

# REMOVAL OF CONGO RED FROM AQUEOUS SOLUTION USING LIGNOCELLULOSIC BIOSORBENT AND SOLID STATE FERMENTATION OF DYE-ADSORBED BIOSORBENT

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## Introduction

Discharge of highly coloured industrial wastewaters, containing synthetic dyes, is considered to be an important source of water contamination. The common characteristics of synthetic dyes are physicochemical, thermal and optical stability, which enable their prolonged persistence in the environment (Li et al., 2014). Furthermore, they exhibit recalcitrance towards biodegradation, thus making their removal by conventional biological treatment technologies inefficient. Congo red (CR) is a diazo dye derived from benzidine, widely used in textile and paper industries. Wastewater containing CR is not highly biodegradable, since this dye is toxic to many organisms (Li et al., 2014).

Among the methods employed for dye removal from wastewaters, adsorption has been found to be superior to other methods because of its capability to efficiently adsorb a whole range of structurally different dyes, low operational cost, ease of design and insensitivity to toxic substances (Li et al., 2014). Activated carbon is the most commonly used adsorbent. However, its use in wastewater treatment is somewhat limited due to the high price and therefore the need for regeneration and reuse (Rafatullah et al., 2010).

In recent years, there has been an increasing amount of literature on the use of low-cost lignocellulosic materials, arising from agricultural or industrial residues as biosorbents for dye removal (Velić et al., 2015; Li et al., 2014). In order to be considered “low-cost”, the adsorbent has to be abundant in nature or has to be an industrial by-product or waste material that requires little or no processing (Rafatullah et al., 2010). The brewers’ spent grain meets all the above-mentioned criteria. Once the waste material has been used for adsorptive removal of dyes, dye-loaded material should not be directly discarded to the environment. Subsequent treatment using microorganisms that can degrade or mineralize both dye and lignocellulosic material would represent an efficient solution to the problem.

White-rot fungi are a group of fungi that degrade lignin and lignin-like substances. In addition, the specific extracellular enzyme systems of white-rot fungi enable them to degrade or mineralize a broad spectrum of different environmental pollutants, including many synthetic dyes (Jayasinghe et al., 2008).

The objectives of this study were: a) to use the brewers’ spent grain as a biosorbent for the CR removal from aqueous solutions and to evaluate its adsorption properties by batch adsorption experiments; b) to screen different white-rot fungi for their CR decolourisation ability; c) to evaluate the CR decolourization ability of the selected white-rot fungus

cultivated under solid-state conditions using dye-adsorbed brewers' spent grain as a substrate.

## Material and Methods

**Biosorbent.** Brewers' spent grain (BSG), kindly donated by "Osječka pivovara d.d." brewery was dried (oven dried at 60 °C for 48 h) and milled using standard laboratory knife mill with 1 mm screen (MF10 basic, IKA Labortechnik, Germany), sieved and 100-500 µm fraction was used for the experiments. No other chemical or physical treatments were applied prior to adsorption experiments.

**Adsorption experiments.** Batch adsorption experiments were carried out by adding a fixed amount (5, 10 and 15 g L<sup>-1</sup>) of adsorbent to 100 mL CR (Kemika d.d., Zagreb, Croatia) solution (30, 50, 100 and 150 mg L<sup>-1</sup>) taken in Erlenmeyer flask. The pH was adjusted to 7 (or higher for pH dependency experiments) and measured using MP230 pH-meter (Mettler Toledo, Switzerland). The flasks were placed in the incubator shaker (INNOVA 4340, New Brunswick Scientific, New Jersey, USA) at 25 °C and 150 rpm for 240 min. The samples were taken at different intervals (5, 10, 20, 30, 60, 90, 120, 150 and 240 min) for spectrophotometric determination of colour removal. Dye solution samples taken at various intervals were centrifuged for 5 min at 10 000 rpm (Tehtnica Centric 322A, Domel d.o.o., Slovenia). The dye concentrations in clarified supernatants were determined at 498 nm (Lambda 25, Perkin Elmer, USA). The percentage removal of dye was calculated by equation:

$$\% \text{ dye removal} = 100 (\gamma_0 - \gamma) / \gamma_0$$

where  $\gamma_0$  and  $\gamma$  are the initial dye concentration and dye concentration after certain contact time, respectively.

**Screening fungi for CR decolourisation ability.** Prior to solid-state fermentation experiment, four white rot fungi strains, *Phanerochaete chrysosporium* CCBAS 570, *Trametes versicolor* CCBAS AG613 (both The Culture Collection of Basidiomycetes, Prague, Czech Republic), *T. versicolor* TV6 and *Ceriporiopsis subvermispota* (both The Microbial Culture Collection of the National Institute of Chemistry, Ljubljana, Slovenia), were screened for their ability to decolourise CR during cultivation (9 days at 27 °C) on agar plates containing CR at final concentrations of 50, 100 and 150 mg L<sup>-1</sup>. Radial growth and the zone of colour change on agar plates were measured (at two perpendicular directions) every three days. The results were expressed as decolourisation index (decolourization diameter/mycelial diameter) (Jayasinghe et al., 2008).

**Biological treatment of dye-adsorbed biosorbent.** White-rot fungus *T. versicolor* CCBAS AG613 (The Culture Collection of Basidiomycetes, Prague, Czech Republic) was cultivated on potato dextrose agar (Liofilchem, Italy) in Petri dishes for 7 days at 28 °C. Mycelial discs (diameter of 6 mm) were used as inoculum for solid-state cultivation that was carried out in a horizontal cylindrical glass jar (bioreactor, volume 4.25 L) equipped with appropriate sensors. Aeration was carried out continuously (air flow rate was set at 30 L h<sup>-1</sup>) using sterile air filter and air compressor, while the mixing was intermittent and manual (every 24 h for 5 minutes). The bioreactor was filled with 250 g BSG and 350 mL dye solution (150 mg L<sup>-1</sup>), mixed and sterilized. After cooling, the bioreactor was inoculated with 25 mycelial discs. The experiment was conducted at ambient temperature for 21 days. Abiotic control containing only dye-adsorbed BSG and biotic control containing BSG and fungus were run for comparison. The samples were taken every seven days and analysed for proteins and colour. The temperature, relative humidity and mass loss in the bioreactor were monitored on-line. The temperature was measured at different points (headspace, inlet, outlet and bioreactor

surroundings) and in the substrate bed (bottom) using stainless steel thermocouple penetration probes (type T), connected to the 8-channels Pico A/D converter and a PC application PicoLog (Pico Technology Limited, England) for the data acquisition. Testo 635 and 350 devices (Testo Inc., Sparta, New Jersey, USA) were used for additional temperature and relative humidity monitoring. The bioreactor was placed on the balance (EOD120, OHAUS, Switzerland) for continuous mass loss monitoring. Sample *water content determination* was conducted thermogravimetrically using Halogen Moisture Analyzer HR73 (Mettler Toledo, Switzerland). The *colour* of samples was measured using chromameter (Konica Minolta CR 400) in *Lab* colour space system. Colour changes of samples during the experiment were expressed as total colour changes ( $\Delta E$ ) and the colour intensity change (chroma) ( $C^*ab$ ). The samples' *protein content* was determined by standard Kjeldahl procedure.

All analytical results were expressed as means of three replicates.

## Results and Discussion

### *The adsorption experiments*

The adsorptive efficiency of BSG was investigated and the results are presented in Figure 1. The effect of contact time and initial dye concentration are given in Figure 1 a. It can be seen that CR adsorption on BSG (i.e. CR concentration decrease) followed three-step process, a rapid initial adsorption within the first 60 min of the experiment, followed by a period of slower uptake and no significant uptake at the end of experiment. This is consistent with Ahmad and Kumar (2010) using low-cost adsorbent bael shell carbon for CR removal. The initial dye concentration has a significant effect on the adsorptive removal of dyes from aqueous solutions (Ahmad and Kumar, 2010). The percentage adsorption of CR decreased with the increase in the initial dye concentration, which is expected taken into account that at constant adsorbent concentration at higher initial dye concentration there are fewer available adsorption sites. As shown in Figure 1b the percentage removal of CR increased with the increasing adsorbent concentrations, which is in accordance with the results reported by Salleh et al (2011). However, at later stages of the experiment (contact time 120 min) the removal was similar for all runs. The effect of pH on adsorption was studied in the range of 6.5-10 (data not shown), which is just slightly above the usual wastewater pH range (6.5-9). It was found that the removal efficiency increased from 69.03 % to 93.88 % due to change in pH from 10 to 6.5, which is consistent with literature data (Dawood and Sen, 2012).

### *Screening fungi for CR decolourisation ability*

The results of screening the four fungal strains for CR decolourisation ability on agar plates revealed that only three strains were able to decolourise CR and CR did not strongly inhibit the mycelial growth of the investigated fungi (data not shown). *P. chrysosporium* CCBAS 570 did not exhibit CR decolourisation ability, while both *T. versicolor* strains, as well as *C. subvermispora* were able to decolorize CR to some extent. However, based on the highest decolourisation index ranging from 1.05 to 1.13 for CR concentrations ranging from 50 to 150 mg L<sup>-1</sup>, *T. versicolor* CCBAS AG613 was chosen for further SSF experiments.

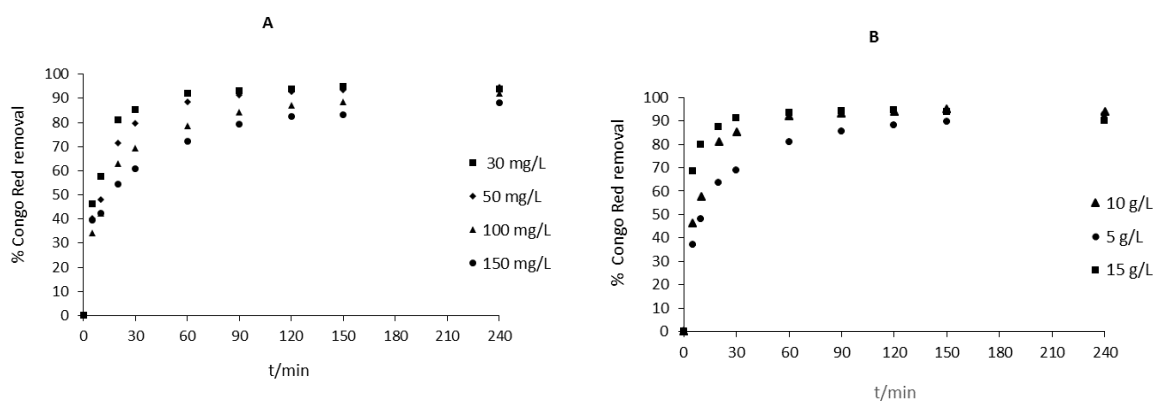


Figure 1 A) the effect of contact time and the initial dye concentration on the CR adsorption on BSG ( $t = 25\text{ }^{\circ}\text{C}$ ,  $150\text{ rpm}$ ,  $\text{pH} = 7$ ,  $V = 100\text{ mL}$ ,  $\gamma_{\text{adsorbent}} = 10\text{ g L}^{-1}$ ), B) the effect of adsorbent concentration on the CR adsorption on BSG ( $t = 25\text{ }^{\circ}\text{C}$ ,  $150\text{ rpm}$ ,  $\text{pH} = 7$ ,  $V = 100\text{ mL}$ ,  $\gamma_{\text{dye}} = 30\text{ mg L}^{-1}$ )

#### Biological treatment of dye-adsorbed biosorbent

*T. versicolor* CCBAS AG613 grew readily under solid-state conditions using dye-loaded BSG as a substrate and carrier. Apart from visible confirmation of fungal colonisation of the substrate, the increase in substrate bed temperature, compared to ambient temperature, also indicated actively growing culture (Figure 2). No significant changes in relative humidity were observed during the cultivation.

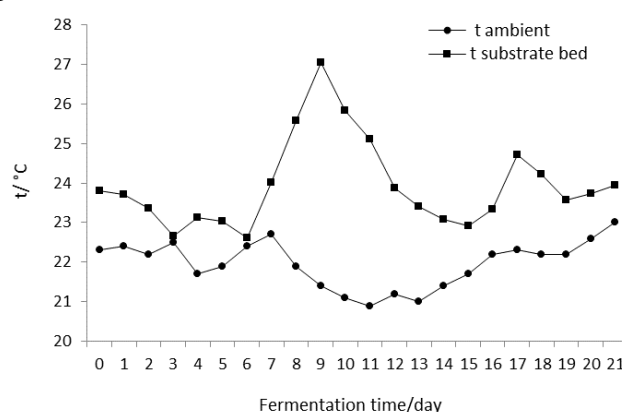


Figure 2. Temperature profiles during fermentation

Furthermore, the protein content results (data not shown) for biotic control and bioreactor sample show continuous increase (from 23.02 to 26.00 and from 23.02 to 24.90, respectively) after 7, 14 and 21 days of cultivation, which is in accordance with the literature (Peksen et al., 2011). However, the higher increase for biotic control is probably the result of slight inhibition of fungal growth by CR. The protein content of abiotic control remained the same throughout the experiment. Even though the substrate was not completely decolourised, CR decolourisation (expressed as total colour change and colour intensity change of fermented dye-adsorbed BSG samples compared to their abiotic controls after 7, 14 and 21 days) increased with the extension of cultivation time (Figure 3a). The same was observed for mass loss (Figure 3b).

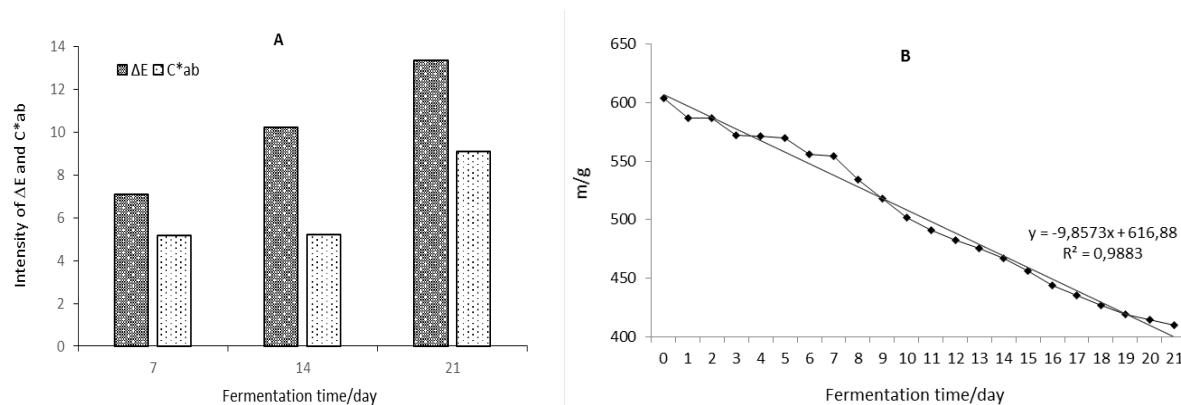


Figure 3 a) total colour change and colour intensity change of fermented samples compared to their abiotic controls after 7, 14 and 21 days of fermentation, b) time course of substrate mass loss

## Conclusion

Brewers' spent grains proved to be highly efficient biosorbent for Congo red removal from aqueous solutions. The resulting dye-adsorbed BSG was successfully used as substrate for *T. versicolor* CCBAS AG613 cultivation under solid-state conditions. These findings provide the following insights for future research: detailed characterization of BSG as possible commercial adsorbent and elucidation of the mechanisms involved in the decolourisation of dye-adsorbed biosorbents by *T. versicolor* CCBAS AG613.

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