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A COMPARATIVE STUDY ON THE ACCUMULATION OF ACID RED 18 AND REACTIVE BLACK 5 DYES BY GROWING *SCHIZOPHYLLUM COMMUNE* AND *TRAMETES VERSICOLOR*

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ABSTRACT

The accumulation of acid red 18 and reactive black 5 by growing *Schizophyllum commune* and *Trametes versicolor* was studied with respect to the initial pH varying from 1 to 6 and initial dye concentration (10 to 100 mg/litre) at 25°C. The initial pH of the dye solution strongly affected the growth of the fungus and the accumulation of dyes. The effective pH was observed as 2 for both; growth and color removal of these azo dyes. Increasing the concentration of azo dyes inhibited the growth of *S. commune.* It was observed that both fungi were capable of removing acid red 18 and reactive black 5 with a maximum specific uptake capacity of 82.1 and 179.1 (mg/g) for *S. commune* and 76.1 and 178.3 (mg/g) for *T. versicolor*, respectively for an initial dye concentration of 100 mg/litre. Maximum percentage color removal was observed at lower concentrations of both the dyes. Finally, it was observed that the percentage color removal was found to be more in reactive black 5 dye when compared to the acid red 18 dye studied in the present investigation.

Key words: *Schizophyllum commune*, *Trametes versicolor,* Accumulation, Reactive black 5, Acid red 18

INTRODUCTION

Dyes are extensively used in textile, paper, printing industries and dye houses. The effluents produced by these industries detoriates the water quality and causes damage to the human health[1]. These dyes are often recalcitrant to microbial degradation because they

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contain substitution such as azo and sulpho groups[2].

Dyes are usually classified as anionic (Acid and reactive dyes), cationic (Basic dyes) and non-ionic (Disperse dyes)[3]. These synthetic dyes are usually treated by physical or chemical treatment processes include flocculation, floatation, membrane filtration, electrochemical destruction, ion exchange and ozonation. But these methods are involved with high initial investment and operations costs.

Activated carbon is the most widely used adsorbent for the removal of color from the effluent but due to its high cost, it is not useful for a large-scale operation[4]. So this leads to search for cheap and alternative sources such as eucalyptus barks[5], agricultural residues[6] and barley husks[7] and rice husk[8].

These low cost adsorbents are characterized by low adsorption capacity[9]. Recently, the use of fungal, bacterial and algae biomass has been investigated as a potential adsorbent. Large amount of fungal biomass waste produced by industries can also be used as biosorbent and the disposal of these adsorbent can be solved by recycling after regenerating the biosorbent using sodium hydroxide and organic solvents[10-12]. Many research works reported in literature are concerned with living fungi for the bioaccumulation and biodegradation of dyes[13] and dead fungal biomass for the biosorption of synthetic dyes[14, 15].

The main aim of viable culture in color removal process is to avoid the need of separate biomass production process, for instance activation, harvesting, drying, processing and storage when compared to usage of non-viable microbial biomass.

Bioaccumulation mechanism plays an important role in the color removal of dye by living fungi. *S. commune*[16, 17] and *T. versicolor*[18] are the white rot fungal strain, which have been used for the decolorization of lignin and textile dyes. In the present investigation, *S. commune* and *T. versicolor* fungal biomass has been used for the removal of textile azo dyes in a batch process. The main advantage of the *S. commune* is that it is readily available waste wood rot fungal biomass. The scope of the present work was to investigate the growth and bioaccumulation properties of *S. commune* and *T. versicolor* as a function of the initial pH and initial acid red 18 and reactive black 5 dye concentration.

EXPERIMENTAL

Materials and methods

Materials

Acid red 18 and reactive black 5 dyes were obtained from Sd Fine–Chem limited, Mumbai, India. The chemical structures of the dyes are shown in Fig. 1. All other chemicals and solvents used were analar grade and were purchased from Glaxo India Limited, Mumbai, India.

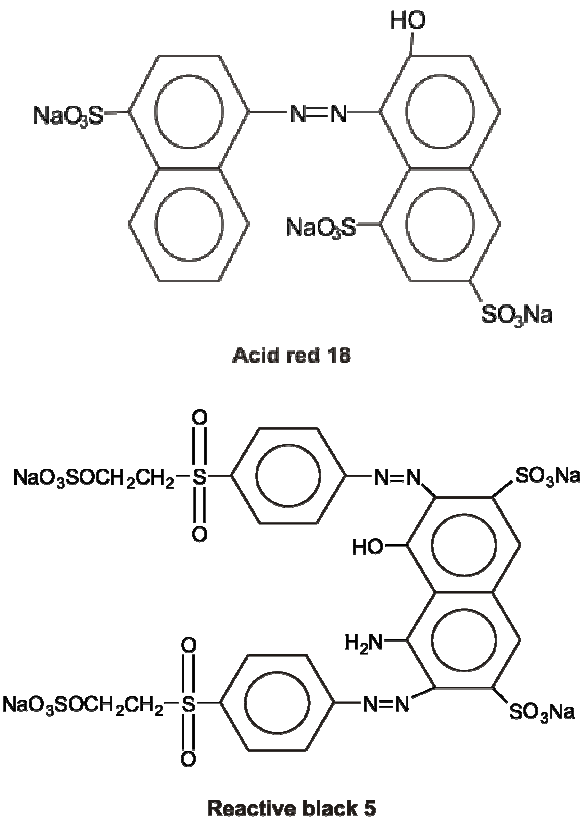


Fig. 1: The chemical structure of the acid red 18 and reactive black 5

Fungal isolation and cultivation

The *S. commune* (white rot fungus) grown on the bark of *Tamarindus indica* (Tamarind tree) was isolated through serial dilution and spread plating methods. The strain

was characterized at the Center for Advanced Studies, Botany, University of Madras, Chennai, India. The *T. versicolor* (white rot fungus) was obtained from the IMTECH (Institute of Microbial Technology), Chandigarh, India for color removal study. Both strains were grown aerobically in potato dextrose broth medium in a temperature controlled shaker maintained at 30°C and at 180 rpm. After 72 h, the cultures were transferred into the azo dye solution.

Accumulation experiment

The growth and dye uptake by *S. commune* and *T. versicolor* were carried out in 250 mL Erlenmeyer flask containing 100 mL of acid red 18 and reactive black 5 dye solution. For the pH studies, 30 mg/litre of acid red 18 and reactive black 5 dye solutions were prepared and the pH of the individual dye solutions were adjusted to 1, 2, 3, 4, 5 and 6 using tartaric acid. Dye solutions of different concentration (10, 20, 30, 50, 70 and 100 mg/litre) were prepared and the solution pH was maintained at 2 for the initial dye concentration studies. All the solutions were sterilized. 15% (v/v) of the fungal pure culture was inoculated into the dye solutions and the experiment was repeated (same as that used for the growth of the fungus). Samples were withdrawn for every 12 h interval, during the growth phase. It was then centrifuged at 12000 rpm for 10 min and the absorbance of the supernatant liquid was determined using U.V. spectrophotometer (Hitachi, U3210, Japan). Color removal values were calculated as the ratio between the bio-accumulated concentrations of dye on the fungal biomass to the initial dye concentration. The dry weight of the biomass was determined by drying the cell pellet at 70°C for 24 h. At every 12 h interval, pH of the dye solution was measured and it was observed that the deviation from the fixed value was marginal. Hence, change in pH values was not accounted for during the color removal process. All experiments were carried out with suitable control and the values used in the calculations were the arithmetical average of at least two experimental values.

Statistical analysis

Mathematical and statistical analyses were executed in duplicate and the results were found to be within 5%[19]. The initial dye concentrations were employed with 6 replicate equilibrium uptake tests, which have been used in the batch color removal studies. Standard deviation was calculated for the final dye concentration values using the statistical analysis and the values were observed to be less than 5% of the mean value. The experimental results obtained from the present investigation were analyzed using randomized block design on the SPSS by analysis of variance (ANOVA)[20].

RESULTS AND DISCUSSION

The effect of acid red 18 and reactive black 5 dyes on growth and bioaccumulation properties of *S. commune* and *T. versicolor* was investigated as a function of initial pH and initial dye concentration. The dried cell biomass (X), specific growth rate of the microorganisms (μ), bio-accumulated dye concentration at any time (C_{acc}), bio-accumulated dye concentration at the end (72 h) of growth (C_{accm}), maximum specific dye uptake (q_m) and specific uptake rate (q_r) were determined. The uptake yield (uptake %) was also investigated as the ratio of the bio-accumulated concentration of dye at the end of the growth phase to the initial dye concentration.

The specific growth rate of both fungi is presented by the following equation

$$\mu = \frac{1}{X}\frac{dX}{dt} \qquad \ldots(1)$$

The specific growth rate was determined from the slope of the ln X versus time (t) plot at the exponential growth phase.

The maximum specific dye uptake (q_m) is obtained by the following equation

$$q_m = \frac{V(C_o - C_{reg})}{V_m X_m} \qquad \ldots(2)$$

where, V, C_o, C_{reg}, V_m and X_m are volume of the dye solution (L), initial dye concentration (mg/L), residual dye concentration at the end of fungal growth (mg/L), volume of the fungal suspension (L) and maximum dried fungal biomass concentration at the end of fungal growth (g/L), respectively.

The specific uptake rate (q_r) is defined by the expression

$$q_r = -\frac{1}{X}\frac{dC_{acc}}{dt} \qquad \ldots(3)$$

where, X, C_{acc} and t are fungal biomass concentration, dye concentration and time, respectively.

The influence of initial pH on the maximum dried fungal biomass concentration of the acid red 18 and reactive black 5 dyes are presented in Table 1. It was observed from Fig. 2, that the specific growth rate of the fungus was found to be increased with increase

in pH from 1 to 6 and the maximum specific dye uptake was found to decrease with an increase in pH from 2 to 6.

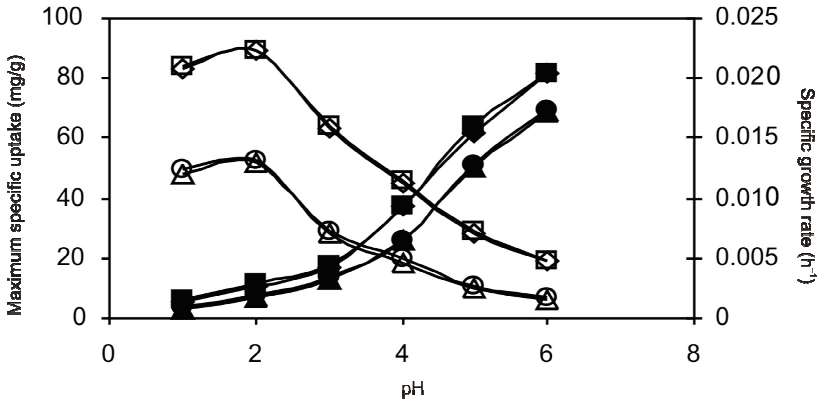


Fig. 2: The effect of pH on the maximum specific uptake of acid red 18 (*S. commune* (○) and *T. versicolor* (Δ)) and reactive black 5 (*S. commune* (□) and *T. versicolor* (◊)) and the specific growth rate of both fungi in dye solutions acid red 18 (*S. commune* (●) and *T. versicolor* (▲)) and reactive black 5 (*S. commune* (■) and *T. versicolor* (♦)) at 25^{O} C, 180 rpm and 30 mg/litre of initial dye concentration.

Table 1. Effect of initial pH on the maximum dried *S. commune* and *T. versicolor* concentration at the end of microbial growth in acid red 18 and reactive black 5 solution (T = 25°C, S_R = 180 rpm, C_o = 30 mg/litre)

pH	*S. commune*		*T. versicolor*	
	$X_m \times 10^2$ in acid red 18 (g/litre)	$X_m \times 10^2$ in reactive black 5 (g/litre)	$X_m \times 10^2$ in acid red 18 (g/litre)	$X_m \times 10^2$ in reactive black 5 (g/litre)
1	19.6 ± 0.2	20.5 ± 0.1	19.4 ± 0.1	20.3 ± 0.2
2	20.9 ± 0.2	22.2 ± 0.2	20.7 ± 0.1	22.0 ± 0.3
3	23.3 ± 0.1	24.8 ± 0.1	23.0 ± 0.2	24.6 ± 0.2
4	28.9 ± 0.2	35.2 ± 0.2	28.6 ± 0.3	34.9 ± 0.3
5	44.4 ± 0.4	53.6 ± 0.4	43.9 ± 0.2	53.2 ± 0.2
6	61.1 ± 0.3	75.6 ± 0.4	60.4 ± 0.3	74.9 ± 0.4

Maximum specific uptake of acid red 18 and reactive black 5 dyes were determined as 52.6 and 89.5 mg/g for *S. commune* and 51.7 and 89.1 mg/g for *T. versicolor*, respectively at the pH 2. At lower pH, negatively charged dye anions present in the dye solution are being adsorbed by positively charged cell surfaces due to the electrostatic attraction[12,14,19]. The fungal cell wall is composed of polysaccharides, proteins, lipids and melanin with several functional groups (such as amino, carboxyl, thiol and phosphate groups) capable of binding the dye molecules[13].

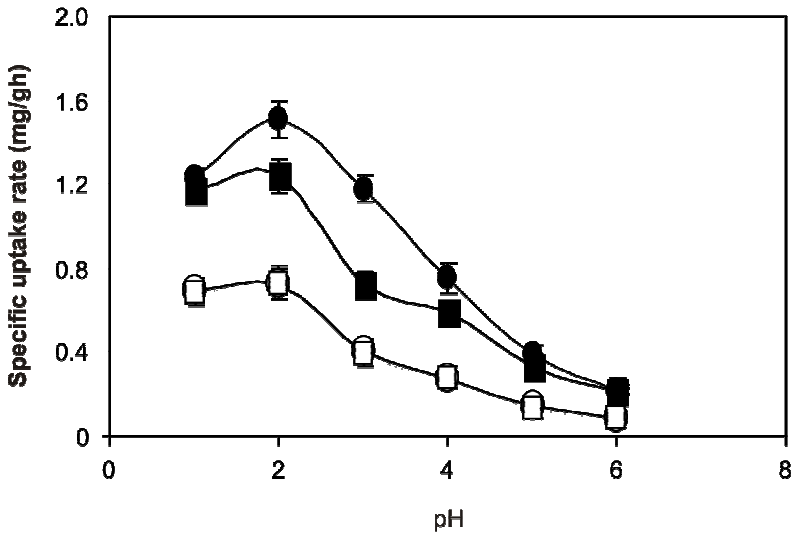


Fig. 3. The effect of pH on the specific uptake rate of the acid red 18 and reactive black 5 dye using *S. commune* (acid red 18 (○) and reactive black 5 (•)) and *T. versicolor* (acid red 18 (□) and reactive black 5 (■)) at 25^{O} C, 180 rpm and 30 mg/L initial dye concentration.

The influence of initial pH on the specific uptake rate of acid red 18 and reactive black 5 using *S. commune* and *T. versicolor* are presented in Fig. 3. It was observed that the specific uptake rate was fond to be increased with increase in pH upto a value of 2 and then declined with further increase in pH value above 2. Maximum specific uptake rate of acid red 18 and reactive black 5 dyes were determined as 0.73 and 1.51 mg/g h for *S. commune* and 0.72 and 1.24 mg/g h for *T. versicolor,* respectively at the pH 2. The effect of pH on percentage color removal of acid red 18 and reactive black 5 dye solution using *S. commune* and *T. versicolor* are shown in Fig. 4. The maximum percentage color removal was obtained at pH value 2 and then decreased gradually with increasing pH.

Effect of initial dye concentration on growth

Different concentrations (10, 20, 30, 50, 70 and 100 mg/litre) of acid red 18 and reactive black 5 dye solutions were prepared and the solution pH was adjusted to the optimal value of 2 by the addition of tartaric acid. Bioaccumulation experiments were carried out with *S. commune* and *T. versicolor* and the results are presented in Table 2 for acid red 18 and reactive black 5. The bioaccumulated acid red 18 and reactive black 5 dye concentration at the end of fungal growth was found to be increased with increase in initial dye concentration. From Fig. 5, it was observed that the specific growth rate and maximum fungus concentration decreased with increasing initial dye concentration in the dye solutions containing tartaric acid. This was due to the inhibition effect of azo dyes at higher initial concentrations on the growth of the both fungal strains.

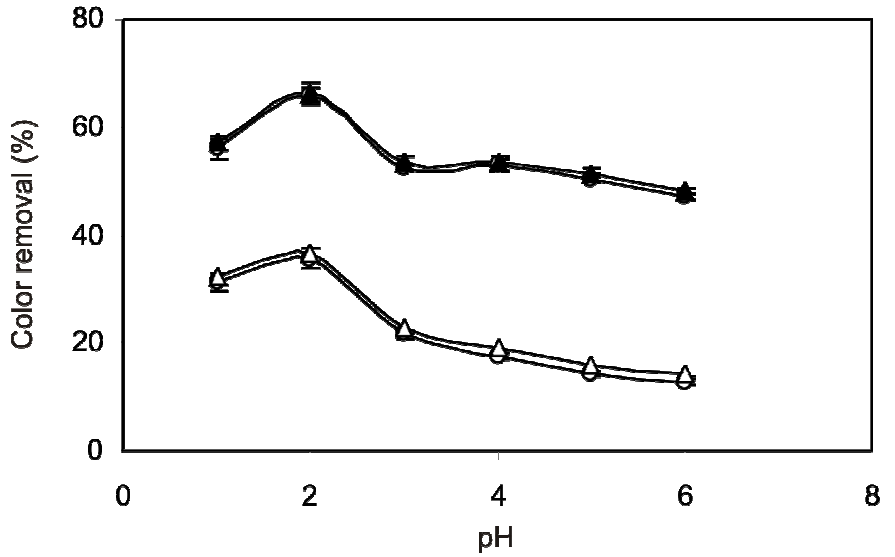


Fig. 4: The effect of pH on percentage color removal of acid red 18 and reactive black 5 dyes using *S. commune* (acid red 18 (Δ) and reactive black 5 (▲)) and *T. versicolor* (acid red 18 (○) and reactive black 5 (•)) at 25^{O} C, 180 rpm and 30 mg/L initial dye concentration.

In the absence of azo dye, the maximum specific growth rate for *S. commune* and *T. versicolor* were recorded as 0.0071 (h^{-1}) and 0.0053 (h^{-1}) (Fig. 5) and fungus concentration for *S. commune* and *T. versicolor* were recorded as 0.298 (g/litre) and 0.268 (g/litre) (Table 2), respectively. The increase of acid red 18 dye concentration from 10 mg/litre to 100 mg/litre led to an increase in the specific dye (acid red 18) uptake capacity

of the fungus from 27.1 to 82.1 mg/g for *S. commune* and from 27.1 to 76.1 mg/g for *T. versicolor*, respectively It was also observed that the uptake yields were found to be higher at a lower dye concentrations.

The growth and specific dye uptake capacity of both fungi were strongly influenced by the initial dye concentration. The specific growth rate of the both fungi decreased from 0.0034 to 0.0018 (h^{-1}) (reactive black 5) and from 0.0025 to 0.0009 (h^{-1}) (acid red 18) for *S. commune* and from 0.0032 to 0.0017 (h^{-1}) (reactive black 5) and from 0.0023 to 0.0007 (h^{-1}) (acid red 18) for *T. versicolor* when the dye concentrations were increased from 10 to 100 mg/litre, respectively.

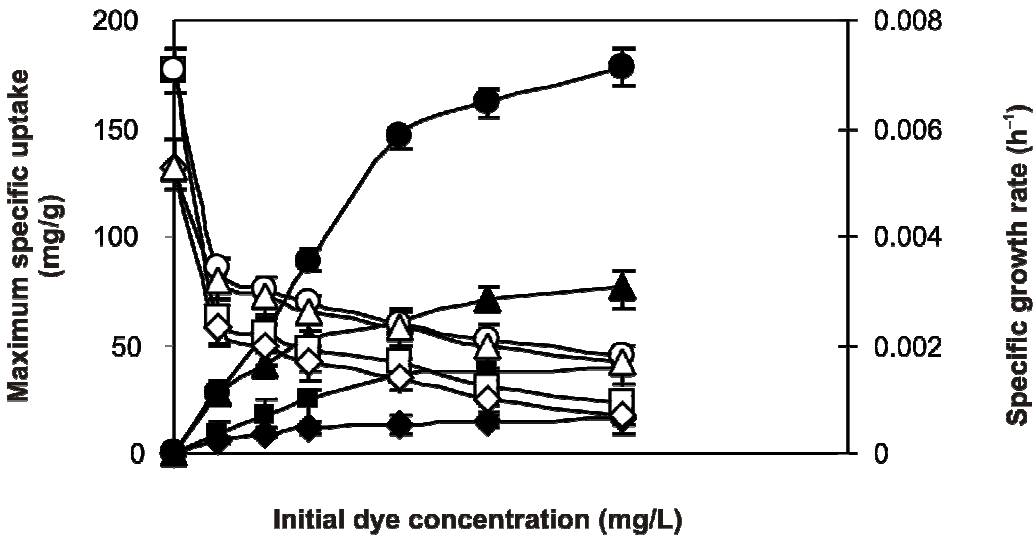


Fig. 5: The effect of initial dye concentration on the maximum specific uptake of acid red 18 (*S. commune* (□) and *T. versicolor* (◊)) and reactive black 5 (*S. commune* (○) and *T. versicolor* (Δ)) and the specific growth rate of both fungi in dye solutions of acid red 18 (*S. commune* (■) and *T. versicolor* (♦)) and reactive black 5 (*S. commune* (●) and *T. versicolor* (▲)) at 25^{O} C, 180 rpm and pH value of 2.

With 100 mg/litre initial dye concentration, the maximum specific uptake capacity was obtained from the bioaccumulation experiment as 82.1 mg/g (acid red 18) and 179.1 mg/g (reactive black 5) for *S. commune* and 76.1 mg/g (acid red 18) and 178.3 mg/g (reactive black 5) for *T. versicolor*. With the influence of initial dye concentration, the specific uptake rate of acid red 18 and reactive black 5 using *S. commune* and *T. versicolor* are presented in Fig. 6.

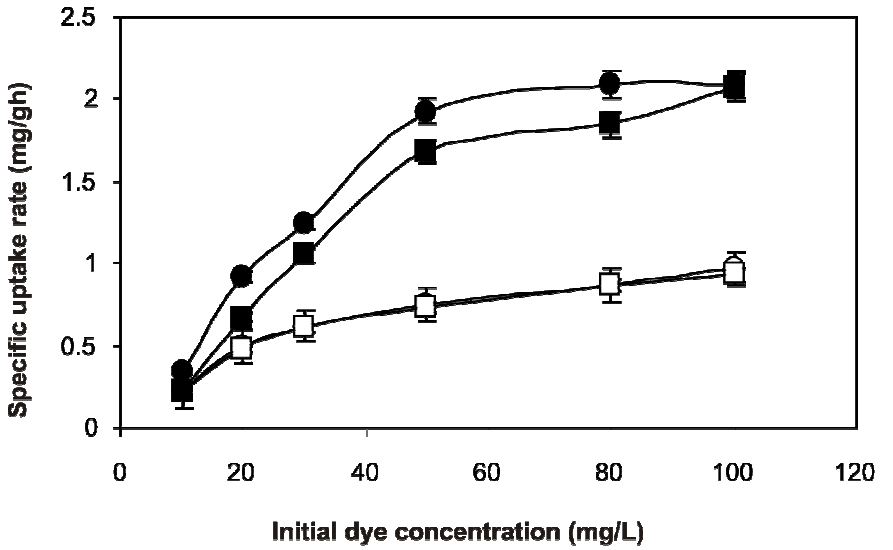


Fig. 6: The effect of initial dye concentration on the specific uptake rate of the acid red 18 and reactive black 5 dye using *S. commune* (acid red 18 (○) and reactive black 5 (●)) and *T. versicolor* (acid red 18 (□) and reactive black 5 (■)) at 25^{O} C, 180 rpm and pH value of 2.

The specific uptake rate was found to increase with increase in initial dye concentration in the dye solutions containing tartaric acid. The specific uptake rate of reactive black 5 using *S. commune* and *T. versicolor* was found to be more when compared to the acid red 18 dye. The maximum specific uptake rate value of reactive black 5 dye was determined as 2.09 (mg/g h) for *S. commune* and 2.07 (mg/g h) for *T. versicolor*, respectively. Abundantly and cheaply available wood rot fungus *S. commune* has been successfully used for the dye removal process and the result was found to be comparable with other result obtained by other fungal cultures available in the literature. Hence, the removal of textile azo dyes using waste fungal biomass *S. commune* has been considered as an important result.

The maximum dye uptake capacity of reactive black 5 dye was reported as 44 (mg/g) using *Endothiella aggregata,* 60 (mg/g) using *Geotrichum fici,* 92 (mg/g) using *Tremella fuciformis and* 36 (mg/g) using *Dekkera bruxellensis by* Polman and Breckenridge[22]. However in thes present investigation it has been observed that the reactive black 5 dye uptake capacity was found to be 179.1 (mg/g) using *S. commune* and 178.3 (mg/g) using *T. versicolor* waste fungal biomass.

Table 2. Comparison of the *S. commune* and *T. versicolor* maximum dried fungus and bio-accumulated acid red 18 and reactive black 5 dyes concentration at the end of microbial growth obtained at different initial dye concentrations (T = 25°C, S_R = 180 rpm, pH = 2)

C_o (mg/litre)	Acid red 18		Reactive black 5	
	$X_m \times 10^2$ (g/litre)	C_{accm} (mg/litre)	$X_m \times 10^2$ (g/litre)	C_{accm} (mg/litre)
		S. commune		
0	29.8 ± 0.3	Nil	29.8 ± 0.3	Nil
10	21.9 ± 0.3	6.02 ± 0.03	23.2 ± 0.4	8.98 ± 0.5
20	21.4 ± 0.2	8.97 ± 0.02	22.7 ± 0.4	17.95 ± 0.07
30	20.9 ± 0.2	11.00 ± 0.09	22.2 ± 0.2	23.98 ± 0.08
50	20.5 ± 0.2	12.97 ± 0.08	21.8 ± 0.4	35.93 ± 0.08
70	20.0 ± 0.1	14.97 ± 0.10	21.3 ± 0.2	37.90 ± 0.09
100	19.5 ± 0.2	15.99 ± 0.09	20.8 ± 0.3	38.89 ± 0.08
		T. versicolor		
0	26.8 ± 0.2	Nil	26.8 ± 0.3	Nil
10	21.6 ± 0.2	5.95 ± 0.03	23.0 ± 0.2	8.97 ± 0.04
20	21.1 ± 0.2	8.69 ± 0.04	22.5 ± 0.3	17.93 ± 0.06
30	20.7 ± 0.1	10.70 ± 0.05	22.0 ± 0.3	23.82 ± 0.05
50	20.3 ± 0.2	12.37 ± 0.06	21.6 ± 0.2	35.74 ± 0.08
70	19.7 ± 0.2	14.10 ± 0.08	21.1 ± 0.1	36.34 ± 0.07
100	19.3 ± 0.1	14.66 ± 0.09	20.6 ± 0.2	36.63 ± 0.08

Microbial growth and dye accumulation kinetics

The result obtained from the growth and dye accumulation of both fungi inoculated with 10, 20, 30, 50, 70 and 100 mg/litre initial dye concentrations of acid red 18 and reactive black 5 are analysed. From Fig. 7 it was observed that the percentage color removal decreased with increase in dye concentration due to the inhibitory effect of dyes on the growth of the fungus and caused a reduction in final biomass concentration. The percentage color removal of reactive black 5 by *S. commune* in 120 h was observed as 64.5%, 6.3.7%, 62.2%, 61.2%, 49.3% and 37.2% at 10, 20, 30, 50, 70 and 100 mg/litre initial dye concentrations, respectively.

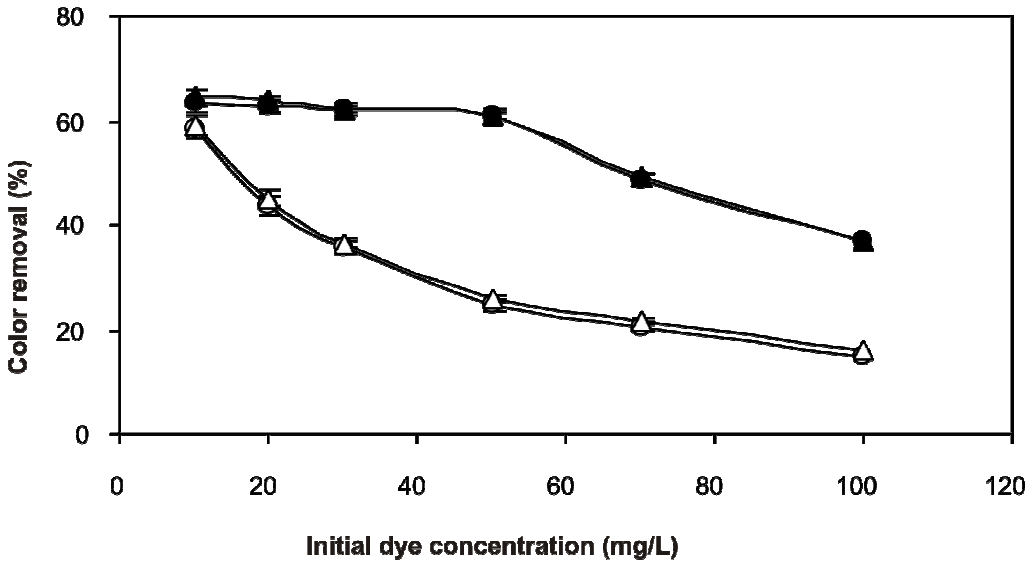


Fig. 7: The percentage of color removal of acid red 18 and reactive black 5 dye using *S. commune* (acid red 18 (Δ) and reactive black 5 (▲)) and *T. versicolor* (acid red 18 (○) and reactive black 5 (●)) at 25^{O} C, 180 rpm and pH value of 2.

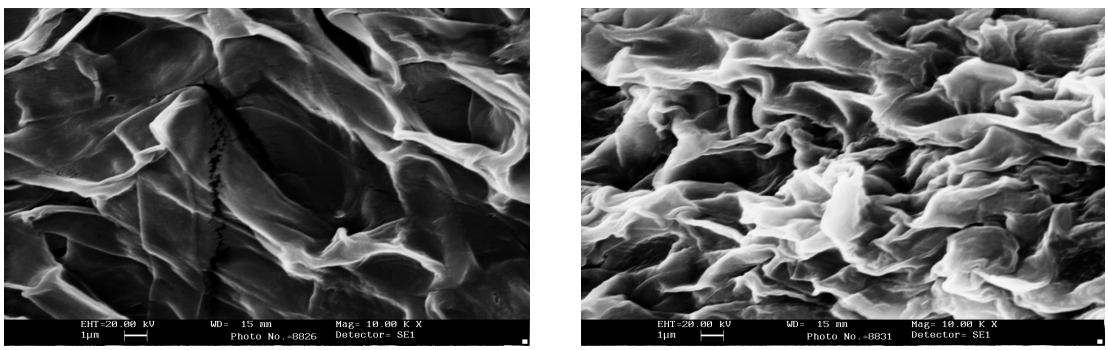
For reactive black 5, the percentage color removed by *T. versicolor* was found to be 63.3%, 62.5%, 62.0%, 61.0%, 48.6%, and 36.7% for initial dye concentrations of 10, 20, 30, 50, 70 and 100 mg/litre, respectively in 120 h. The same trend was observed for acid red 18 and the percentage color removal for 10, 20, 30, 50, 70 and 100 mg/litre initial dye concentrations was observed to be 59.1%, 44.8%, 36.6%, 25.9%, 21.4%, and 16.0%, respectively by *S. commune*. The growth of the fungus had been affected by toxicity of acid red 18. The presence and increase of the dye concentration in the bioaccumulation medium caused an increase in the lag phase and a sharp decrease in microbial growth

because of the acid red 18 dye inhibition effect. The percentage color removalwas found to be 58.3%, 43.7%, 35.6%, 24.7%, 20.1%, and 14.7% for 10, 20, 30, 50, 70 and 100 mg/litre initial dye concentration in 120 h, respectively by *T. versicolor*.

From the experimental results it was revealed that the percentage color removal of dyes using *S. commune* was found to be more when compared to the values obtained using *T. versicolor*. The accumulation rate of reactive black 5 with one naphthalene ring, two benzene ring, four sulfonic acid substitutions, a primary amino and two azo groups was found to be more when compared to the acid red 18 with two naphthalene rings and three sulfonic acid group substitutions at lower pH values. Dye decolorization depends on the dye structure[23] and different dyes have different molecular structures. So a fungus capable of decolorizing dyes with small structural differences can markedly affect the rate of decolorization.

Scanning electron microscope studies

Scanning Electron Microscopy (SEM) is a novel technique that was increasingly used to examine biological specimens. The surface morphology of the *T. versicolor* and *S. commune* sorbent is exemplified by the scanning electron micrograph without dye adsorption (Fig. 8).



(a) *S. commune* **(b) *T. versicolor.***

Fig. 8: Scanning electron microscope image of the (a) *S. commune* and (b*) T. versicolor.*

As shown in the SEM micrograph, the rough and porous surface size was found to be more on the surface of *S. commune* when compared to *T. versicolor* species. This surface property should be considered as a factor providing an increase in the total surface

area; thereby, increasing the percentage of color removal of acid red 18 and reactive black 5 dyes.

CONCLUSION

Bioaccumulation of ascid red 18 and reactive black 5 dyes present in aqueous solutions using both the white rot fungi *S. commune* and *T. versicolor* were found to be effective during the growth phase of the fungus. When the initial dye concentration was 100 mg/litre, maximum uptake was obtained at the initial pH of 2. In the absence of azo dyes, the specific growth rate of the fungus was found to be maximum. Increasing the dye concentration inhibited the growth of the fungus and decreased the biomass concentration and reduced the percentage of color removal. In the present investigation, percentage color removal of reactive black 5 using *S. commune* was observed to be more when compared to other dye studied using *T. versicolor.*

NOMENCLATURE

C_o Initial acid red 18 and reactive black 5 dye concentration (mg/litre).

C Residual acid red 18 and reactive black 5 dye concentration in the bioaccumulation medium at any time (mg/litre).

C_{reg} Residual acid red 18 and reactive black 5 dye concentration in the bioaccumulation medium at the end of fungal growth (mg/litre).

C_{acc} Bioaccumulated acid red 18 and reactive black 5 dye concentration at any time (mg/litre).

C_{accm} Bioaccumulated acid red 18 and reactive black 5 dye concentration at the end of fungal growth (mg/litre).

q_m Bioaccumulated acid red 18 and reactive black 5 dye quantity per g of dried fungal biomass at the end of microbial growth (g/litre).

X Dried fungal biomass concentration in the bioaccumulation medium at any time (g/litre).

X_m Maximum dried fungal biomass concentration the end of microbial growth (g/litre).

V_m Volume of the fungal suspension (L)

M_{fb} Maximum dried fungal biomass at the end of fungal growth (g).

μ Specific growth rate of the fungus (h^{-1}).

q_r Specific uptake rate of the fungus (mg/g h).

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