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Development and optimization of the dye removal process by *Trichoderma reesei* using starch effluent as a growth supplement

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ABSTRACT

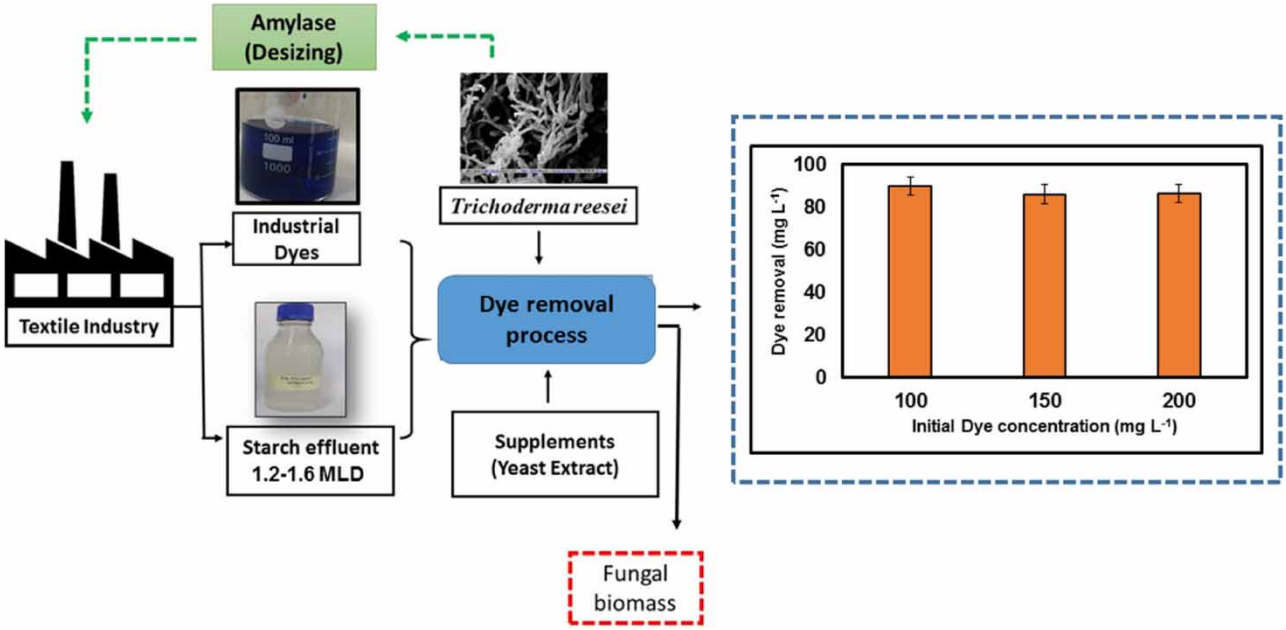
The textile industry generates enormous starch effluent from the desizing process that can be utilized as a nutrient source for fungal growth and simultaneous dye decolorization. In the present study, *Trichoderma reesei* was used as a potential fungal isolate for the decolorization of reactive dyes using a minimal salt media for growth. The dye removal of Reactive blue 13, Reactive red 198, Reactive yellow 176, and Reactive black 5 were 95.35, 88.17, 86.01, and 94.84 mg L^{-1}, respectively, by fungal biomass at 100 mg L^{-1} of initial dye concentration in 48 h was achieved. *T. reesei* showed decolorization of dyes at initial concentrations upto 500 mg L^{-1} with high dye uptake capacity. The glucose (5 g L^{-1}) and yeast extracts (2.5 g L^{-1}) were optimal for maximum dye decolorization. The utilization of starch effluent as an alternative nutrient source supplemented with 3.5 g L^{-1} glucose as growth media by *T. reesei* showed >85% of decolorization of Reactive blue 13 (100–200 mg L^{-1}). Thus, starch effluent could be partially supplemented with glucose to support fungal growth and dye decolorization, eliminating the requirement of minimal salts for dye decolorization that follows a sustainable approach.

Key words: growth kinetics, starch effluent, sustainable, textile dyes, *Trichoderma reesei*

HIGHLIGHTS

- *T. reesei* showed decolorization of reactive dyes at 48 h of incubation.
- Specific growth rate of the fungus is 0.03 h^{-1} and the doubling time is 23.10 h.
- Optimum glucose and yeast extracts are 5 and 2.5 g L^{-1} for maximum dye decolorization.
- Process optimized with starch effluent as a cheap carbon-rich nutrient source.
- Starch effluent spiked with 3.5 g L^{-1} glucose showed >85% of dye removal.

GRAPHICAL ABSTRACT



1. INTRODUCTION

Textile industries are one of the leading industries and contribute a significant share of India's GDP. Textile industries involve various wet process operations such as desizing, bleaching, scouring, and dyeing, which leads to a large volume of effluent discharge in the environment (Saha *et al.* 2018). According to the reports, a large part of dyes do not bind to the fabric and wash out as a pollutant in water streams causing damage to the aquatic ecosystems (Madhushika *et al.* 2020). The effluent of different operating units in the textile industry is collected in separate storage tanks prior to treatment through conventional methods. The effluent generated from the dyeing operation generally contains high color, acids, alkalis, and surfactants (Khan *et al.* 2023), whereas the starch effluent produced from the desizing operation is colorless with high organic content (Shahzad *et al.* 2021). The textile industry generates 1.2–1.6 million liters per day (MLD) starch effluent after the desizing process (Samuchiwal *et al.* 2021). It contains starch, glucose, fabric residue, NaOH, and various salts. Being a rich source of organic content, it opens a great opportunity to utilize it as a carbon supplement for microbial growth in the dye decolorization process.

Biological processes have emerged as an eco-friendly choice for effluent treatment due to less sludge generation in contrast to the physiochemical methods (Verma *et al.* 2017). Some fungi possess the ability to grow in extreme environmental conditions containing high dye load and other toxic chemicals (Ning *et al.* 2018) and play an active role in dye accumulation (Singh 2017).

In the recent past, several studies have been performed on the decolorization of textile dyes using fungal species. *Aspergillus tamarii* achieves maximum decolorization of Reactive dyes in 72 h using glucose (1 g L^{-1}), yeast extract (0.5 g L^{-1}) and minimal salts (Sharma *et al.* 2017). Biomass of *Trichoderma virens* and *Trichoderma viride* showed high efficiency in Congo red decolorization (50 mg L^{-1}) in 24 h (Argumedo-Delira *et al.* 2021). Abd El-Rahim *et al.* (2017) showed decolorization of methyl red and direct violet dye by *Aspergillus* species on media containing glucose, yeast extract and various minimal salt concentrations. *Aspergillus nidulans* showed 80–95% decolorization of Synozol red HF-6BN and Synozol black B after 4 days on a basal media containing glucose (10 g L^{-1}), peptone (5 g L^{-1}) and yeast extract (1 g L^{-1}) (Khan *et al.* 2020). However, the above studies required additives such as minimal salts and nutrient sources to decolorize the textile dyes. The requirement of additives would lead to an increase in the cost and make the process unfit for scale-up commercialization.

The utilization of industrial waste as an alternate supplement could be an approach to achieve our targets for the decolorization process. However, limited studies were conducted on the use of industrial wastes as a carbon source for microorganism growth in dye decolorization studies. *Candida tropicalis* showed decolorization of Acid blue 28, Basic

violet and 3 Direct red 93 while grown on sugarcane bagasse extract as a medium (Charumathi & Das 2010), *Aspergillus niger* and *Aspergillus versicolor* actively growing on molasses medium have been shown to decolorize various dyes including Reactive black 5 (RB 5), Maxilon red GRL, Brillant blue R and Everdirect fast black VSF (Taskin & Erdal 2010; Taştan *et al.* 2012). However, the above studies showed the utilization of different industrial wastes which was not generated within the premises of the textile industry. Hence, it would cause unnecessary dependence of the decolorization process upon other industrial waste including transportation costs for transferring waste to the treatment site.

The starch effluent generated from the textile wet processing (containing starch and salts) could serve as a medium for actively growing fungal culture used in decolorization studies. So, utilization of textile industry-generated effluent itself could eliminate the use of minimal salts and carbon sources for the fungal growth in the dye decolorization process. This would not only eliminate the cost of media for fungal growth but also solve the problem of effluent treatment/disposal. Our previous studies showed the production of amylase (using starch effluent as a growth supplement) by *Trichoderma reesei* and its application in the desizing of grey fabric (Kalia *et al.* 2021). In the current study, authors explored the potential of fungal culture (having amylase producing capability) in decolorization of textile dyes using starch effluent generated from textile industries as an alternate carbon source. Accordingly, the present study focuses on (1) study of growth kinetics of fungal biomass in the presence of dye, (2) study of dye decolorization of different textile dyes at a broad range of initial dye concentration, (3) optimization of nutritional parameters such as glucose concentration, yeast extract concentration and (4) utilization potential of starch effluent as a carbon source for decolorization process.

2. MATERIALS AND METHODS

2.1. Fungal culture and its growth conditions

T. reesei F-2084 used in the study was obtained from Enzyme Technology Lab, Department of Biochemical Engineering and Biotechnology, Indian Institute of Technology Delhi, India. The fungal strain was grown on 2.5% malt extract slants (1215, TM-Media, India) for 7 days at 30 °C and stored at 4 °C in the refrigerator.

2.2. Textile dyes and chemicals

The textile dyes used in the experimental studies, i.e., Reactive blue 13 (RB 13), Reactive red 198 (RR 198), Reactive yellow 176 (RY 176) and RB 5 were obtained from the textile industry in India. These dyes were selected as they are extensively used in the leading textile industry for dyeing cotton fabrics. The stock solution of 10,000 mg L^{-1} of dye concentration was prepared in deionized water and sterilized for dye decolorization studies. The absorption maxima of dyes were obtained by scanning the dye solution over 300–700 nm wavelength using UV-vis spectrophotometer (Lambda 35, PerkinElmer, USA). Standard curve plotting was done using absorbance at absorption maxima of each dye to calculate the concentration of dye. All the biological media and chemicals were procured from TM-Media, Hi-Media and Sigma–Aldrich.

2.3. Decolorization of dyes by *T. reesei* in submerged media

Flasks containing 100 mL of growth media consisting of (g L^{-1}): NH_4NO_3 (0.5), $MgSO_4{\cdot}7H_2O$ (0.1), NaCl (1.0), K_2HPO_4 (0.5), yeast extract (2.5), glucose (10) was adjusted to pH 6.5. The flasks were sterilized at 121 °C for 20 min in an autoclave. The flasks were then mixed with dye solution at 100 mg L^{-1} concentration in a sterile condition. For inoculation, spore suspension of *T. reesei* was prepared by adding 5 mL of sterilized Tween 80 (0.1% *v/v*) in a 7-day-old fungal slant. The sterilized flasks were inoculated with 1 mL of spore suspension having ~10^6 spores (hemocytometer Neubauer, TOUFF) and incubated at 30 ± 1 °C (Orbitek, Scigenics Biotech, India) for 5 days at 150 rpm. Samples were withdrawn at every 24 h interval and centrifuged (Spinwin microcentrifuge MC-01, Tarson) at ~9,000 g for 10 min. The absorbance was taken at absorption maxima of each dye to calculate dye decolorization (%).

2.3.1. Desorption study of RB 13

Biomass obtained after decolorization of RB 13 (100 mg L^{-1}) (as described above) was separated and washed using distilled water. Desorption study of RB 13 from dye laden fungal biomass was done by adding 0.01 M NaOH as an eluant. In the flask containing 4.5 g L^{-1} of dye-laden fungal biomass, 100 mL of 0.01 M NaOH was added and kept at 30 ± 1 °C, 150 rpm for 30 min (Patel & Suresh 2008). Samples were withdrawn at every 10-min interval and absorbance was taken at absorption maxima of dye to estimate the desorption efficiency. The dye desorption (%) from the dye-laden fungal biomass was

calculated using the following equation (Munagapati *et al.* 2021):

$$Dye\ desoprtion\ (\%) = \frac{Amont\ of\ dye\ desorbed}{Total\ dye\ removed} \times 100 \quad (1)$$

2.4. Growth kinetics of *T. reesei* in the presence of RB 13

Growth kinetics of *T. reesei* was studied with RB 13 at 100 mg L^{-1} of initial dye concentration. One mL of spore suspension was added into the flasks containing sterile growth media of initial pH 6.5 and RB 13 (100 mg L^{-1}). Flasks were incubated at 30 ± 1 °C with agitation at 150 rpm. The flasks were withdrawn at every 6-h interval and samples were analyzed for biomass productivity and dye decolorization. Abiotic control (growth media with dye but without inoculum) and biotic control (growth media without dye and inoculated with fungal spores) were also studied under similar conditions. The growth of fungal isolate was described using cube root kinetic model (Papagianni 2004):

$$M^{1/3} = M_0^{1/3} + kt \quad (2)$$

where M is the concentration of biomass at time 't', M_0 is the concentration of biomass at time $t = 0$ and k is the constant.

Specific growth rate (μ) of *T. reesei* was calculated by plotting a graph between the natural log of biomass produced and time throughout the exponential growth phase.

Doubling time (t_d) is the time required for a fungal population to double and is calculated using the following equation:

$$Doubling\ time\ (t_d) = \frac{\ln 2}{\mu} \quad (3)$$

2.5. Effect of different concentration of dyes on decolorization

Effect of dye concentrations ranging from 100 to 500 mg L^{-1} of four textile dyes (RB 13, RR 198, RY 176 and RB 5) on dye decolorization efficiency by *T. reesei* was investigated. Flasks containing sterilized growth media and different dye concentrations were inoculated with spore suspension (1 mL) of *T. reesei* and incubated at 30 ± 1 °C. Flasks were withdrawn at 72-h intervals and samples were processed to determine dye decolorization efficiency. The dry weight of fungal biomass was estimated at the end of the experiment.

2.6. Effect of different concentrations of glucose and yeast extracts on decolorization of RB 13

The batch experiments were performed using RB 13 at 100 mg L^{-1} of dye concentration. The effect of initial glucose concentration 0, 1, 2.5, 5 and 10 g L^{-1} on decolorization of RB 13 by *T. reesei* was studied. Other components in media were kept the same as mentioned in Section 2.3. Flasks were withdrawn at 72-h interval and samples were processed to determine dye decolorization efficiency. Residual glucose was estimated by the dinitrosalicylic acid (DNS) method. The dry weight of fungal biomass was estimated at the end of the experiment.

The effect of initial yeast extract concentrations at 0, 1, 2.5, 5 and 10 g L^{-1} on decolorization of RB 13 by *T. reesei* was studied. The glucose concentration was kept at 10 g L^{-1} and other media components were the same as mentioned in Section 2.3. Flasks were withdrawn at 72-h interval and samples were processed to determine dye decolorization efficiency. The dry weight of fungal biomass was estimated at the end of the experiment.

2.7. Characterization of starch effluent

The starch effluent generated after desizing the fabric in the textile industry was characterized for various physico-chemical parameters such as pH, chemical oxygen demand (COD), total dissolved solids (TDS), sulfate, alkalinity and color (hazen value) upon arrival. Procured effluents were kept at 4 °C in cold chamber until further use.

2.7.1. Utilization of starch effluent, as a carbon source for decolorization of RB 13

Starch effluent has a high value of TDS due to the presence of sodium hydroxide, sodium carbonate, potassium and sodium chloride. Thus, the presence of salts in the starch effluent eliminates the need to add analytical-grade minimal salts in the medium. Also, the liquid state of starch effluent eliminates the addition of distilled water. Hence, the study was performed

using starch effluent as a carbon as well as a nutrient source. The decolorization experiments were conducted in sterilized flasks containing 100 mL of starch effluent and yeast extract (2.5 g L^{-1}) maintained at initial pH 6.5. The flasks were sterilized and the experiment was carried out using RB 13 at concentrations of 100, 150 and 200 mg L^{-1}. The flasks were inoculated using 1 mL of fungal spore suspension and incubated at 30 ± 1 °C for 72 h at 150 rpm. Flasks were withdrawn at 72-h interval and samples were processed to determine dye decolorization efficiency. The dry weight of fungal biomass was estimated at the end of the experiment.

2.7.2. Starch effluent spiked with glucose for decolorization of RB 13

The experiments were also performed by adding initial glucose concentrations 1.5, 2.5 and 3.5 g L^{-1} in starch effluent along with yeast extract (2.5 g L^{-1}) to estimate dye decolorization. The experiment was carried out using RB 13 at different dye concentrations, i.e., 100, 150 and 200 mg L^{-1}. The experiment was carried out as mentioned in Section 2.7.1.

2.8. Analytical technique

2.8.1. Dye decolorization analysis

The concentration of dyes was measured by taking the absorbance at the absorption maxima of the respective dyes using spectrophotometer. The absorption maxima of RB 13, RY 176, RR 198 and RB 5 were at 600, 420, 520 and 600 nm, respectively. The calibration curve was plotted between the optical density and concentration of dyes to determine the respective dye concentration.

The dye decolorization (%) was estimated using the equation:

$$Dye\ decolorization\ (\%) = \left(\frac{A_i - A_t}{A_i}\right) * 100 \tag{4}$$

where A_i is the absorbance at time = 0 h, A_t is the absorbance at time = t.

The dye uptake capacity of the fungal biomass was calculated using the equation:

$$Dye\ uptake\ capacity\ \left(\frac{mg}{g}\right) = \frac{C_i - C_t}{m} \tag{5}$$

where C_i is the initial dye concentration, C_t is the final dye concentration and m is the weight of fungal biomass (g L^{-1}).

2.8.2. Reducing sugar estimation

Reducing sugar in the samples was analyzed by adding 3 mL of DNS reagent in 100 µL of sample and then keeping it at 95 ± 1 °C for 10 min in a water bath (Miller 1959). The sample absorbance was taken using a spectrophotometer at 540 nm. The standard curve for glucose was prepared for the estimation of residual glucose left after dye decolorization.

2.8.3. Estimation of fungal biomass

The dry cell weight of fungal biomass was determined by filtering the fungal biomass using Whatman no. 1 filter paper which was pre-weighted. The filter paper containing biomass was kept in the oven at 60 ± 1 °C overnight for drying. The dry weight was expressed as a gram of dried fungal biomass per liter of growth media.

$$Dry\ fungal\ biomass\ \frac{g}{L} = \frac{W_f - W_i}{100} \times 1,000 \tag{6}$$

where W_i is the weight of filter paper, W_f is the dry weight of biomass on the filter paper.

2.9. Statistical analysis

All the data sets were taken in triplicates and respective mean values and standard deviations were determined. One-way analysis of variance (ANOVA) was done using SPSS (version 21.0) software. Duncan's multiple range test (DMRT) was applied with a significance level of $p < 0.05$. The comparison of different parameters was performed using Student's t-test.

3. RESULTS AND DISCUSSION

3.1. Decolorization of different textile dyes by *T. reesei*

Reactive azo dyes are widely used in textile industry for dyeing cotton and cellulosic fibers. However, during dyeing process, 50% of reactive dyes do not get bound to the fabric and washout in dye bath effluent (Rai *et al.* 2005). The presence of azo dye causes toxicity and salinity in the effluent leading to several detrimental effects on the environment. There is a need to treat the dye contaminants before discharging it into the waterstreams. In the present study, some commonly used reactive dyes in dyeing process, i.e., RB 13, RR 198, RY 176 and RB 5 were procured from the concerned textile industries. The decolorization of individual dyes was done using grown fungal biomass of *T. reesei*. The results (Figure 1(a)) show that decolorization of targeted dyes at 100 mg L^{-1} concentration occurred within 48 h of incubation. The highest absolute dye removal was observed in RB 13 (95.35 $\pm$ 4.80 mg L^{-1}) and RB 5 (94.84 $\pm$ 4.78 mg L^{-1}) followed by RR 198 (88.17 $\pm$ 4.47 mg L^{-1}) and RY 176 (86.01 $\pm$ 4.42 mg L^{-1}) in 48 h. After 48 h of interval, similar dye removal was observed for different textile dyes. The higher removal of RB 13 and RB 5 shows that these dyes have a higher affinity for fungal biomass than RY 176 and RR 198. The affinity of the dyes varies with the difference in dye structure (Robinson *et al.* 2001). Other studies have also reported better decolorization of textile dyes by fungal biomass (Gül & Dönmez 2013; Omar 2016). Mathur *et al.* (2018) reported that *Aspergillus lentulus* showed maximum decolorization of Reactive yellow (80%), Reactive remazole red (>85%) and Reactive blue (>95%) at initial dye concentrations of 50 and 100 mg L^{-1} after 72 h of incubation. According to another study, 96–97% decolorization of Reactive red, Reactive blue, Reactive orange and Reactive black (200 mg L^{-1} concentration) was achieved by *Trametes versicolor* on 6th day (Yang *et al.* 2017). *Trichoderma asperellum* showed 66.80% decolorization of malachite

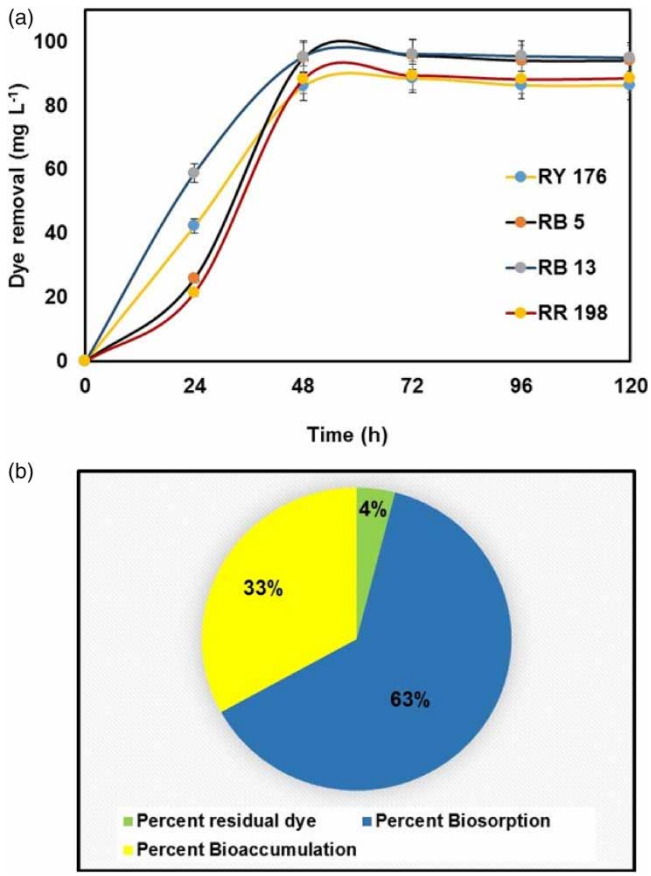


Figure 1 | (a) Removal of various textile dyes by *Trichoderma reesei* (dye concentration: 100 mg L^{-1}, pH: 6.5, temperature: 30 $\pm$ 1 °C and agitation: 150 rpm). (b) Mechanism of textile dye removal by *T. reesei* (Reactive blue 13 at 100 mg L^{-1} initial concentration). The results were reported as a mean $\pm$ SD for triplicates (RY 176: Reactive Yellow 176, RB 5: Reactive Black 5, RB 13: Reactive Blue 13, RR 198: Reactive Red 198).

green and 62.50% decolorization of cotton blue at 100 mg L^{-1} concentration after 8 days of incubation (Marcharchand & Ting 2017). However, the incubation time of these studies was long for efficient decolorization. Our results show a lesser time interval (48 h) for efficient decolorization of target textile dyes using grown fungal biomass of *T. reesei*.

In the present study, the mechanism of dye decolorization could be through the initial attachment of dye molecules to the surface of fungal biomass (biosorption) and then transportation of those molecules across the cell membrane resulting in bioaccumulation (Hansda & Kumar 2016). To measure the relative contribution of biosorption and bioaccumulation of the dye onto the fungal biomass, a desorption study was performed. It was observed that from total 96% dye decolorization, 63% dye was biosorbed onto the surface of the biomass and 33% dye was accumulated inside the cell (Figure 1(b)). Similarly, Kaushik *et al.* (2014) showed that 50.83% of Acid blue 120 dye was biosorbed on *A. lentulus* biomass. Also, 4.45% biosorption of Congo red dye on *Trametes versicolor* biomass was reported (Munagapati *et al.* 2021). This result showed that the dye was not firmly bound to the fungal biomass. In the present study, desorption of a fraction of dye from the fungal biomass shows that both biosorption and bioaccumulation were involved in dye removal.

3.2. Growth kinetics of RB 13 by *T. reesei*

The kinetics of fungal growth and dye decolorization of RB 13 (Figure 2) show that dye decolorization occurred with the growth of fungal biomass. In the uninoculated flask (abiotic control), no dye decolorization was observed proving that it occurred due to fungal biomass and not due to any media component interaction. Almost complete dye decolorization was found within 48 h of incubation with maximum dye decolorization occurring between 24 and 42 h. The growth of fungal biomass in the presence of dye was almost similar to that observed in the absence of dye (biotic control). During the exponential growth phase of *T. reesei* from 12 to 54 h, dye removal and biomass production showed a positive correlation ($R^2 = 0.90$), indicating the role of growing fungal biomass in dye decolorization. The overall dye uptake rate of fungal biomass was 1.33 $\pm$ 0.06 mg h^{-1}. The cube root kinetics model was applied to the biomass of *T. reesei* as the fungal biomass increases with respect to the cubic function of time (t) (Tough & Prosser 1996). The graph was plotted between the cube root of fungal biomass ($M^{1/3}$) and time (T) till maximum dye decolorization occurred and equilibrium was achieved at 48 h (Appendix, Supplementary Material, Figure S1). The graph shows a straight line with a slope showing value of growth constant 0.24 h^{-1}. Appendix, Supplementary Material, Figure S2 shows the graph between the natural log of fungal biomass for *T. reesei* and time. The specific growth rate (μ) of fungus during the log phase was 0.03 h^{-1} with a doubling time 23.10 h. A similar study by Kaushik & Malik (2010) showed

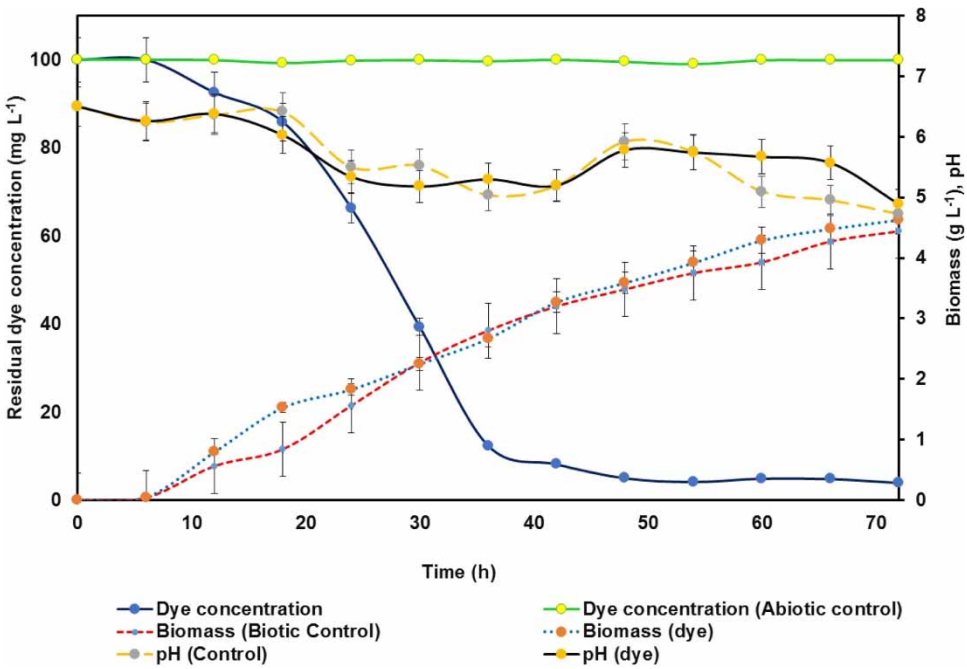


Figure 2 | Kinetics of fungal growth and removal of Reactive blue 13 by *Trichoderma reesei* with time (initial dye concentration: 100 mg L^{-1}, temperature: 30 $\pm$ 1 °C, pH: 6.5 and agitation: 150 rpm). The results were reported as a mean $\pm$ SD for triplicates.

decolorization of textile dyes by *A. lentulus*. The specific growth rate of *A. lentulus* during the log phase was 0.19 h^{-1} and doubling time 2.35 h. Overall, the fungal biomass played a salient role in decolorization of dye.

3.3. Effect of different concentration of dyes on decolorization

The effect of initial dye concentration on biomass production is shown in Table 1. It has been observed that the amount of biomass production decreased with increasing concentration of dyes. The biomass production in the presence of RB 13, RY 176, RR 198 and RB 5 at 100 mg L^{-1} concentration (3.95 ± 0.19–4.46 ± 0.22 g L^{-1}) was near to that of control biomass, i.e., in the absence of dye (4.4 ± 0.22 g L^{-1}). This shows that lower concentrations of dyes did not hinder biomass production. However, on increasing the concentration of dyes from 200 to 500 mg L^{-1}, a significant decrease ($p < 0.05$) in biomass production was observed. This showed that increasing concentrations of dye might be causing a toxic effect on the growth of microorganism. RB 13, RY 176, RR 198 and RB 5 showed almost 17, 18, 22 and 30% reduction, respectively, in biomass

Table 1 | Effect of different dye concentrations on fungal biomass production (pH 6.5, 30 ± 1 °C, 150 rpm)

Dye	100 mg L^{-1}	200 mg L^{-1}	300 mg L^{-1}	500 mg L^{-1}
Reactive blue 13	4.46 ± 0.22	4.28 ± 0.19	3.88 ± 0.16	3.69 ± 0.15
Reactive red 198	4.11 ± 0.21	3.59 ± 0.17	3.28 ± 0.20	3.18 ± 0.14
Reactive yellow 176	3.99 ± 0.17	3.66 ± 0.16	3.45 ± 0.15	3.28 ± 0.14
Reactive black 5	3.95 ± 0.19	3.27 ± 0.16	3.15 ± 0.15	2.75 ± 0.13

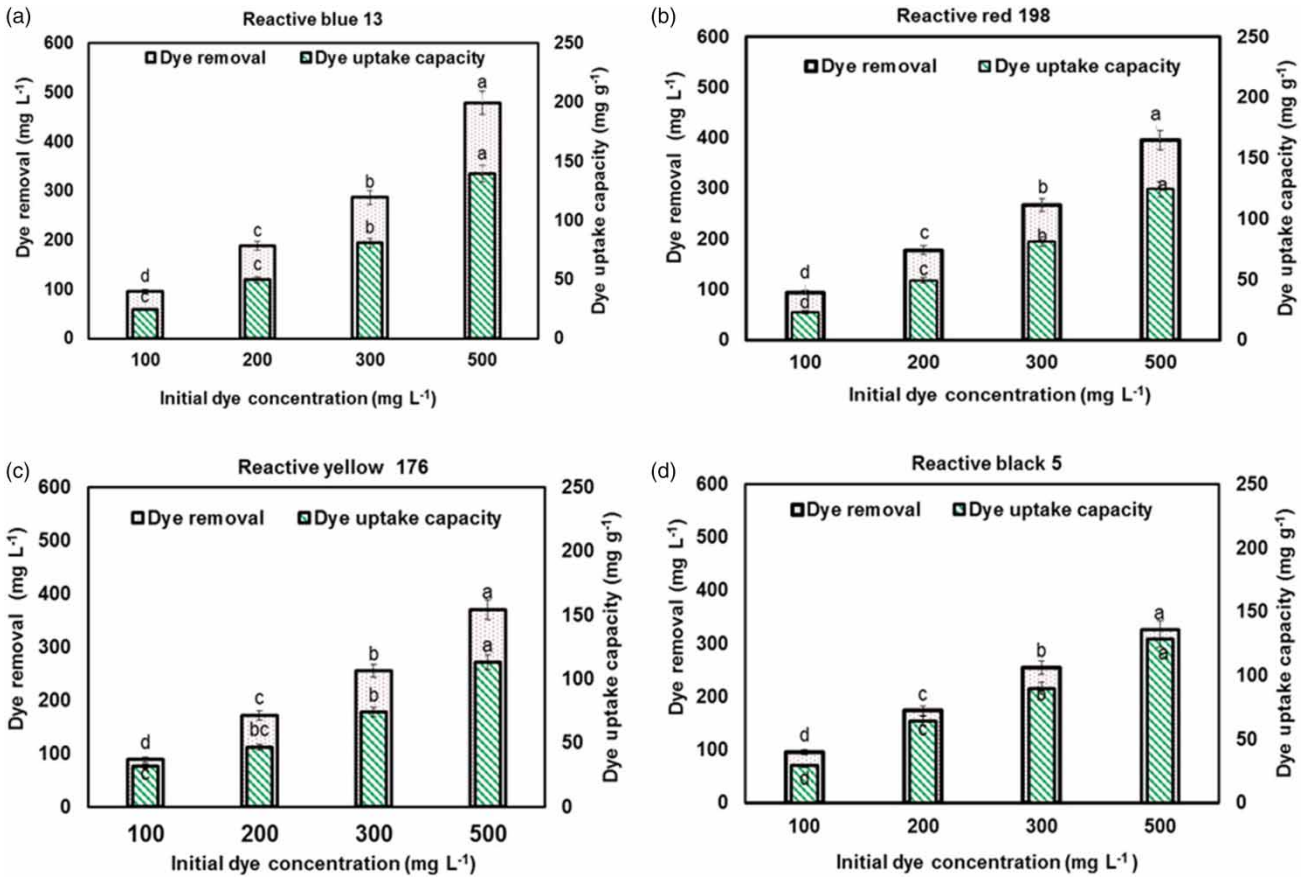


Figure 3 | Dye removal and dye uptake capacity of *Trichoderma reesei* obtained at different concentrations of (a) Reactive blue 13, (b) Remazole red 198, (c) Reactive yellow 176 and (d) Reactive black 5 (pH: 6.5, temperature: 30 ± 1 °C, agitation: 150 rpm, glucose: 10 g L^{-1} and yeast extract: 2.5 g L^{-1}). The results were reported as a mean ± SD for triplicates. Different alphabets on the bars indicate significant difference from each other ($p < 0.05$).

Table 2 | Comparison of dye removal (%) of various at higher concentration by fungus

Dyes	Concentration (mg L^{-1})	Dye removal (%)	References
Reactive blue 21	1,000	47.5	Omar (2016)
Reactive orange 122		34.6	
Reactive red 198		46.3	
Reactive blue 19		45.8	
Reactive yellow 160		11.5	
Congo red	1,000	78.18	Singh & Divedi (2022)
Acid orange 86	1,000	42	Ghosh *et al.* (2014)
Reactive yellow	500	85	Mathur *et al.* (2018)
Reactive remazole red		67	
Reactive blue		75	
Reactive black 5	50	87.07	Adnan *et al.* (2014)
Reactive blue	100	100	Ekramul Karim *et al.* (2017)
Reactive brilliant blue	200	82	Sing *et al.* (2017)
Reactive green	250	97.9	Sinha & Osborne (2016)
Reactive black 5	100	93.56	Hadibrata *et al.* (2012)
Vat novatic grey	100	100	Mathur *et al.* (2015)
Reactive remazole red		89.2	
Reactive yellow		75.9	
Reactive S blue		100	
Reactive blue 13	500	92.60	Present study
Reactive yellow 176		80.03	
Reactive remazole red 198		79.06	
Reactive black 5		66.97	

production at 500 mg L^{-1} compared to 100 mg L^{-1} concentration. RB 5 showed the highest reduction in fungal biomass. This might be due to diazo bonds in the dye structure that increases the toxicity level (Saratale *et al.* 2009).

Also, the effect of increasing dye concentration on its removal is depicted in Figure 3. As the dye concentration increases, the dye removal increases significantly ($p < 0.05$). Almost complete dye removal was observed in RB 13, i.e., 95.31 $\pm$ 4.76 mg L^{-1}, 188 $\pm$ 9.4 mg L^{-1}, 286.71 $\pm$ 14.3 mg L^{-1} and 479 $\pm$ 23.95 mg L^{-1} at dye concentrations of 100, 200, 300 and 500 mg L^{-1}, respectively. Other dyes such as RY 176, RR 198 and RB 5 showed almost complete dye removal at 100–300 mg L^{-1}. However, at 500 mg L^{-1}, less dye removal was observed in dyes compared to RB 13, RY 176, RR 198 and RB 5 showed 370 $\pm$ 18.5 mg L^{-1}, 395 $\pm$ 19.75 mg L^{-1} and 326 $\pm$ 16.3 mg L^{-1} dye removal, respectively, at 500 mg L^{-1} concentration.

As the biomass concentration is reduced with increasing concentration of dye, the uptake capacity of dye per unit biomass gets affected. The dye uptake capacity increases significantly ($p < 0.05$) with increasing initial concentration of the dyes (Figure 3). The dye uptake capacity of fungal biomass was higher for RB 13 followed by RR 198, RB 5 and RY 176. Highest dye uptake capacity of fungal biomass for dyes, i.e., RB 13 (139.28 $\pm$ 6.31 mg g^{-1}), RY 176 (113.02 $\pm$ 5.62 mg g^{-1}), RR 198 (124.40 $\pm$ 5.10 mg g^{-1}) and RB 5 (128.67 $\pm$ 5.43 mg g^{-1}) was observed at 500 mg L^{-1}. Similarly, the dye uptake capacity of *A. carbonarius* and *Penicillium galbrum* biomass increased with increasing the Congo red dye concentration from 25 to 100 mg L^{-1} (Bouras *et al.* 2017). Also, the uptake capacity of *A. fumigatus* for dyes such as Reactive remazole red, Reactive blue and Reactive yellow at 500 mg L^{-1} was 72, 63 and 95 mg g^{-1}, respectively, which increased to 89.6, 69.5, and 108.7 mg g^{-1} at 2,000mg L^{-1}, respectively (Mathur *et al.* 2018). Therefore, it is evident that with increasing concentration of dye, the dye uptake capacity of fungal biomass increases.

Comparison of dye decolorization (%) of textile dyes in the present study with the literature is shown in Table 2. In the present study, RB 13 showed almost similar dye removal (around 95%) at all dye concentrations. In other dyes, the dye removal efficiency of fungal biomass decreases with increasing dye concentration. RB 5 showed almost complete dye

decolorization (85 ± 4.25%–95 ± 4.75%) at 100–300 mg L^{-1}. However, a significant ($p < 0.05$) decrease in dye removal efficiency was observed at 500 mg L^{-1}, i.e., 66.97 ± 3.37%. According to Omar (2016), *A. niger* showed 87% decolorization at 100 mg L^{-1} of Reactive yellow and at 1,000 mg L^{-1} dye concentration, decolorization sharply reduced to 11.5%. Also, decolorization efficiency of *A. terreus* at 100 mg L^{-1} initial dye concentration was 94.5%. On further increasing the concentration of dye to 1,000 mg L^{-1}, the decolorization efficiency was reduced to 74.45% at 168 h (Singh & Dwivedi 2020). *Aspergillus sp.* showed a decrease in color removal of Acid orange 86 from 98.2 to 42% by increasing the dye concentration from 50 to 10,000 mg L^{-1} (Ghosh *et al.* 2014).

3.4. Effect of glucose and yeast extract concentration

The effect of different glucose concentrations from 0 to 10 g L^{-1} on dye decolorization by *T. reesei* was studied to minimize the inputs. Figure 4(a) shows that dye removal increases significantly ($p < 0.05$) with increasing glucose concentration upto 5 g L^{-1}. In the absence of glucose, low dye removal 36.91 ± 1.84 mg L^{-1} (36% dye decolorization) was observed with biomass concentration 0.8 ± 0.04 g L^{-1} in 72 h showing the importance of carbon source for fungal growth. At 5 g L^{-1} glucose concentration, the highest dye removal, i.e., 95.05 ± 4.75 mg L^{-1} (95% decolorization) was observed with higher biomass concentration (3.5 ± 0.17 g L^{-1}). As shown in Figure 4(b), with the increased concentration of glucose, a significant ($p < 0.05$) increase in biomass production was observed. Glucose acts as a primary carbon source for fungal growth as it forms a structural element of fungal cells. It is responsible for the energy required for growth and metabolism (Jasińska *et al.* 2015). Glucose consumption led to the formation of pellets which absorbs and internalize the dye. However, at tested dye concentration, glucose concentrations 5 and 10 g L^{-1} showed almost complete dye decolorization at 72 h incubation with residual glucose 0.59 and 2.6 g L^{-1}, respectively. With increasing concentration of glucose from 5 to 10 g L^{-1} insignificant increase in dye removal was observed. After 48 h, at 5 g L^{-1} initial glucose concentration almost complete glucose consumption (86%) was observed showing 94.1 ± 4.70 mg L^{-1} dye removal (94% dye decolorization). However, at 10 g L^{-1} initial glucose concentration, 52% of the glucose was consumed showing 95.47 ± 4.77 mg L^{-1} dye removal (95.47% dye decolorization) and the rest was left unutilized. This shows that 5 g L^{-1} glucose was optimum to achieve maximum dye decolorization.

At 5 g L^{-1} glucose concentration, glucose consumption by the fungal biomass was directly proportional to dye decolorization (Figure 4(c)). At 72 h, the residual glucose was almost nil showing maximum dye decolorization. The dye consumed per gram of glucose consumption was 218.18 ± 10.9 mg g^{-1} on 24 h, 218.57 ± 10.9 mg g^{-1} on 48 h and 95 ± 4.75 mg g^{-1} on 72 h. In previous studies, *A. fumigatus* showed maximum decolorization of methyl red at 0.5% glucose (Abd El-Rahim *et al.* 2017). *A. niger*, *Aspergillus fumigatus* and *Aspergillus flavus* showed maximum decolorization of Trypan blue at a media supplemented with 2% glucose concentration (Madhuri & Vijayalakshmi 2014). Hence, the optimal glucose requirement would vary depending on the organism and nature of the dye being removed.

Effect of different concentrations of yeast extract from 0 to 10 g L^{-1} on dye decolorization was studied. Results in Figure 5(a) shows that maximum dye decolorization was observed in 48 h with increasing yeast extract concentration from 0 to 2.5 g L^{-1}. In the absence of yeast extract, 10.07 ± 0.50 mg L^{-1} of dye removal was observed whereas 77.65 ± 3.88 mg L^{-1} of dye removal was observed in the presence of 1 g L^{-1} yeast extract concentration. An increase in dye removal in the presence of yeast extract suggests that yeast extract plays a crucial role in enhancing fungal growth. After 48 h of incubation, dye decolorization was almost the same showing an insignificant difference ($p < 0.05$) from 2.5 to 10 g L^{-1} yeast extract concentration.

With the increasing yeast extract concentration, fungal biomass also increases (Figure 5(b)) thereby reducing the dye load on the individual fungal pellet. The enhancement of dye decolorization with yeast extract concentration was due to the presence of amino acids and vitamins as required growth factors (Abd El-Rahim *et al.* 2003). *A. niger* showed 54% decolorization of direct violet and 90% decolorization of Congo red dye in a media supplemented with yeast extract as a nitrogen source (Mohan *et al.* 2015; Abd El-Rahim *et al.* 2017). Thus, 5 g L^{-1} of glucose and 2.5 g L^{-1} of yeast extract was the optimum concentration to achieve complete dye decolorization of RB 13 at a tested concentration.

3.5. Use of starch effluent as a growth medium

Large volume of starch effluent (around 1.2–1.6 MLD) is generated from the desizing unit operation of the concerned textile industry. It shows alkaline nature (pH 9.0) and had 2,500 mg L^{-1} of starch and 1,500 mg L^{-1} of glucose concentration, respectively. The physiochemical parameters of starch effluent are mentioned in Table 3. This showed the presence of various

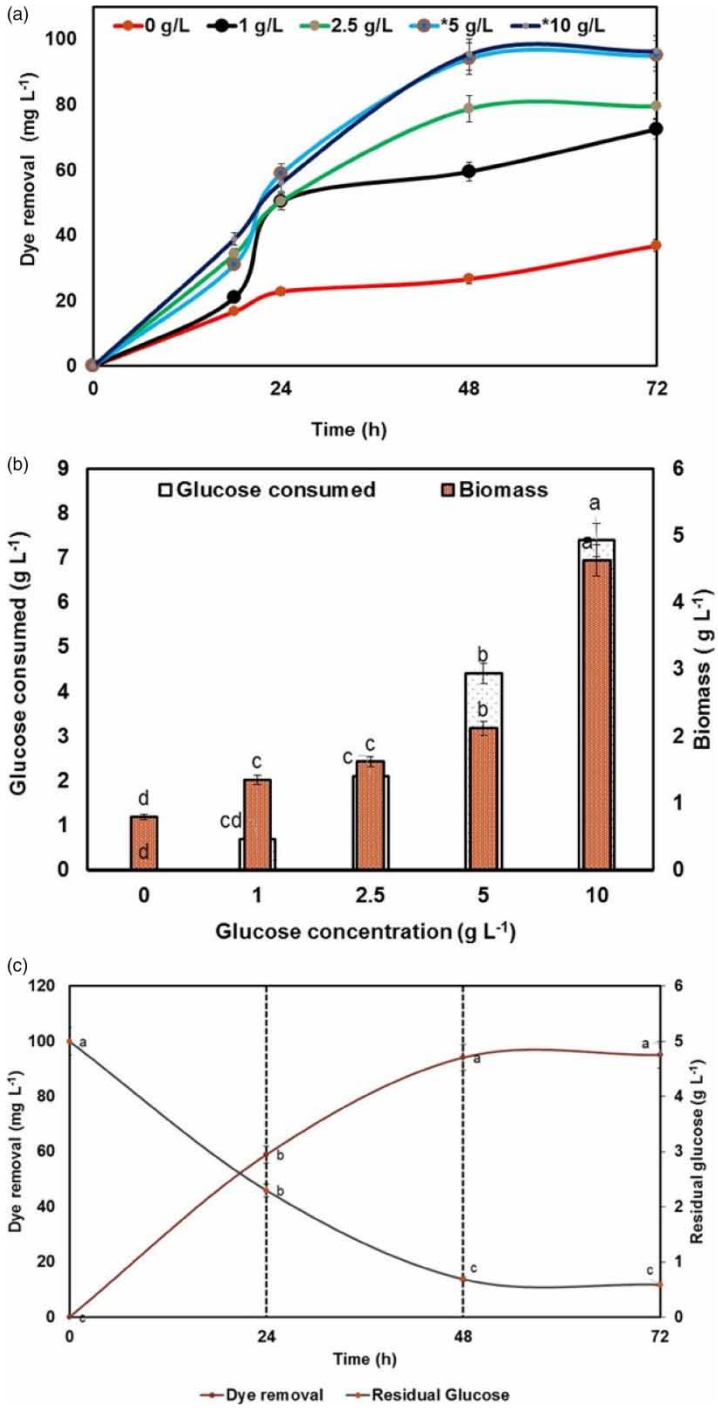


Figure 4 | (a) Effect of initial glucose concentration on dye consumption by *Trichoderma reesei*, (b) effect of glucose consumption on fungal growth and (c) relationship between dye removal and glucose (5 g L^{-1}) consumption by *T. reesei* (initial concentration of Reactive blue 13–100 mg L^{-1}, 30 °C, pH 6.5, yeast extract 2.5 g L^{-1}). The error bars are reported as a mean ± SD for triplicates. Values under different parameters that do not have same alphabet are significantly different ($p < 0.05$). * represents no significant change between means of the tested by Student's *t*-test (significance $p < 0.05$).

salts in the effluent which could also be utilized as a replacement for minimal salt requirement during biomass growth. Moreover, the generation of a huge volume of starch effluent also leads to several adverse environmental effects. Therefore, the use of starch effluent was studied to evaluate the production of fungal biomass and its dye decolorization efficiency. Dye

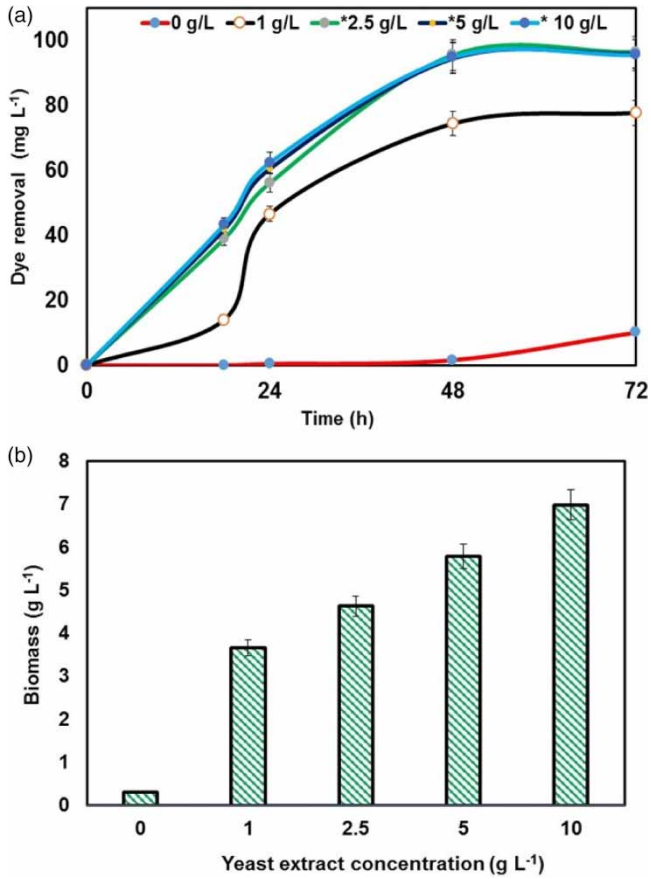


Figure 5 | (a) Effect of initial yeast extract concentration on dye removal by *Trichoderma reesei* and (b) effect of initial yeast extract concentration on fungal growth (initial concentration of Reactive blue 13–100 mg L^{-1}, temperature: 30 ± 1 °C, agitation: 150 rpm, pH: 6.5 and glucose: 10 g L^{-1}). The results were reported as a mean ± SD for triplicates. * represents no significant change between means of tested and optimized concentration by Student's *t*-test (significance $p < 0.05$).

Table 3 | Characterization of starch effluent

Parameters	Concentration
pH	9
Glucose (mg L^{-1})	1,500
Starch (mg L^{-1})	2,500
TDS (mg L^{-1})	3,338
Total nitrogen (mg L^{-1})	14.35
Ammonical nitrogen (mg L^{-1})	0.978
Phosphate (mg L^{-1})	8.38
Colour (Hazen)	1,211
Total hardness (mg L^{-1})	60
Calcium hardness (mg L^{-1})	38
Magnesium hardness (mg L^{-1})	22
Alkalinity (mg L^{-1})	1,480
COD (mg L^{-1})	4,128
TSS (mg L^{-1})	356

Note: TDS, total dissolved solids; COD, chemical oxygen demand; TSS, total suspended solids.

decolorization at different initial concentrations (100–200 mg L^{-1}) of RB 13 was studied using starch effluent as a carbon source. According to Figure 6(a), the dye removal by fungal biomass also increases with increasing dye concentration. At lowest dye concentration, i.e., 100 mg L^{-1}, 64.56 ± 3.22 mg L^{-1} of dye removal (64% dye decolorization) was observed by fungal biomass. Further increasing the dye concentration to 200 mg L^{-1}, dye removal was 104.44 ± 2.61 mg L^{-1} (52% dye decolorization). According to the literature, Dönmez (2002), reactive textile dyes showed >90% decolorization using *C. tropicalis* grown in 10 mL molasses medium (containing 10 g L^{-1} sucrose). Similarly, *A. versicolor* grown on molasses medium (containing 10 g L^{-1}) showed decolorization of Maxilon red GRL (88.3%), Brilliant blue R (99.5%) and Everdirect fast black VSF (100%) at 100 mg L^{-1} concentration (Taştan *et al.* 2012). Charumathi & Das (2010) revealed that *C. tropicalis* grown on a medium containing 30% sugarcane bagasse extract (containing 24 g L^{-1} sugar content) decolorizes Acid Blue 93, Basic Violet and Direct Red 28. However, on increasing the dye concentration decolorization decreases along with growth rate. In the present study, less dye decolorization was observed compared to the studies mentioned above. The medium used in the above studies is a rich source of carbon whereas starch effluent used in the current study has less amount of carbon. Also, starch effluent contains surfactants and alkalis that hinder the growth of fungal biomass. This results in less dye decolorization compared to the studies reported above. In the present study, comparing the dye decolorization and biomass produced with starch effluent to that of glucose, it was found that the dye decolorization efficiency of fungal biomass with starch effluent at 100 mg L^{-1} of initial concentration of dye was (64% decolorization) less than that of fungal biomass with glucose (95% decolorization). These results are contradictory to previous reports where 30% (*v/v*) addition of starch effluent to dye effluents resulted in ~70% decolorization by anaerobic bacterial consortium (Samuchiwal *et al.* 2021). This

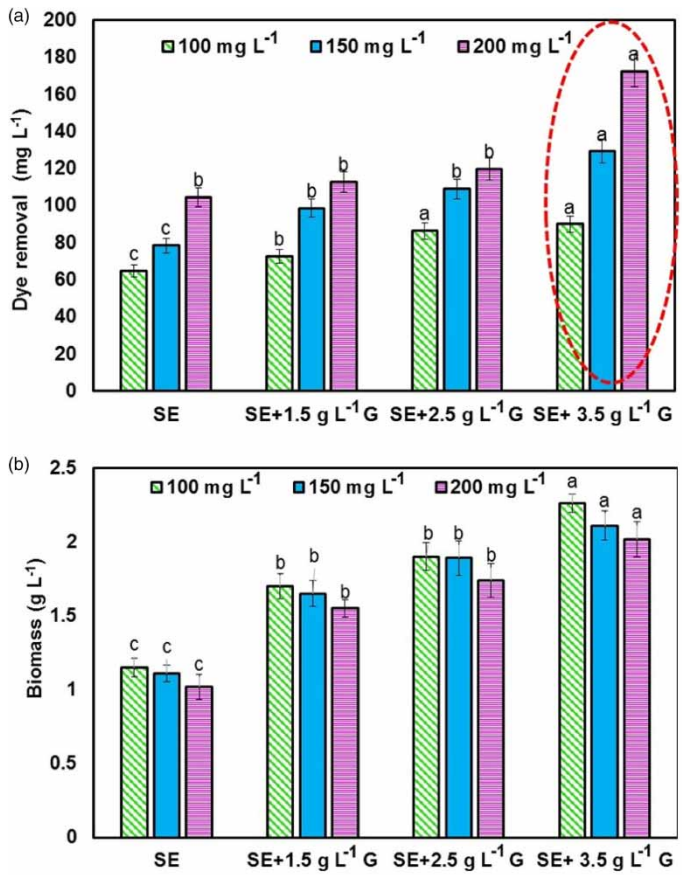


Figure 6 | (a) Effect of starch effluent and glucose-spiked starch effluent on dye removal at different initial dye concentration, (b) effect of starch effluent and glucose-spiked starch effluent on production of biomass by *Trichoderma reesei* after 72 h (temperature: 30 ± 1 °C, pH: 6.5, agitation: 150 rpm and yeast extract: 2.5 g L^{-1}). The results were reported as a mean ± SD for triplicates. Values under different dye concentration bars that have different alphabets indicate significant difference from each other ($p < 0.05$) (SE, starch effluent; G, glucose).

could be attributed to the fact that the bacterial consortium was native to the textile effluent and would have been adopted to utilize starch effluent as a carbon source as well as to the salinity and alkalinity of starch effluent. *T. reesei* did not show such adaptation to starch effluent.

Further, starch effluent was spiked with different concentrations of glucose to improve the dye decolorization efficiency of *T. reesei*. In Figure 6(a), it was depicted that with the increasing concentration of glucose in starch effluent, the dye removal by the fungal biomass also increases. At 100 mg L^{-1} dye concentration, a significant increase ($p < 0.05$) in dye removal was observed in glucose concentration from 2.5 g L^{-1} in starch effluent. However, at 150–200 mg L^{-1} dye concentration, a significant ($p < 0.05$) increase in dye removal was observed with increasing the glucose concentration to 3.5 g L^{-1} in starch effluent. More than 85% of dye decolorization was achieved in all dye concentrations (100–200 mg L^{-1}) with 3.5 g L^{-1} of glucose spiked in starch effluent.

As shown in Figure 6(b), a significant ($p < 0.05$) increase in fungal biomass concentration was observed from starch effluent with no glucose to glucose-spiked starch effluent at different dye concentrations. On increasing the spiked glucose concentration from 1.5 to 2.5 g L^{-1} in starch effluent, no significant increase in biomass concentration was observed. However, at 3.5 g L^{-1} of spiked glucose concentration a significant increase in biomass production was observed. Hence, 3.5 g L^{-1} of glucose spiked in starch effluent was the optimum condition for the removal of dyes at different concentrations. It can be concluded that the presence of glucose is necessary for the decolorization process but starch effluent addition can help in reducing the glucose input.

3.6. Feasibility of using starch effluent for textile process

Based on the present study, starch effluent procured from the leading textile firm could be used as a fungal growth medium and simultaneous dye decolorization. In a laboratory scale, 100 mL of starch effluent along with 3.5 g L^{-1} of glucose and 2.5 g L^{-1} of yeast extract as a medium for growth of *T. reesei* showed >85% decolorization of RB 13 (100–200 mg L^{-1}). However, more studies need to be done at a large scale to establish the use of starch effluent as a medium for fungal growth and decolorization of textile effluent. Also, a life cycle assessment of the process can be done to study the impact of the process on the environment. The utilization of waste procured from the textile industry as a medium for fungal growth to decolorize textile dyes and produce amylase (Kalia *et al.* 2021) leads to a reduction in decolorization cost and enzyme production process, respectively. Using starch effluent as a medium would also reduce the amount of carbon source, mineral salts and water requirement needed for fungal growth. Hence, this provides an alternate package to deal with the problems associated with the textile industry.

4. CONCLUSIONS

The present study showed the decolorization potential of *T. reesei* for various industrial-grade dyes. The decolorization process was optimized to minimize the inputs of external supplements and to maximize the decolorization output. The novelty of this study lies in utilizing starch effluent generated from textile wet processing as a carbon-rich medium for *T. reesei* cultivation during dye decolorization. Partial supplementation of starch effluent with glucose not only reduces carbon input but also eliminates the requirement of minimal salts for the decolorization process. Overall, the developed decolorization process could lead to a more sustainable practice suitable for industrial applications.

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DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

AUTHOR CONTRIBUTION

S.K. conceptualized the study, wrote the original draft, performed the methodology, reviewed, investigated, and did data curation. S.S. wrote the original draft, investigated, and did data curation. V.D. reviewed and edited. A.M. reviewed, edited, investigated, did data curation and acquired funds.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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