Low cost CaCl₂ pretreatment of sugarcane bagasse for enhancement of textile dyes adsorption and subsequent biodegradation of adsorbed dyes under solid state fermentation

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Highlights

- Dye adsorption capacity of SCB was enhanced by using different pretreatments.
- Among pretreatments studied CaCl₂ pretreated SCB shows maximum adsorption capacity.
- Adsorbed dyestuff on CaCl₂ pretreated SCB was decolorized under SSF using Providensia stuartii.
- Tray bioreactor was studied for decolorization of adsorbed dyes on CaCl₂ treated SCB.
- Analysis of product carried out using FTIR, HPLC and HPTLC confirmed degradation.

Abstract

Pretreatments to sugarcane bagasse (SCB) such as CaCl₂, alkali, ammonia, steam and milling showed 91%, 46%, 47%, 42% and 56% adsorption of Solvent Red 5B (SR5B); 92%, 57%, 58%, 56% and 68% adsorption of simulated dyes mixture (SDM), and 86%, 45%, 49%, 44% and 56% adsorption of a real textile effluent (RTE), respectively. However, the untreated SCB showed 32%, 38% and 30% adsorption of SR5B, SDM and RTE, respectively. Adsorption of SR5B on CaCl₂ pretreated SCB follows pseudo-second order kinetics. SEM and FTIR analysis reveals the delignification of CaCl₂ pretreated SCB. SR5B, SDM and RTE adsorbed on CaCl₂, alkali, ammonia, steam and milling pretreated SCB were decolorized under solid state fermentation using isolated Providensia staurti strain E3SPG. Tray bioreactor study showed 86% American Dye Manufacturers Institute (ADMI) removal of RTE in 72 h. Biodegradation of adsorbed SR5B was confirmed using FTIR, HPLC and HPTLC.

1. Introduction

Environmental pollution caused by the rapid industrialization is one of the major and most important problems of the modern world. Wastewater released from the textile industry was...
considered as most polluting among all industrial sectors (Vandevivere et al., 1998). The huge growth in the textile industries has been resulted in an immense increase in the complexity and volume of the dye containing wastewater. Several textile dyes are reported to be carcinogenic and mutagenic in nature (Eichlerova et al., 2006). Hence, it is necessary to provide effective treatment for textile effluent.

Several chemical, physical and biological methods are used for the removal of dyes from wastewater. However, the chemical and physical methods have several drawbacks such as high cost and release of hazardous secondary waste (Saratale et al., 2009). While, in comparison with various technologies available for textile wastewater treatment, adsorption is an inexpensive, fast and universal method (Gupta et al., 2006). Adsorption using the activated carbon is a commercially applicable and effective method for textile dyes removal from textile wastewater. Although, activated carbons are good materials for adsorption of different dyes but their use is restricted in the view of high cost, regeneration of adsorbent after each sorption cycle and environmental problems such as release of zinc and eutrophication (Crini, 2006; Gupta and Suhas, 2009). Each reactivation process of activated carbon results in 10 ± 15% loss of the sorbent (Robinson et al., 2001). In order to overcome these problems, low cost adsorbents were always preferred over the activated carbon for textile dye adsorption (Mittal et al., 2012).

Sugarcane bagasse (SCB) is an agro-industrial waste produced in sugar and alcohol industries. It is known to be the largest natural fiber resources because it contains high cellulose content, high yield and annual regeneration capacity (Huang et al., 2012). Thousands of tons of SCB are produced daily by the sugarcane processing industries, leading to a big environmental problem (Darani and Zoghi, 2008). Use of SCB for adsorptive textile dye removal has been reported earlier (Chen et al., 2001). It is true that, textile dyes removal using adsorption process is economical but still this method does not remove these hazardous compounds from the environment and they remain persistent in the environment (Robinson and Nigam, 2008).

On the other hand, dyes adsorbed after the adsorption treatment were decolorized under solid state fermentation (SSF) which combines, physical method-adsorption and biological method-SSF to ensure safe, economical and ecofriendly removal of textile dyes completely from the environment (Robinson and Nigam, 2008; Kadam et al., 2011, 2012). Textile wastewater is generated per day in huge volumes. Therefore, it is not practically feasible to apply submerged culture conditions for textile wastewater treatment. Hence, adsorption of dyes on agricultural wastes from textile wastewater and its bioremediation under SSF was preferred than submerged fermentation conditions (Murugesan et al., 2007). Use of various pretreatments to agriculture waste substrate was found to improve textile dye adsorption due to break down of complex lignin structures (Robinson et al., 2002; Batzias and Sidiras, 2004). Moreover, enhancement of textile dye adsorption performance of substrates using various pretreatment methods and its subsequent bioremediation under SSF significantly improves the treatment technology. An extensive literature on removal and kinetics of individual textile dye by adsorption methods is available (Gupta and Suhas, 2009). However, there is lack of promising treatment approach for adsorptive removal of different textile dyes from simulated dyes mixture (SDM) and real textile effluent (RTE).

The present study investigates the effect of various pretreatments to SCB in order to enhance the adsorption capacity for textile dye SR5B, SDM and RTE. Textile dyestuffs adsorbed on pretreated SCB were decolorized under the SSF using isolated Providencia staurti strain EbSPG.

2. Methods

2.1. Dyestuffs and chemicals

All chemicals were of highest purity and of an analytical grade. Microbiological medium nutrient broth and CaCl$_2$ was obtained from Hi-media laboratory, India. The textile dyes Solvent Red 5B and real textile effluent (RTE) was obtained from local textile mill, Ichalkaranji, India. The effluents were collected in airtight plastic container and filtered through ordinary filter paper to remove large suspended particles. The pH of the filtered effluent was adjusted to 7.0 and stored at 4 ± 1°C until use. The simulated dyes mixture (SDM) were prepared by adding various textile dyes (each 20 mg L$^{-1}$) such as SR5B, Remazol Red, Orange 3R, Scarlet RR, Navy Blue HE2R, Golden Yellow HE2R, Direct Red 5B and Blue GL Solo.

2.2. SCB as substrate

SCB were obtained from local sugar industry Sugar mill, Kolhapur. The SCB was ground to get the particles size of 2 mm. SCB particles were then washed with distilled water and dried in oven at 80°C overnight to get constant weight and was preserved in desiccators for further use.

2.3. Different pretreatments to SCB

2.3.1. CaCl$_2$ pretreatment

SCB (2 g) was added separately in the 0.2% concentrations of CaCl$_2$ and incubated at 100°C for 24 h. The dried SCB was used for further adsorption study. Batzias and Sidiras (2004) reported the activation of beech sawdust by using 20% CaCl$_2$ and then it was incubated for 1 h at 100°C. SCB pretreated with above method was compared with the CaCl$_2$ treatment of SCB used in this study.

2.3.2. Alkali pretreatment

SCB (2 g) was added to 3 M NaOH solution and kept it for 3 h. After this treatment the samples were neutralized with HCl. Then it was washed thoroughly with distilled water and dried at 80°C in an oven to obtain a constant weight (Robinson et al., 2002).

2.3.3. Ammonia steeping

Two grams of SCB was added to a solution containing 4 M NH$_4$OH and kept it in an orbital shaker 120 rpm for 24 h at 22°C. Then it was filtered through Whatman filter paper No. 1 and the treated SCB were washed with distilled water and dried to constant weight at 80°C in oven.

2.3.4. Steam pretreatment

Two grams of SCB was added in 50 mL of distilled water and autoclaved for 40 min at 121°C. Then it was filtered through Whatman filter paper No. 1 and the residues were dried in an oven at 80°C to obtain the constant weight.

2.3.5. Milling of SCB

SCB was milled separately in a commercial blender. After blending the material was sieved through the Micro-Mesh sieves (Industrial Netting, USA) having pore size of 0.002 mm. The sieved SCB particles were used for adsorption study.

2.4. Adsorption studies

Adsorption of dyes on SCB (treated as well as untreated) was performed in a batch technique. Two grams of SCB was taken in a 50 mL solution of dye SR5B (150 mg L$^{-1}$) in 250 mL Erlenmeyer flasks and kept at shaking condition (120 rpm) for 20 min. After
shaking it was filtered through Whatman filter paper No. 42. The filtrate obtained was centrifuged at 5000 rpm for 15 min. The intensity of supernatant obtained was measured at maximum absorbance wavelength (530 nm) of SR5B using Hitachi U-2800 spectrophotometer (Kadam et al., 2011, 2012). Similar process was used for SDM and RTE. The percent of adsorbed dye was calculated from the following equation.

\[
\text{% Adsorption} = \left(\frac{A_0 - A}{A_0}\right) \times 100
\]

Where, \( A_0 \) is the absorbance of control sample and \( A \) is the absorbance of sample after adsorption on SCB. The environmental characteristics such as total organic carbon (TOC) chemical oxygen demand (COD) and biological oxygen demand (BOD) of SDM solution and RTE were studied before and after the adsorption process (APHA, 1998). The COD of the textile effluent was measured by using automated COD analyzer (Spectralab CT 15, India). The total organic carbon (TOC) was measured using Hach DR 2700 spectrophotometer (Hach Co., USA) (Lade et al., 2012).

\( \text{CaCl}_2 \) pretreated SCB showed maximum adsorption capacity for SR5B, SDM and RTE when compared with the other pretreatments studied. Therefore, adsorption kinetics of \( \text{CaCl}_2 \) on \( \text{CaCl}_2 \) pretreated SCB (2 g) was studied by taking SR5B at the concentrations of 40, 80, 120, 160 and 200 mg L\(^{-1}\) in 50 mL distilled water and with agitation time 10, 20, 30, 40, 50 and 60 min (Lin and Wang, 2009). Adsorption kinetic models such as pseudo-first order kinetic and pseudo second order kinetic were studied using non linear regression method (Khambhaty et al., 2008; Lin and Wang, 2009). The dye adsorbed SCB was used further for depolarization study.

2.5. Isolation, screening and identification of SCB adsorbed dye degrading microorganism

SCB adsorbed dyes SR5B was taken in 250 mL Erlenmeyer flasks and autoclaved at 121 °C and 15 min. After adjustment of moisture content to 85–90%, these flasks were inoculated with 0.5 g of soil sample collected from the waste disposal site of textile processing and dye manufacturing units in and around Ichalkaranji (India). The flasks are incubated at 30 ± 2 °C at static and shaking conditions. From the different flask showing decolorization, 0.5 g of decolorized medium was serially diluted and poured on nutrient agar plates by four quadrant spread plate technique. The isolated pure colonies were screened for decolorization of SR5B adsorbed on SCB. The colony showing decolorization consistently was selected and transferred on nutrient agar (g L\(^{-1}\): peptone 10, sodium chloride 10 and beef extract 2) slants and stored at 4 °C for further use. The 16S rRNA sequence analysis was used for identification of isolate. 16S rRNA gene sequencing of isolated bacteria was carried out at Bangalore Genie, Bangalore, India. The alignment of nucleotide sequence was with the available sequences was done at Blastn site at NCBI server [http://www.ncbi.nlm.nih.gov/BLAST2/blast.cgi] and [http://www.ncbi.nlm.nih.gov/BLAST/blast.cgi]. The phylogenetic tree was constructed using the aligned sequences by the neighbor joining method using Kimura-2-parameter distances in MEGA 4 software. The 16S rRNA sequence was deposited in Gene Bank databases. Isolation, screening and identification of distillery industry waste yeast biomass (DIW-YB) adsorbed dye degrading microorganism was reported earlier by Kadam et al. (2012).

2.6. Decolorization experiment

Inoculums for decolorization experiment were prepared by transferring loop full of \( P. \) staurti culture aseptically into 2 ml nutrient broth (g L\(^{-1}\): peptone 10, sodium chloride 10 and beef extract 2) and incubated at 30 °C under static condition. Textile dye SR5B adsorbed SCB (2 g) was added into 250 mL Erlenmeyer flasks. pH was adjusted to (6–7) and flasks were sterilized at 121 °C for 15 min. pH measurement was done with a Horiba pH meter (M13, Japan). These flasks were inoculated by 2 mL of 24 h grown \( P. \) staurti having absorbance 1.0 at the wavelength of 530 nm. The moisture content of the medium was maintained to 85–90%. All flasks were incubated at 30 °C under static condition. Similar process was used for SDM and RTE (Kadam et al., 2012). Decolorization of adsorbed textile dyes were measured by using desorption method (Kadam et al., 2011). For desorption 50 mL of dimethyl sulphoxide (DMSO) was added into 250 mL Erlenmeyer flask containing 2 g of dye adsorbed SCB and kept under shaking condition (120 rpm) for 20 min. The solution was filtered through Whatman filter paper No. 42 and filtered solution was then centrifuged at 7000 rpm for 10 min. The clear supernatant was used for color measurement and the intensity of color was measured at maximum absorbance wavelength of respective dyes using spectrophotometer (Hitachi U-2800 Japan). Initial and final absorbance values were used to calculate the decolorization percentage.

In order to measure, the decolorization of mixture of dyes and textile effluent the % ADMI removal values were measured. The characteristics of mixture of dyes and textile effluent are highly erratic in both, the hues and the concentration of color. Due to presence of complex mixture of dyes, it did not show well defined peaks in the visible region hence the decolorization of the mixture of dyes was determined in terms of the ADMI value (Lade et al., 2012). Initial and final ADMI values were calculated for the measurement of ADMI removal ratio (APHA, 1998). All decolorization experiments were performed in three sets. Abiotic (without microorganism) controls were always included.

2.7. Optimization of pH, temperature and moisture content for degradation of SR5B

In order to optimize the pH for decolorization of SR5B adsorbed on \( \text{CaCl}_2 \) pretreated SCB by \( P. \) staurti, the pH of the medium was adjusted to 2, 4, 6, 8 and 10. After pH adjustments the flasks were inoculated with \( P. \) staurti and incubated at 30 °C at static condition. The effect of temperature on decolorization was studied at different temperatures such as 20, 30, 40, and 50 °C at static condition by keeping the pH 6.5–7. Moisture content of the medium was adjusted to 75%, 80%, 85%, 90% and 95% in order to study its effect on the decolorization. After adjustment of moisture content pH was adjusted to 6–7 and incubated at 30 °C static conditions.

2.8. Analysis of modified surface of \( \text{CaCl}_2 \) pretreated SCB and biodegraded metabolites of SR5B under SSF

After adsorption, the solution retained was scanned between 400 and 800 nm using spectrophotometer (Hitachi U-2800 Japan), for qualitative analysis of dye removal. Surface modifications of SCB caused by \( \text{CaCl}_2 \) pretreatment were analyzed by fourier transform infrared spectroscopy (FTIR) and scanning electron microscope (SEM). SEM analysis was carried out using JEOL-JSM-6360, Japan and the FTIR (Perkin Elimmer Spectrum one, USA) analysis was carried out in the mid IR region of 450–4000 cm\(^{-1}\) with 16 scan speed.

For the extraction of metabolites, the decolorized medium was added with 100 mL of distilled water and kept at shaking condition 120 rpm for 1 h. The medium was centrifuged at 10,000 rpm for 20 min. The supernatant obtained was used to extract metabolites with an equal volume of ethyl acetate and extract was then evaporated in vacuum over anhydrous Na₂SO₄ and dried (Kadam et al., 2011). High performance liquid chromatography (HPLC) analysis (Waters model No. 2690, USA) was carried out with C₁₈ column (symmetry, 4.6 × 250 mm) using isocratic method with 10 min run time. The mobile phase used was HPLC grade methanol with
a flow rate 0.50 mL min⁻¹. Biodegradation of SR5B was also confirmed by analyzing the obtained metabolites with HPTLC system (CAMAG, Switzerland) as reported earlier (Lade et al., 2012). Control SR5B and obtained metabolites (10 µl) were applied on the pre-coated silica gel plates (HPTLC Lichrospher silica gel 60 F254S, Merk, Germany) by micro syringe using spray gas nitrogen sample applicator (Linomat V, CAMAG, Switzerland). The sample application for plate were set as 6 mm bands, 10 mm apart from Y-axis, and 10 mm from the lower edge of the plate, first application position 20 mm from left edge. The solvent system used was toluene: methanol (7:3 v/v). The twin trough chamber was pre-equilibrated with developing solvent for a period of 20 min prior to plate development. HPTLC plate was developed by placing in the trough chamber containing pre-conditioning solvent until the desired running distance is reached and then oven dried at 120 °C for 20 min. After development, densitometric evaluation of spots was carried out at 254 and 530 nm wavelength using deuterium and tungsten lamp respectively with slit dimension of 5 × 0.45 mm using CAMAG TLC Scanner-3 (CAMAG, Switzerland). The chromatograms were integrated using HPTLC WinCats evaluation software (Version 1.4.4.6337).

2.9. Tray bioreactor study

CaCl₂ pretreated SCB (200 g) was added in the 5 L of RTE for adsorption of dyes with continuous stirring. The dye adsorbed SCB separated by passing the mixture through the muslin cloth. Then the slurry of dye adsorbed SCB (without autoclaved) was spread uniformly in a tray having dimensions 48 × 33 cm (length × width) and thickness 3 cm. The pH and moisture content was adjusted to 7 and 90%, respectively. This tray was inoculated with 100 mL of 24 h grown Providencia stuartii culture (O.D. 1.0 at 530 nm) and incubated at room temperature and non sterile conditions. Two gm of sample was removed after 12 h interval from inoculated test tray and control tray (without inoculation) and extracted with 20 ml DMSO for color measurement (Kadam et al., 2012).

2.9.1. DGGE analysis of samples from tray bioreactor

As the tray reactor was incubated in non sterile conditions, the change in microbial flora was studied after the 0, 24, 48 and 72 h using DGGE technique. One gm of SCB were removed from trays at 0, 24, 48 and 72 h and grown in nutrient medium at 30 °C for 24 h. The genomic DNA of these samples was extracted according to the method described by Ausubel et al. (1997). Polymerase chain reaction (PCR) amplification of the 16S rRNA genes was carried out from the extracted DNA using the forward primer RDB1-GC clamped (F58-CGCCGCAGCAGAGTGGGAGTGTAGTT GATCTCTGCTCA) and reverse primer RDB2 (GGACTACGAGG-TATCTAAAT). The composition of 50 µl reaction mixture was 1X PCR buffer, 1 µM, 1 nM of dNTPs, 2 mM MgSO₄, 1 unit Taq DNA polymerase, 0.25 pM of forward and reverse primers and 2 µl of template DNA. The PCR amplification included initial denaturation at 95 °C for 5 min, 35 cycles of 95 °C for 15 s, 50 °C for 15 s, and 72 °C for 15 s, followed by 10 min final extension at 72 °C. The purity and amplification of PCR product was checked on 1% agarose gel. Amplified product was purified using column method and denaturing gradient gel electrophoresis (DGGE) of concentrated product was performed using Decode Universal Mutation Detection System (BioRad). Samples were loaded onto 8% (w/v) polyacrylamide gels (37.5:1, acrylamide: bisacrylamide) in 1× TAE buffer with a denaturing gradient ranging from 35% to 70% denaturant run at 60 °C, 80 V for 14 h. Gel was stained with silver stain and visualized (Joshi et al., 2013).

2.10. Phytotoxicity study

SR5B was removed from solution by the adsorption on CaCl₂ pretreated SCB. Hence, it is important to carry out phytotoxicity of SR5B adsorbed SCB. In order to study toxicity effects of the control dye SR5B, dye adsorbed on SCB and its degradation products at the concentration of 500 ppm phytotoxicity tests were performed (Saratele et al., 2010). Plants Sorghum vulgare and Phaseolus mungo used for the toxicity analysis. The phytotoxicity study of control SR5B, SR5B adsorbed SCB and its biodegraded product was carried out at room temperature (10 seeds of each) by watering separately 5 ml sample per day. At the same time control set was carried out using water. Lengths of plumes (shoot) and radicle (root) were recorded on 12th day.

2.11. Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) with Tukey-Kramer multiple comparison test using the software GraphPad InStat version 3.06.

3. Results and discussion

3.1. Adsorption studies

3.1.1. Adsorption of dyestuffs on SCB

SCB without pretreatments showed 32% adsorption of SR5B. However, alkali, ammonia, steam and milling pretreatments showed 46%, 47%, 42% and 56% adsorption of SR5B (Table 1). Pretreatment of SCB using CaCl₂ showed 91% adsorption of SR5B (Table 1). Various chemical and physical treatments have been applied to help break down the complex lignin complex in order to improve the adsorption performance of many lignocellulosic materials (Robinson et al., 2002; Chun et al., 2004). Pretreatments of sugarcane bagasse (SCB) such as CaCl₂, alkali, ammonia, steam and milling showed 92%, 57%, 58%, 56% and 68% adsorption of SDM, and 86%, 45%, 49%, 44% and 56% adsorption of RTE, respectively (Table 1). These adsorption studies indicate enhanced adsorption performance of CaCl₂ pretreated SCB for SR5B, SDM and RTE. A number of earlier reports suggest the kinetics performance and removal of individual dyes by adsorption on various substrates (Demirbas, 2009). In this contest, adsorption of dyes from direct SDM and RTE has significant importance because the effluent present in environment contains complex mixture of dyes (Kadam et al., 2012). Pretreatment of SCB using 20% CaCl₂ as per the method reported by Batzias and Sidiras (2004) showed 84% adsorption of SR5B. While, 0.2% CaCl₂ pretreatment showed 91% adsorption. The use of lesser CaCl₂ concentration for pretreatment than the earlier reported method designates the reduction in treatment cost with better adsorption performance. Once the dye has been adsorbed to the substrate it will became more treatable, and then these adsorbed dyes biodegrade under SSF (Robinson et al., 2001).

3.1.2. Reduction in TOC, COD and BOD of SDM and RTE after adsorption process

Adsorption of dyes from SDM solution and RTE on pretreated SCB (CaCl₂, alkali, ammonia, steam and milling) and control SCB showed reduction in environmentally important parameters such as TOC, COD and BOD. Treatment of textile effluents is very important as they are characterized by high BOD, COD and TOC (Phugare et al., 2010). Removal of dyes from effluent reduces TOC, COD and BOD values (Cristóvão et al., 2010). In this study, TOC reduction in the SDM solution and RTE after adsorption on control SCB was found to be 30% and 24%, respectively. However, adsorption of dyes on CaCl₂, alkali, ammonia, steam and milling pretreated SCB showed TOC reduction of 87%, 48%, 44%, 43% and 59% for SDM
and, 86%, 51%, 50%, 51% and 60% for RTE, respectively. Above results suggest that significant increase in percentage reduction of TOC of SDM and RTE takes place by CaCl₂ pretreated SCB (Table 2). Similarly, percent reduction of COD and BOD in the SDM solution and RTE also enhanced significantly after adsorption on CaCl₂ pretreated SCB (Table 2). Hence, CaCl₂ pretreated SCB showed improved TOC, COD and BOD removal after adsorption of dyestuffs from SDM and RTE when compared to other pretreatments.

### Table 1
Effect of pretreatments of SCB on adsorption of textile dyestuffs and subsequent biodegradation of adsorbed dyes under SSF.

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Control SCB (A)</th>
<th>CaCl₂ (B)</th>
<th>Alkali (C)</th>
<th>Ammonia (D)</th>
<th>Steam (E)</th>
<th>Milling (F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>32 ± 0.88</td>
<td>86 ± 0.80</td>
<td>91 ± 0.75</td>
<td>47 ± 2.08</td>
<td>46 ± 0.57</td>
<td>56 ± 0.57</td>
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<tr>
<td>2</td>
<td>86 ± 0.80</td>
<td>80 ± 1.4</td>
<td>80 ± 1.4</td>
<td>78 ± 1.08</td>
<td>78 ± 1.4</td>
<td>75 ± 2.50</td>
</tr>
<tr>
<td>3</td>
<td>38 ± 0.33</td>
<td>92 ± 0.33</td>
<td>57 ± 0.33</td>
<td>58 ± 0.66</td>
<td>56 ± 0.33</td>
<td>68 ± 1.66</td>
</tr>
<tr>
<td>4</td>
<td>86 ± 0.57</td>
<td>72 ± 0.88</td>
<td>68 ± 1.32</td>
<td>68 ± 2.50</td>
<td>69 ± 0.98</td>
<td>70 ± 1.85</td>
</tr>
<tr>
<td>5</td>
<td>30 ± 0.57</td>
<td>86 ± 0.57</td>
<td>45 ± 1.20</td>
<td>49 ± 1.32</td>
<td>44 ± 3.12</td>
<td>56 ± 2.40</td>
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</tbody>
</table>

Values are mean of three values ± SEM.
A – % adsorption.
A# – % ADMI removal after adsorption process.
B – % decolorization of adsorbed dyes by *P. stuarti* under SSF in 72 h.
B# – % ADMI removal after decolorization of adsorbed dyes by *P. stuarti* under SSF in 72 h.

### Table 2
Reduction in TOC, COD and BOD of simulated dye mixture (SDM) solution and real textile effluent (RTE) after the adsorption process.

<table>
<thead>
<tr>
<th>Sr. no</th>
<th>Control SCB (A)</th>
<th>CaCl₂ (B)</th>
<th>Alkali (C)</th>
<th>Ammonia (D)</th>
<th>Steam (E)</th>
<th>Milling (F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30 ± 0.57</td>
<td>87 ± 0.57</td>
<td>48 ± 0.33</td>
<td>44 ± 1.00</td>
<td>43 ± 0.57</td>
<td>59 ± 0.66</td>
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<tr>
<td>2</td>
<td>24 ± 0.33</td>
<td>86 ± 0.33</td>
<td>51 ± 0.85</td>
<td>50 ± 0.33</td>
<td>51 ± 1.52</td>
<td>60 ± 0.88</td>
</tr>
<tr>
<td>3</td>
<td>20 ± 0.57</td>
<td>84 ± 0.66</td>
<td>44 ± 0.33</td>
<td>42 ± 0.48</td>
<td>43 ± 0.33</td>
<td>67 ± 0.57</td>
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<tr>
<td>4</td>
<td>21 ± 0.88</td>
<td>79 ± 0.88</td>
<td>49 ± 0.66</td>
<td>48 ± 0.56</td>
<td>48 ± 0.58</td>
<td>65 ± 0.33</td>
</tr>
<tr>
<td>5</td>
<td>27 ± 1.15</td>
<td>87 ± 1.15</td>
<td>45 ± 1.15</td>
<td>43 ± 2.02</td>
<td>39 ± 1.50</td>
<td>52 ± 1.15</td>
</tr>
</tbody>
</table>

Values are mean of three values ± SEM.

3.1.3. Adsorption kinetics of SR5B on CaCl₂ pretreated SCB

Kinetics for adsorption of amount of SR5B adsorbed *q*ₜ (mg/g) on CaCl₂ pretreated SCB with time *t* (min) was studied (Fig. 1). Non linear regression method was used to solve the pseudo first order and pseudo second order kinetic models for adsorption of SR5B on CaCl₂ pretreated SCB. The rate constants of adsorption were determined from non linear pseudo first order equation (Lin and Wang, 2009).

\[ Q_t = q_e (1 - e^{-kt}) \]

Where, *qₜ* and *qₑ* are amount of dye adsorbed (mg g⁻¹) at equilibrium and at time *t* (min), respectively, and *k₁* is the rate constant of adsorption (min⁻¹). The values of *k₁* and calculated *qₑ* were calculated from software Origin version 9. Pseudo second order kinetics model were expressed using nonlinear equation (Khambhaty et al., 2008).

\[ q_t = k_2 q_e^2 t / 1 + k_2 q_e t \]

Where, *k₂* (g mg⁻¹ min⁻¹) the second order rate constant, *k₂* and *qₑ* were calculated from software Origin version 9. The R² values obtained for pseudo-second-order expressions found to be more correlated to experimental *qₑ* values than the R² values obtained for pseudo first order expression (Table 3). These results suggest that pseudo-second-order model best fit for adsorption of SR5B on CaCl₂ pretreated SCB. Pseudo second-order kinetics suggests that the adsorption of SR5B on CaCl₂ pretreated SCB is a multi-step process involving sorption on the external surface and diffusion into the interior of adsorbent. Pseudo second-order mechanism of adsorption of Methylene Blue on modified Ficus carica fiber was observed earlier (Gupta et al., 2012).

3.1.4. Investigation of surface modifications of SCB after the CaCl₂ pretreatment

CaCl₂ pretreatment of SCB has showed maximum adsorption capacity for dyestuffs. Hence, it is necessary to study the modified surface properties of SCB after the CaCl₂ pretreatment. SEM [SM-1a] represents SEM analysis of the control SCB and CaCl₂ pretreated SCB. SEM [SM-1a (A)] of SCB without pretreatment showed smooth and hard surface indicating the presence of complex lignocellulosic framework. Presence of the complex lignocellulosic material
exposes less functional group which was ultimately required for adsorption. Hence, control SCB shows less adsorption for dyes. However, SEM [SM-1a (B)] of CaCl₂ pretreated SCB showed spitted and distorted surface properties lead to increase in the surface area and degradation of complex lignocellulosic framework. Increase in surface area due to split of lignocellulosic framework leads to enhance the dyestuff adsorption. SEM analysis of SCB after the pretreatments was also reported earlier (Irfan et al., 2011; Huang et al., 2012). FTIR analysis of control bagasse showed single absorption peak at 1629.7 cm⁻¹ [SM-1b (i)]. After the pretreatment FTIR spectrum showed absorption at 896, 1050, 1165, 1245, 1320, 1374, 1425, 1512, 1633, 2920, and 3418 cm⁻¹ [SM-1b (ii)]. Absorption obtained at 896 cm⁻¹ corresponds to glucosidic linkage (Liu et al., 2007). The absorption at 1050 cm⁻¹ is assigned to C–O stretching in cellulose, hemicelluloses, and lignin or C–O–C stretching in cellulose and hemicelluloses. Hydroxyl group stretching and C–H stretching in treated bagasse suggested by 3318 and 2920 cm⁻¹. The bands at 1374 and 1165 cm⁻¹ suggest absorption by C–H and C–O stretching in acetyl group in hemicelluloses and C–O–C stretching in cellulose and hemicelluloses, respectively (Liu et al., 2007). The splitting of highly-ordered and tight crystalline structure of SCB resulted in the increase of amorphous phase, specific surface area, and free OH groups subsequently increased the chemical reactivity of SCB for dye adsorption.

3.2. Decolorization studies

3.2.1. Decolorization of adsorbed dyestuffs

The SCB (without pretreatment) showed 32% adsorption for SR5B (150 mg L⁻¹) and hence SCB adsorbed 48 mg of SR5B dye. Similarly, the CaCl₂, alkali, ammonia, steam and milling pretreated SCB adsorbed 136, 70, 69, 63 and 84 mg of SR5B. This adsorbed dye was taken as the initial dye concentration for decolorization study (Kadam et al., 2011). Isolated adsorbed dye degrading microorganism was identified as P. staurti strain EbtSPG (Fig. 2) and the 16S rRNA sequence was deposited in genebank with accession number JX500693. The adsorbed dyestuff was decolorized under the SSF using P. staurti. SR5B adsorbed on SCB (without pretreatment) was decolorized up to 86% in 72 h by P. staurti (Table 1). While, P. staurti showed 80% decolorization of SR5B adsorbed on CaCl₂ pretreated SCB in 72 h (Table 1). 78%, 78%, 75% and 75% decolorization was observed for SR5B adsorbed on alkali, ammonia, steam and milling pretreated SCB (Table 1). Close investigation of these results revealed that decolorization performance was improved with pretreated SCB compared to control SCB. Similarly, decolorization of dyes adsorbed from SDM solution and RTE on pretreated SCB showed more ADMI removal than control SCB by P. staurti (Table 1). Delignification of SCB might cause increase in cell biomass due to availability of additional nutrient support which might result in the improved decolorization performance. Similar results were reported earlier as pretreatment to corn stocks enhanced the microbial growth and leads to increase in the production of organic acids (Guo et al., 2011). Hence, out of all the pretreatments studied, CaCl₂ pretreatment of SCB shows enhanced adsorption and decolorization under SSF conditions was seen in this study.

3.2.2. Optimization of pH, temperature and moisture content for decolorization of adsorbed SR5B on CaCl₂ pretreated SCB

P. staurti showed 30%, 84%, 72% and 37% decolorization of SR5B at the pH of 4, 6, 8 and 10. However, at the pH 2 no decolorization

![Fig. 2. Phylogenetic analysis of P. staurti.](image-url)
was observed (Fig. 3A). The pH has a major effect on the rate of dye decolorization and pH tolerance of decolorizing bacteria is quite important, as it makes them suitable for biological treatment of effluents (Saratale et al., 2009). Optimum temperature for decolorization of adsorbed SR5B by P. staurti was found to be 30 °C. Above 30 °C, the rate of moisture loss increases and caused decreased rate of decolorization (Kadam et al., 2012). Decolorization rate was decreased below the 30 °C because of cessation of microbial growth. Decolorization of adsorbed SR5B by P. staurti at the moisture content 85, 90 and 95 found to be 55%, 83% and 63%, respectively (Fig. 3B). While, the moisture content below 85% caused reduction in decolorization rate. Decrease in moisture causes reduced microbial growth and minimizes the contact of microbial cell with dye molecules. Moisture content of 90% was found to be optimum for SR5B decolorization (Fig. 3C). Optimization of pH, temperature and moisture content give additional insights to develop strategy for lab scale tray bioreactor development.

3.2.3. Tray bioreactor study

The CaCl₂ pretreated SCB (200 g) showed 87% adsorption of dyes from 5 L of RTE. P. staurti showed 13%, 26%, 37%, 52%, 70% and 86% ADMI removal from adsorbed dyestuff in 12, 24, 36, 48, 60 and 72 h, respectively (Fig 3D). From the overall tray bioreactor study, it can be concluded that the batch scale flask experiments can be successfully implemented at lab scale tray bioreactor. As the trays were incubated at non sterile conditions and in the open system there might be chances of other microbial communities to be present. 16S rRNA PCR amplification of the extracted DNA from P. staurti and samples from tray bioreactor showed amplification product of 750 bps (Fig. 4A). DGGE analysis of 0, 24, 48 and 72 h samples from tray suggest the presence of isolated P. staurti throughout fermentation process (Fig. 4B). The pure culture of P. staurti has shown the biodegradation of adsorbed dyes from RTE in aseptic conditions. Hence, P. staurti plays important role in decolorization of dyes in tray bioreactors which are incubated in the open system. However, change in microflora was observed during SSF at different intervals due to incubation in open system and non sterile conditions. This observed microflora in tray reactor might not be consistent due to incubation in the open system as environmental condition may vary place to place. DGGE for the enumeration of change in microflora was also used by Kolekar et al. (2012) for analysis of sludge granules. This result designates that P. staurti has significant role in degradation of dyes from RTE in open tray reactor system.

3.2.4. Metabolite analysis of SR5B

The sample obtained after decolorization of SR5B showed decreased absorbance at absorbance maxima of 530 nm which suggests 80% decolorization. Differential FTIR spectrum obtained after the decolorization suggests biodegradation of SR5B adsorbed

![Fig. 3](image-url) Effect of pH [A], temperature [B] and moisture content [C] on decolorization of adsorbed SR5B and tray bioreactor study [D].
on CaCl₂ pretreated SCB by *P. staurti* [SEM-2a]. FTIR spectrum of control dye SR5B showed the peaks at 3443, 1638, 970, 803 and 471 cm⁻¹ [SEM-2a (A)]. The peaks at 3444, 1638, 970 and 803 cm⁻¹ represents the presence of N–H stretching, C=O stretching, C–H deformation and C–H deformation in benzene ring with tree adjacent H atoms, respectively. Change in FTIR spectrum of metabolites [SEM-2a (B)] confirmed the biodegradation of SR5B. FTIR of control SDM showed the peaks at 3229, 2958, 2371, 1659, 1521, 1455, 1340, 1124, 1081, 1050, 920, 749 and 636 cm⁻¹ which represents N=O stretching, C–H stretching, NH⁺ stretching, C=O stretching, C=O stretching, C–H deformation, NO₂ stretching, C=O stretching, C=N stretching, C–H deformation, C–Cl stretching, respectively [SEM-3 (a)]. Change in FTIR spectrum of its metabolites suggests biodegradation of SDM [SEM-3 (b)]. The peaks obtained in FTIR spectrum of control RTE are 3682, 2925, 2850, 2675, 1725, 1640, 1600, 1498, 1320, 1089, 980, 760, 880 and 650 cm⁻¹ which represents O–H stretching, C–H stretching, C–H stretching, O–H stretching, C=O stretching, C=N stretching, NH deformation, N=O stretching, C–N vibration, R–O–R stretching, S=O stretching, C–H deformation, C–H deformation and C–Cl stretching, respectively [SEM-3 (c)]. Changed FTIR spectrum after the decolorization of RTE confirms its biodegradation [SEM-3 (d)]. Similarly FTIR analysis for biodegradation of dye Red M5B, SDM and RTE were reported earlier (Telke et al., 2010; Kadam et al., 2012; Lade et al., 2012). HPLC spectrum of control dye SR5B showed retention time of 4.408 and 3.905 min and after the biodegradation the products showed the peaks 2.943, 3.787, 4.137, 4.617 and 5.138 min [SEM-2b (C)]. Hence, change in retention time after the decolorization confirms biodegradation of SR5B adsorbed on CaCl₂ pretreated SCB by *P. staurti* [SEM-2b (D)]. HPLC analysis for Reactive Red 120 biodegradation was reported earlier by Paul et al. (2012). HPLC of control SDM showed the retention time at 3.192, 3.320, 3.424, 3.774, 4.017, 4.232, 4.745, 7.211 and 7.651 min [SEM-4 (a)]. After the biodegradation of SDM, retention time of the products was found to be 1.492, 1.650, 2.096 and 2.354 min which confirm its biodegradation [SEM-4 (b)]. Similarly change in retention time of control RTE after decolorization confirms biodegradation of RTE [SEM-4 (c and d)]. HPTLC chromatogram showed the absence of control SR5B band in the metabolites lane suggested biodegradation of SR5B [SEM-2c (a)]. Lade et al. (2012) also reported biodegradation of Rubin GFL using HPTLC technique. With respect to *Rf* values, control dye SR5B showed a peak of 0.40, where as *P. staurti* biodegraded showed peaks of 0.20, 0.30, 0.39, 0.45, 0.58, 0.66, 0.74 and 0.78 [SEM-2c (b)] indicating formation of several metabolites of SR5B.

### Table 4

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Water</th>
<th>Phaseolus mungo</th>
<th>Sorghum vulgare</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SR5B</td>
<td>SR5B adsorbed SCB</td>
<td>Degraded product of SR5B</td>
</tr>
<tr>
<td>Germination (%)</td>
<td>100</td>
<td>20±0.4</td>
<td>40±0.8</td>
</tr>
<tr>
<td>Plumule (cm)</td>
<td>11.0±0.4</td>
<td>6.4±0.3**</td>
<td>7.5±0.8**</td>
</tr>
<tr>
<td>Radicle (cm)</td>
<td>7.5±0.2</td>
<td>4.6±0.3**</td>
<td>5.0±0.7**</td>
</tr>
</tbody>
</table>

The values are significantly different from control at *P < 0.01, **P < 0.001* by one-way analysis of variance (ANOVA) with Tukey–Kramer comparison test. Values are mean of three experiments ± SEM.

### 3.3. Phytotoxicity study

Plant bioassays are useful tool to study the effect of toxic compounds on seed germination, root elongation and plant growth (Saratale et al., 2010). In the presence of control SR5B solution seeds of *S. vulgare* and *P. mungo* showed 30% and 20% of germination respectively. SR5B adsorbed SCB showed 40% germination for both the plants (Table 4). However, SCB obtained after the

![Fig. 4. PCR amplification (A) and DGGE analysis (B) of (1) *P. staurti* culture (2) 0 h, (3) 24 h, (4) 48 h and (5) 72 h of tray bioreactor samples.](image-url)
Decolorization of adsorbed SR5B under SSF showed enhanced germination (90%) for both S. vulgaris and P. mungo. Above results suggest that, the germination percentage was significantly increased after the biodegradation of adsorbed textile dye than control SR5B as well as dye adsorbed on CaCl2 pretreated SCB. Similarly, in the presence of SR5B metabolites the plumule and radicle lengths of plants were found to be very close to the control plant (in presence of distilled water). While, in presence of control SR5B and SR5B adsorbed on SCB the lengths of plumule and radicle of plants were significantly reduced (Table 4). Germination percentage, shoot lengths and root lengths were reduced in presence of dye and their subsequent increase after the biological treatment was reported earlier (Saratale et al., 2010; Paul et al., 2012). Hence, textile dyes even adsorbed on adsorbents has toxic effect on the plant as they remain persistent in the environment but biodegradation of adsorbed dyes under SSF removes the toxicity of dyes significantly.

4. Conclusion

CaCl2 pretreated SCB shows enhancement in the adsorption of dyes among different pretreatments studied. Adsorbed dyestuffs on pretreated SCB were decolorized under SSF by P. stuarti. Metabolite analysis by FTIR, HPLC and HPTLC confirms SR5B biodegradation by P. stuarti. Phytotoxicity studies revealed significant reduction in the toxicity due to biodegradation of adsorbed dye SR5B under SSF. Decolorization of dyes from RTE adsorbed on CaCl2 pretreated SCB in tray bioreactor gives an additional insight to develop large scale treatment protocol. Enhancement of adsorption capacity of SCB and biodegradation of adsorbed textile dyestuff can be economical and ecofriendly for textile wastewaster treatment.

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References