact time periods on the efficiency of the fungal biomass to absorb the dye. An increase in the biomass concentration of *R. nigricans* from 100 mg to 150 mg had positive effect on the amount of percentage of dye sorption in the solution except when the contact period much longer i.e., 25 to 30 minutes. In the case of *R. nigricans* and *A. Niger*, the increase in biomass from 150 mg to 200 mg biomass resulted in decrease in dye uptake in general. Studied showed that both type of biomasses were quite effective for the biosorption of red dye. As much as 66.50% dye was removed from the effluent by 150 mg biomass of *A. niger* after 20 minutes of contact period. Up to 93.69% of the dye could be removed by 200 mg biomass of *R. nigricans* after 25 minutes of contact period. In general, *R. nigricans* was much more efficient biosorption agent as compared to *A. niger*. The range of adsobtion of dye by *R. nigricans* lied between 50.60% to 93.69% while that of *A. niger* lied between 36.62 to 66.50%.

Keywords: biosorption, *Aspergillus Niger*, *Rhizopus nigricans*, dead biomass, etc

Introduction
Industrial effluents contribute enormously to water deterioration and their treatment is the subject of discussion and regulation in many countries. Industrial effluents of various industries like textile, distillery, paint, finishing, natural and synthetic dyes, paper and pulp industries and oil mills etc. are the major contributors of water pollution. Industrial effluents, contain different types of inorganic and organic contaminants and discharged through the aquatic route either in water soluble or insoluble forms. Textile effluents are among the most difficult to treat wastewaters due to their considerable number of toxic substances (Hai *et al.*, 2017). It is believed that one third of the total water pollution comes in the form of discharge of industrial effluents (Bulgariu *et al.*, 2014) [4]. Adulteration of water is caused by releasing of industrial wastewater into the natural water bodies (Ayangbenro *et al.*, 2017) [1]. Different class of colourant (dyes and pigments) with complex aromatic structure are in use for different applications like food, paper, printing, textiles, pharmacy, cosmetics and plastic (Mohana *et al.*, 2008; Boudechiche *et al.*, 2016) [16, 3]. Globally higher number than 10,000 types of colourants (pigments and dyes) are used in colouring applications (Pandya *et al.*, 2017) [18]. It is estimated that more than 8, 00,000 tons of colourants are produced annually, however 10% of the used colourants lost during the process and dyestuff makes entry into the external surroundings (Kalaiarasi *et al.*, 2012) [12]. The dyes of synthetic origin are of complex aromatic structure and difficult to degrade by conventional biodegradation process like the activated sludge process (Kalkan *et al.*, 2015) [13]. Dyes and pigments cause chronic ecological problems as they are toxic and carcinogenic. Purging of dyes from the coloured waste effluents is a crucial problem which has now become a serious threat to lives depend on it (Kumar *et al.*, 2020) [14].
The biosorption techniques have been used effectively in the effluent treatment process mainly for heavy metals (Carpentor and Price, 1976; Borstad *et al.*, 1992) [5, 2] and dyes (Desa, 2000; Sarangi *et al.*, 2001; Chauhan *et al.*, 2002) [7, 20, 6, 19, 20]. Biosorption can be defined as "A process in which solids of natural origin are employed for sequesteration or seperation and isolation of heavy metal from an aqueous environment" (Santosh *et al.*, 2002). The same is true in the case of dyes also.
During our observations the dead biomass of *Aspergillus niger* and *Rhizopus nigricans* is utilized for biosorption of dyes from effluents emanating from industries. Therefore, it was decided to work out.

a. The ability of dead biomass of *Aspergillus niger* and *Rhizopus nigricans* to sorb dye released in the effluent;
b. Effect of different concentrations of fungal biomass on their efficiency to sorb the dye;
c. Effects of different contact time periods on the efficiency of the fungal biomass to adsorb the dye.

Materials and Methods

Preparation of Fungal Biomass

Aspergillus niger and *Rhizopus nigricans* isolated in laboratory were tried as biosorbents. The fungal cultures were maintained on Potato Dextrose Agar medium (PDA). The spore suspension of each fungus was inoculated in 2000 ml of Yeast Malt extract Sucrose medium (YMS). This 2000 ml of YMS medium were dispensed equally in 16 flasks of 250 ml capacity. Thus, each of the 16 flasks contained 125 ml of the medium per fungus. After inoculation, the flasks were incubated on a shaker for 48-72 h (28-30°C), and then for about 25-30 days with shaking at periodic intervals. 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 minutes. Water was drained off and the wet biomass was dried at 37-40°C for 5 days. The dried fungal biomass so obtained was crushed using a morter and pestle and the powdered biomass so obtained was sieved through a simple sieve. The powered samples were stored at a dry place till further use.

Procurement of Effluents and the Dye

The effluents containing red dye were collected from textile dyeing unit located near Shastri Nagar (Meerut). The red dye powder used originally by the unit was also purchased from the market. It is sold under the brand name “Red Brodex”

Preparation of Standard Solution

Since 5 Kg of Red dye is dissolved in 800 liters of water by the industrial unit, a dye solution of similar concentration was prepared by dissolving 6.25 gm of the dye in 1 liter of water to prepare stock solution. The O.D. of the solution was beyond the range of the spectrophotometer so, its dilutions were prepared. For this, 1ml of the dye solution was thoroughly mixed with 99 ml of distilled water. The solution was found to have maximum absorption wavelength of 485 nm. From the stock solutions dilutions of different concentrations of the dye were prepared by mixing 10 ml, 20 ml, 30 ml, 40 ml, 50 ml, 60 ml, 70 ml, 80 ml, and 90 ml of the dye solution with sufficient water so that the volume of each solution was 100 ml. The O.D. of these solutions were measured and plotted on a graph paper to obtain the line of best fit.

Estimation of the Amount of Dye in the Untreated Effluents

1 ml of the effluent was diluted with 49 ml of water so that the total volume was raised to 50 ml and the effluent was diluted 50 times. O.D. was measured and was observed to be 0.415. When multiplied with the factor 4243.98, it yielded the value 0.415 × 4243.98 = 1761.25 *i.e.*, the concentration of dye in the effluent was 1761.25 ppm.

Estimation of the Dye Concentration in the Effluent Treated with Fungal Biomass

50 ml of diluted effluent as described in the section 2.4. were taken in each of a set of 54 flasks. The set was divided into 3 subsets of 18 flasks. Each flask of subset A were added 100 mg of *Aspergillus niger* biomass. Similarly, each flask of sets B and C received 150 mg and 200 mg of the *Aspergillus niger* biomass. The flasks were kept on mechanical shaker after periodic intervals (5 min, 10 min, 15 min, 20 min, 25 min and 30 min). 3 flasks from subset A, 3 from subset B and 3 from subset C were removed, and their contents were first decanted and 10 ml fractions centrifuged at 4000 rpm for 10 minutes. The O.D of the supernatant fluids were recorded at 485 nm. The concentration of the dye in the supernatant was calculated using the same factor as in the section 2.4.

Percentage reduction in the quantity of dye as a result of the treatment with the biomass was calculated. The dye uptake by the biosorbent was calculated using the formula-

$$Q = \frac{V(C_i - C_f)}{m}$$

Where, Q is the dye uptake (MG dye per g Biosorbent),

V = The liquid sample volume (ml)
C_i = The initial concentration of the dye in the solution (mg/l)
C_f = The final (equilibrium) concentration of the dye in the solution (mg/l) and
M = The amount of added biosorbent on the dry basis (mg)

Note: The similar procedure was repeated using the biomass of *Rhizopus nigricans*.

Result and Discussion

Biosorption of Dye by Fungal Biomass

The capacity of dry biomass of *Aspergillus Niger* and *Rhizopus nigricans* to adsorb red dye (Brodex) was studied. The dry powder of two fungal species were allowed to biosorb dyes for different time period i.e,. 5 minutes, 10 minutes, 15 minutes, 20 minutes, 25 minutes and 30 minutes. Three concentrations i.e., 100mg, 150

mg and 200 mg were tried for each of the two types of biomasses. The results are presented in the tables 3.1, 3.2 and 3.3.

Table 1: The concentration of red dye remaining in the effluent and the Percentage of dye up take in different contact time with different concentration of *Aspergillus niger* (Initial in the effluent =1761.25ppm).

Contact Time	Amount of Fungal biomass.		
	100 mg	**150 mg**	**200 mg**
5min	916.69 ±2.767	1014.31 ±0.064	882.74± 0.047
	47.95%	42.40%	49.87%
10 min	988.84± 0.098	916.69 ± 0.206	997.33± 0.072
	43.85%	47.96%	43.37%
15 min	844.55 ± 0.002	831.82 ± 0.011	819.08 ± 3.081
	52.04%	52.77%	43.49%
20 min	844.55 ± 0.670	589.91 ± 4.816	895.47 ± 0.381
	52.04%	66.50%	49.15%
25 min	855.03 ± 0.165	1116.16 ± 0.186	1068.48 ± 1.536
	51.56%	36.62%	39.27%
30 min	696.01 ± 2.385	899.72 ± 3.106	848.79 ± 0.861
	60.48%	48.91%	41.80%

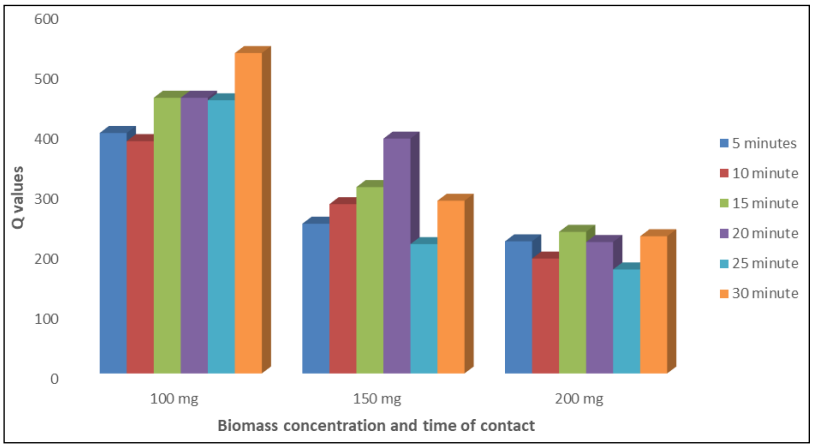
Table 2: The concentration of red dye remaining in the effluent and the Percentage of dye up take in different contact time period with different concentration of *Rhizopus nigricans* (Initial in the effluent =1761.25ppm).

Contact Time	Amount of Fungal biomass		
	100mg biomass	**150 mg biomass**	**200 mg biomass**
5min	568.69 ± 0.104	522.00 ± 0.203	551.71 ± 2.733
	67.71%	70.36%	68.67%
10 min	700.25 ± 0.0895	496.54 ± 0.823	530.49 ± 0.121
	60.24%	71.80%	69.87%
15 min	551.71 ± 0.0348	318.29 ± 0.084	445.61 ± 0.117
	68.67%	81.92%	74.69%
20 min	471.08 ± 0.0257	424.39 ± 0.016	763.91 ± 0.033
	73.01%	77.90%	56.62%
25 min	475.32 ± 0.012	662.06 ± 0.751	110.99 ± 1.358
	73.01%	62.42%	93.69%
30 min	585.66 ± 0.163	870.01 ± 0.016	819.08 ± 0.144
	66.74%	50.60%	53.49%

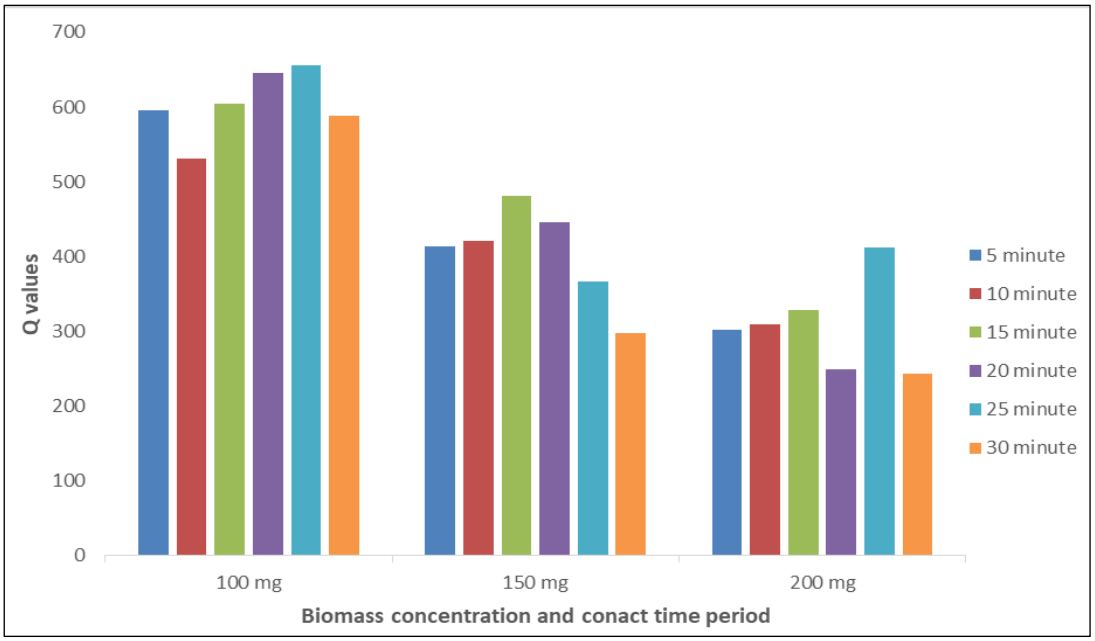
A glance at the tables reveals that

a. Both type of biomasses were quite effective for the biosorption of red dye. As much as 66.50% dye was removed from the effluent by 150 mg biomass of *Aspergillus niger* after 20 minutes of contact period. Up to 93.69% of the dye could be removed by 200 mg biomass of *Rhizopus nigricans* after 25 minutes of contact period.

b. In general, *Rhizopus nigricans* was much more efficient biosorption agent as compared to *Aspergillus niger*.



Graph 1: showing range of adsobtion of dye by Q value (uptake in ppm/mg biomass) of red dye by fungal biomass after different time intervals and with different concentration of biosorption of *A. niger*.



Graph 2: showing range of adsobtion of dye by Q value (uptake in ppm/mg biomass) of red dye by fungal biomass after different time intervals and with different concentration of biosorption of *R. nigricans*.

Rhizopus nigricans lied between 50.60% to 93.69% while that of *Aspergillus niger* lied between 36.62 to 66.50% As can be judge from the table 3.3, of Q Value of *Aspergillus niger* ranges from 172.94 to 532.62. On the other hand, the Q value of *Rhizopus nigricans* ranges from 243.04 to 655.82. As for as metals are concerned the fungi are known to have very good metal uptake system (Gadd 1986) [10]. However, the biomass of mucorales e.g., *Absidia orchidis, Cunninghamella echinulata, mucor* spp., *Rhizopus arrhizus,* and *Rhizopus nigricans* are reported to be efficient biosorbent agent (Vankateswerlu and Stotzky 1989; Luef *et al.,* 1991; Fourest and Roux 1992; Mullen *et al.*, 1992; El-Morsy 2004) [22, 15, 9, 17, 8]. It has been attributed to high chitin and chitosan contents of the cell walls of the fungi (Thezos and Volesky, 1981) [21]. Mullen *et al.,* (1992) [17] also found that *Mucor rouxii* was better adsorber of metal than *Aspergillus niger.*

Conclusion

An increase in the biomass concentration of *Rhizopus nigricans* from 100 mg to 150 mg had positive effect on the amount of percentage of dye sorption in the solution except when the contact period much longer i.e., 25 to 30 minutes. Of course, in case of *Aspergillus niger* even after five minutes there was a lesser dye uptake with 150 mg biomass as compared to 100 mg mass. In the case of *Rhizopus nigricans* and *Aspergillus niger*, the increase in biomass from 150 mg to 200 mg biomass resulted in decrease in dye uptake in general with minor exception. However, if we consider the dye uptake per milligram biomass it can be clearly judge from the graph that the uptake decreased with the increase in the biomass. As reported by others also the presence of higher concentration of biomass in the solution results in reduced distance between biosorbent particles. Thus, many binding sites remain unoccupied. Others also believe that repulsive forces between the dye molecule and the charged surfaces of biosorbents result in decreased in dye adsorption. However further detailed study on saturation kinetics of dye biosorbtions are required through further light on this issue. Barring one exception, the dye uptake per mg was influence by both bioserbent concentration and contact time. It was found that as the biosorbent concentration increase saturation point was reached early.

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