Adsorptive removal of methylene blue from aqueous solutions using lignocellulosic waste materials and biological treatment of dye-adsorbed biosorbent

Antonija Kezerle¹, Tamara Jurić², Natalija Velić³, Damir Hasenay⁴, Tihana Marček³, Darko Velić³

¹Vodovod-Osijek d.o.o., Poljski put 1, Osijek, Croatia
²Osatina grupa d.o.o., Ulica kralja Tomislava 91, Semeljci, Croatia
³Faculty of Food Technology Osijek, Josip Juraj Strossmayer University of Osijek, F. Kuhača 20, Osijek, Croatia, e-mail: natalija.velic@ptfos.hr
⁴Faculty of Humanities and Social Sciences, Josip Juraj Strossmayer University of Osijek, Lorenza Jägera 9, Osijek, Croatia

Abstract

In this study different lignocelluloses waste materials (brewers spent grain and poplar sawdust) were tested as biosorbents for the removal of cationic dye methylene blue from aqueous solutions. The effects of initial dye concentration (5, 15, 25 and 50 mg L⁻¹), adsorbent concentration (5, 10, 15 and 20 g L⁻¹), temperature (25 and 35 °C) and solution pH (3.5, 5.5 and 7.5) on dye removal were investigated. For both tested biosorbents dye removal was rapid within the first 60 min of contact time. The percentage dye removal increased with increase in adsorbent concentration and decrease in the initial dye concentration. The percentage of methylene blue adsorptive removal from 15 mg L⁻¹ aqueous solution concentration (V = 100 mL, g⁻¹ adsorbent = 10 g L⁻¹, t =25 °C, contact time 300 min) was over 90% for both tested biosorbents. The temperature had no significant effect on the percentage removal while the decrease in pH caused reduction of percentage removal of methylene blue when brewers’ spent grain was used as biosorbent. Solid-state fermentation of dye-adsorbed brewers’ spent grain was carried out for 30 days using white-rot fungus T. versicolor CCBAS AG613. The visible decolourisation of samples was observed, i.e. a continuous increase of total colour change and colour intensity change of fermented samples compared to their abiotic controls was determined.

Key words: methylene blue, adsorption, biosorbents, T. versicolor, solid-state fermentation

Introduction

Synthetic dyes are the most common water pollutants. Discharge of highly colored effluents to surface waters reduces the quantity of dissolved oxygen and restricts the light penetration leading to alterations in photosynthesis process, thereby detrimentally affecting the aquatic life (Annuar et al., 2009). Due to prolonged persistence in the environment as a result of their high stability to light and temperature, dyes can endanger human health through consumption of aquatic organisms (Annuar et al., 2009). Furthermore, synthetic dyes exhibit recalcitrance towards removal/
biodegradation by conventional biological wastewater treatment methods. In order to remove
dye residues from aqueous solution, many alternative methods have been developed towards
highly cost conventional approaches, among which adsorption using conventional adsorbents is
the most often employed (Hameed, 2009). Activated carbon is the most widely used adsorbent
in wastewater treatment. Despite its great adsorption capacity, its application is often limited due
to high price. Therefore, it is necessary to include some other inexpensive biosorbents, arising
from industrial or agricultural residues, in dyes elimination (Bhatnagar and Sillanpää, 2010).

Cellulose, hemicellulose and lignin are the main components of agricultural wastes responsible
for adsorption. These waste products are present in nature in large quantities and represent
highly perspective sustainable and renewable natural materials. Besides being cheap, their use
as biosorbents contributes to the reduction of waste disposal cost, thus contributing to envi-
rionmental protection (Bhatnagar and Sillanpää, 2010). Although adsorptive removal of dyes
using lignocellulosic waste materials is very effective, dye-loaded biomass cannot be directly dis-
carded. Therefore, subsequent processing of this biomass using microorganisms that can degra-
de or mineralize both dye and lignocellulosic material would be appreciable. The extracellular
enzyme systems of white-rot fungi enable them to break down carbohydrate polymers: lignin
(being the most recalcitrant towards microbial degradation), cellulose and hemicellulose, as well
as effectively degrade synthetic dyes (Li et al., 2014).

The aim of this study was to investigate the adsorptive capacity of two lignocellulosic waste ma-
terials (poplar sawdust and brewers’ spent grain) to remove cationic dye methylene blue from
aqueous solutions. Furthermore, the biological treatment of dye-adsorbed brewers’ spent grain
using white-rot fungus *Trametes versicolor* was performed in order to explore its possible *in vivo*
decolourisation over time.

**Material and methods**

*Biosorbents.* Two lignocellulosic waste materials poplar sawdust (PS) (*Populus alba* L.) and
brewers’ spent grain (BSG) were used. Prior to adsorption experiments, biosorbents were dried
(first at room temperature and oven dried at 60 °C for 48 h) and milled using standard laboratory
knife mill with 1 mm screen (MF10 basic, IKA Labortechnik, Germany) to ensure the particle size
of adsorbent below 1 mm. No other chemicals or physical treatments were applied prior to ad-
sorption experiments.

*Adsorbates.* Methylene blue (MB) used in this work was purchased from Merck (Darmstadt, Ger-
many). Stock solutions of 1.5 g L\(^{-1}\) were used.

*Adsorption experiments.* Batch adsorption experiments were carried out by adding a fixed amo-
unt of adsorbent (5, 10, 15 and 20 g L\(^{-1}\)) to 100 mL dye solution (5, 15, 25 and 50 mg L\(^{-1}\)) ta-
ned in a 250 mL Erlenmeyer flask. The flasks were placed in the incubator (BD 53#04-63769,
Binder, Tuttlingen, Germany) and kept at constant temperature of 25 °C (and 35 °C for tem-
perature dependence experiments). Adsorbents were soaked in dye solutions for 300 min
and samples were taken at 30 min intervals for spectrophotometric determination of color
removal. The pH was not adjusted (except for pH dependence experiments), but it was mo-
itored using pH-meter (SevenEasyTM pH, Mettler Toledo, Switzerland). Dye solution sam-
pples taken at various intervals were centrifuged for 5 min at 10,000 rpm using a centrifuge
(Heraeus, Multifuge 3L/3L-R, Kendro laboratory Products, London, UK). The dye concentrati-
ons in clarified supernatants were determined at 665 nm using spectrophotometer (UV-1700
PharmaSpec, Shimadzu, Japan). The percentage removal of dye was calculated by equation:

\[
\% \text{ dye removal} = 100 \left( \frac{\gamma_0 - \gamma}{\gamma} \right)
\]
where $\gamma_0$ and $\gamma$ are the initial dye concentration and dye concentration after certain contact time, respectively.

**Biological treatment of dye-adsorbed biosorbent.** White-rot fungus *Trametes versicolor* CCBAS AG613 (The Culture Collection of Basidiomycetes, Prague, Czech Republic) was cultivated on potato dextrose agar in Petri dishes for 7 days at 28 °C. Mycelial plugs (diameter of 6 mm) were used as inoculum for solid-state cultivation that was carried out in 1000 mL glass flasks covered by the sterile paper to ensure aeration. Five mycelial plugs were transferred to each flask containing 50 g of sterilized dye-adsorbed BSG (50 g BSG and 120 mL MB solution concentration of 50 mg L$^{-1}$). Abiotic control was prepared in the same manner, except for the mycelial plugs. Moisture in all samples was between 62 – 65 % (determined using HR73 Moisture Analyzer, Mettler Toledo, Switzerland). Incubation was carried out at 25 °C for 30 days. The samples were taken every ten days and analysed for colour using chromameter (Konica Minolta CR 400, Osaka, Japan) and appropriate equations for total colour change and colour intensity change calculations. Each experiment was conducted in triplicate.

**Results and discussion**

Basic components of agricultural and food industry lignocellulosic waste materials include cellulose, hemicellulose, lignin, lipids, proteins, simple sugars, water, hydrocarbons and starch, containing a variety of functional groups available for adsorption of various pollutants (Bhatnagar and Sillanpää, 2010).

The adsorptive efficiency of selected biosorbents was investigated and the results are presented in Graph 1 and 2. The effect of contact time and initial dye concentration at 25 °C and adsorbent concentration of 10 g L$^{-1}$ are given in Graph 1.

**Graph 1. Effect of initial MB concentration on the adsorption on BSG and PS**

It can be seen that dye uptake was rapid for the first 30-60 min, decreasing in later stages till saturation is allowed. A series of different dye concentrations (5, 15, 25 and 50 mg L$^{-1}$) was selected on the basis of usual dye concentrations reported in actual textile effluents that range from 10 to 50 mg L$^{-1}$ (Nigam et al., 2000). The percentage dye removal decreased with increase in the initial dye concentration for both biosorbents. This is consistent with other authors also reporting that MB removal is highly concentration dependent (Khattri and Singh, 2000). The effect of adsorbent concentration on dye removal was evident only in the early stages of the
experiments (first 60 min) and showed that dye removal increased with the increase of adsorbent concentration (data not shown). At later stages, the removal was similar for all runs.

The effect of temperature was investigated at 25 and 35 °C and had no significant influence on the percentage removal (data not shown) while the decrease in pH caused reduction of percentage removal of MB when BSG was used as biosorbent (Graph 2).

The initial pH of the solution is an important parameter affecting the cationic dye adsorption. Since MB is a basic dye, acidic conditions result in the positively charged surface, thus making H⁺ ions compete with dye cations causing a decrease in the amount of dye adsorbed (Hameed, 2009).

Preliminary investigation of the biological treatment of dye-adsorbed BSG using white-rot fungus *T. versicolor* resulted in visually decolourised fermented samples compared to their abiotic counterparts. Graph 3. shows a continuous increase in total colour change and colour intensity change of fermented samples after 10, 20 and 30 days of solid state fermentation by *T. versicolor*. *T. versicolor*, as well as white-rot fungi in general, have been known to effectively decolorize synthetic dyes because of their extracellular ligninolytic enzymes, namely laccase (Li et al., 2014).
Conclusion

The selected lignocellulosic waste materials had proved to be highly efficient for adsorptive MB removal from aqueous solutions. Dye-adsorbed BSG was also a good substrate for in vivo decolourisation of MB with T. versicolor. Further research is needed to better characterize the used biosorbents as possible commercial adsorbents, as well as to elucidate the mechanisms involved in the in vivo decolourisation of dye-adsorbed biosorbents by T. versicolor.

Literature


Uklanjanje metilenskog modrila iz vodenih otopina adsorpcijom na lignocelulozne otpadne materijale i biološka obrada obojenog biosorbensa

Sažetak

U ovom radu ispitana je mogućnost uklanjanja kationskog bojila metilenskog modrila iz vodenih otopina adsorpcijom na različite lignocelulozne otpadne materijale (pivski trop i piljevina topole) kao biosorbense. Istraživan je utjecaja početne koncentracije bojila (5, 15, 25 i 50 mg L⁻¹), koncentracije adsorbensa (5, 10, 15 i 20 g L⁻¹), temperature (25 i 35 °C) i pH (3.5, 5.5 i 7.5) na učinkovitost uklanjanja bojila adsorpcijom. Uklanjanje bojila najintenzivnije je u prvih 60 min kontakta za oba istraživana biosorbensa. Smanjenjem početne koncentracije bojila i povećanjem mase adsorbensa došlo je do povećanja postotka uklanjanja, što je najbolje vidljivo u prvih 30 min eksperimenta kada je uklanjanje bojila najintenzivnije. Postotak uklanjanja metilenskog modrila iz vodene otopine bojila koncentracije 15 mg L⁻¹ (V = 100 mL, γ_adsorbent =10 g L⁻¹, t = 25 °C, vrijeme kontakta 300 min) iznosio je više od 90% za oba korištena biosorbensa. Temperatura nije imala značajnijeg utjecaja na postotak uklanjanja bojila, dok je smanjenje pH dovelo do smanjenja postotka uklanjanja metilenskog modrila kada je kao adsorbens korišten pivski trop. Biološkom obradom obojenog pivskog tropa pomoću gljive bijelog truljenja T. versicolor CCBAS AG613 uzgajane u uvjetima fermentacije na čvrstim nosačima u trajanju od 30 dana došlo je do vidljivog obezbojenja uzorka, koje se očituje u kontinuiranom porastu ukupne promjene boje i promjene intenziteta boje fermentiranih uzoraka u odnosu na abiotičke kontrole.

Ključne riječi: metilensko modrilo, adsorpcija, biosorbensi, T. versicolor, fermentacija na čvrstima nosačima