Assessment of the efficiency of U-tube continuous bioreactor and immobilized enzyme beads for dye decolourization

Rakesh Kumar Sharma¹ · Richa Sharma² · Daljit Singh Arora²

Received: 9 August 2017 / Accepted: 3 May 2018 / Published online: 7 May 2018
© Springer-Verlag GmbH Germany, part of Springer Nature 2018

Abstract

Two bioreactors (column and U-tube) were compared for continuous dye decolourization efficiency using a laccase-producing white rot fungus, *Phlebia radiata*. Column bioreactor containing immobilized crude enzyme beads and U-tube continuous bioreactor containing actively growing fungal biomass were established. Synthetic dye (coracryl blue C5G) solution treated with immobilized crude enzyme on alginate beads showed a maximum net decolourization up to 55% (flow rate 1 ml/min). The U-tube bioreactor was more efficient in decolorizing the dye, which showed a net decolourization up to 64% at faster flow rate (2.5 ml/min). The decolorization efficiency in both the systems was positively influenced by the slower flow rate. Thus, the study presents designing and operations of two continuous small-scale bioreactors one with immobilized enzyme while the another one with direct fungal contact.

Keywords Coracryl blue · Immobilized enzyme beads · Laccase · *Phlebia radiata* · Textile dye · U-tube bioreactor

Introduction

Textile industry effluent contains heavy load of dyes and is generally treated by physical and chemical methods, i.e., adsorption, coagulation, flocculation, filtration, irradiation, etc. (Robinson et al. 2001). Although these treatments are effective, they have certain limitations to solve the environmental problems. Biological treatment of industrial effluents is an emerging environment friendly approach. It is important to develop an efficient continuous bioreactor for dye decolourization for laboratory-level experiments to ensure the efficiency of different biological systems, which can be further up-scaled as per the requirements. Different white rot fungi have been reported to produce ligninolytic enzymes, capable

to decolourize several dyes (Chander and Arora 2007). The crude enzymes produced can be immobilized on alginate beads to prevent the loss during processing and increase the reusability. Besides enzymatic degradation, fungal biomass has also been used for bioadsorption of toxic materials and dyes (Gimenez et al. 2014). A further advantage is that these fungi can be grown on economic and abundantly available agro-residues for continuous biomass and enzyme production (Sharma and Arora 2010). Several methods based on batch culture have been used to study the decolourization potential of various white rot fungi (Kale et al. 2014; Vantamuri and Kaliwal 2017), while the present study is focused on the development of a laboratory-level continuous bioreactor. Two bioreactor designs have been compared: one of these is based on immobilized enzymes, while the other one is based on direct contact with fungal biomass and continuously produced enzymes in a U-tube. *Phlebia radiata* was used as a model fungus to decolourize coracryl blue C5G dye.

White rot fungus and dye

Phlebia radiata (MJL-1198) and coracryl blue C5G dye (basic blue) were received from T. W. Jeffries (Forest Product Laboratories, Madison, USA) and Colourtex Ltd.,

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s13205-018-1275-2>) contains supplementary material, which is available to authorized users.

✉ Rakesh Kumar Sharma
r.18sharma@gmail.com;
rakeshkumar.sharma@jaipur.manipal.edu

¹ Department of Biosciences, Manipal University, Jaipur, Rajasthan 303007, India

² Department of Microbiology, Guru Nanak Dev University, Amritsar 143005, India

India, respectively. Dye solution was prepared in tap water (50 mg/l) and the decolourization was measured by reading its absorbance at 580 nm.

Decolourization using immobilized enzyme beads

A glass column (60 cm length, 12 mm inner diameter) was filled with immobilized crude enzyme beads. *P. radiata* was grown in 100 ml [0.5% (w/v)] malt extract medium, which showed maximum laccase activity on 9th day, i.e., 0.084 CU/ml as assayed using guaiacol as substrate (Sharma and Arora 2010). The contents of the flask were vacuum filtered and then lyophilized (at -50°C under vacuum) to powder. This lyophilized powder was mixed with 100 ml sodium alginate solution (2% w/v). Sodium alginate solution was added drop-by-drop with the help of 10-ml glass pipette into the 100 ml cross-linking solution [2% (w/v) CaCl_2 in acetate buffer (0.1M, pH 5)]. Calcium alginate beads (3–4 mm diameter) were separated and the process was repeated. Control beads were also prepared using only the malt extract medium instead of cell-free enzyme extract. These beads were separately filled in the column up to 45 cm length (Fig. 1a). A volume of 1500 ml dye solution was passed through the column at a flow rate of 1 ml/min and 100 ml fractions were collected to measure the absorbance. The experiment was run for 25 h and repeated thrice along with control.

Maximum decolourization (70%) was observed in 10–12th fractions (16–20 h) by cell-free immobilized enzyme beads (CFIEB), while in control column it was almost constant (15–19%) throughout the experiment (Fig. 1b). The decolourization observed in the control column was due to the adsorption of the dye on the alginate beads. Thus, the net decolourization was calculated as follows:

Net decolourization = decolourization in CFIEB containing reactor – decolourization in control reactor

On an average, the net decolourization recorded was about 45% (1 ml/min). Slower flow rate (0.5 ml/min) was more effective for dye decolourization (average dye decolourization ~50%) as it allowed a longer holding time, for better contact between dye and the enzyme. Previously, during the dye decolourization study using immobilized enzyme, majority of dye decolourization (79%) was due to adsorption and very minor (4%) decolourization due to enzymatic activity (Zille et al. 2003). The present results also indicate that the adsorption plays an important role during the decolourization studies. This interfering effect can be normalized by using the same setup with control, which contains only the support material without enzyme.

Fig. 1 **a** Schematic representation of immobilized enzyme beads column, **b** decolourization of coracryl blue C5G in cell-free enzyme extract immobilized onto alginate beads (CFIEB) at a flow rate of 1 ml/min

Most of the studies on immobilized enzyme did not mention about the adsorption of dye on the support material, thus making it difficult to calculate the net dye decolourization (Iqbal and Asgher 2013). An earlier study on *Trametes hirsuta* immobilized onto alginate beads showed good decolourization efficiency but the time taken for the operation was too long as compared to the present study (Domínguez et al. 2005). Immobilized biomass of the white rot fungus *Trametes pubescens* was also explored for adsorption of dye. The biomass immobilized by sodium alginate enhanced the sorption capacity for Congo red (Si et al. 2015). Thus, the scientists are continuously trying to improve the system using different fungi or conditions.

Decolourization using U-tube continuous bioreactor

A U-tube reactor of 400 ml capacity (30 cm height, 3 cm inner diameter, 60 cm of U-tube length) along with inlet and outlet (0.5 cm) was designed (Fig. 2a). Polyurethane foam cut into pieces (2 cm²), so as to fit in the U-tube, was sterilized by soaking in 95% ethanol for 48 h and washed thoroughly with sterilized distilled water. Twenty-five foam pieces were packed into the sterilized reactor, then 50 ml of 5-day-grown fungal culture (in mineral salt broth at 25 °C and 200 rpm) was poured along with the sterilized wheat straw-based medium (wheat straw (2.5% w/v) boiled in distilled water for 10 min, cleared by 0.5% activated charcoal, then filtered and sterilized). The U-tube was incubated at 25 ± 2 °C for 8 d to allow the growth of fungus on polyurethane foam. The polyurethane foam provides a porous environment for uniform mycelia growth throughout the tube.

Fig. 2 **a** Schematic representation of U-tube continuous bioreactor. **b** Decolourization of coracryl blue C5G in U-tube continuous bioreactor at the flow rate of 2.5 ml/min

On 9th day, a total volume of 1500 ml dye solution was passed through the tube containing medium and fungal culture, growing at a rate of 2.5 ml/min, with the help of a peristaltic pump and fractions of 100 ml were collected; thus, the experiment was run for 10 h (Supplementary Fig. 1). A control reactor under similar setup without fungus was also run to get the net dye decolourization. The whole experiment was repeated thrice.

Initially, the absorbance showed higher level of decolourization (85–90%) as the fraction contained decolorized dye solution along with some amount of medium. After two fractions it gave a constant decolourization of about 70–60% up to 1000 ml dye solution. Afterwards the decolourization efficiency declined and at the end of the experiment it was 50% (Fig. 2b). In the control experiment, initially it gave about 30–35% of decolourization, which was due to the dilution of dye solution with uninoculated medium. After few fractions it showed only 10–12% of decolourization and at the end of the experiment it was only 5%. The decolourization shown in the control tubes can either be attributed to the dilution of dye solution or auto-oxidation. The final results were calculated by subtracting the control decolourization from the inoculated one, which gave a net decolourization of about 60–45% under continuous system. At the end of the experiment, the left over dye solution in the U-tube or column (after 12 h) showed 100% decolourization as compared to control. During the decolourization studies it was observed that flow rate significantly affects the process. At slower flow rate (1 ml/min) it gave higher decolourization (> 80%), while faster flow rate (2.5 ml/min) decreased the rate of decolourization (comparative data not shown). Earlier study reported that coracryl blue C5G dye was difficult to degrade under batch system (Chander and Arora 2007). Similarly several azo dyes have been evaluated in batch culture, which showed fastest decolourization (~ 40%) in 5 min, while the complete decolorization took 24 h (Akpınar and Urek 2017). On the other hand, continuous U-tube system is quite effective and may be used for longer time as it continuously produces enzyme and decolourizes the dye simultaneously.

Laccase production was maximum on 9th day of incubation, thus this incubation time was decided for enzyme extraction or fungal growth in U-tube. Both the parameters, i.e., performance of the immobilized laccase on epoxy-activated Sepabeads acrylic polymers and immobilized fungal biomass on a laboratory-scale rotating biological contactor (RBC) showed good dye decolourization, which suggests application of the fungal enzyme and mycelium for bioremediation processes (Kunamneni et al. 2008; Pakshirajan et al. 2011). Thus, U-tube reactor seems to be a promising approach to decolourize toxic dyes. In this system, both fungal biomass as well as its enzyme system is involved in dye decolourization, which ultimately enhanced the efficiency of the process. The successful exploitation of lab-level studies

points towards potential of the reactor to provide an environment friendly technique for effluent treatment.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest in the publication.

References

- Akpınar M, Urek RO (2017) Induction of fungal laccase production under solid state bioprocessing of new agroindustrial waste and its application on dye decolorization. *3 Biotech* 7:98
- Chander M, Arora DS (2007) Evaluation of some white-rot fungi for their potential to decolourise industrial dyes. *Dyes Pigments* 72:192–198
- Domínguez A, Couto SR, Sanromán M (2005) Dye decolorization by *Trametes hirsute* immobilized into alginate beads. *World J Microb Biot* 21:405–409
- Gimenez GG, Ruiz SP, Caetano W, Peralta RM, Matioli G (2014) Biosorption potential of synthetic dyes by heat-inactivated and live *Lentinus edodes* CCB-42 immobilized in loofa sponges. *World J Microb Biot* 30:3229–3244
- Iqbal HMN, Asgher M (2013) Decolorization applicability of sol–gel matrix immobilized manganese peroxidase produced from an indigenous white rot fungal strain *Ganoderma lucidum*. *BMC Biotechnol* 13:56
- Kale SK, Deshmukh AG, Dudhare MS (2014) Decolorization and wood degrading ability of locally isolated basidiomycetes. *J Sci Ind Res* 73:735–739
- Kunamneni A, Ghazi I, Camarero S, Ballesteros A, Plou FJ, Alcalde M (2008) Decolorization of synthetic dyes by laccase immobilized on epoxy-activated carriers. *Process Biochem* 43:169–178
- Pakshirajan K, Sivasankar A, Sahoo NK (2011) Decolourization of synthetic wastewater containing azo dyes by immobilized *Phanerochaete chrysosporium* in a continuously operated RBC reactor. *Appl Microbiol Biotechnol* 89:1223–1232
- Robinson T, McMullan G, Marchant R, Nigam P (2001) Remediation of dyes in textile effluent: a critical review on current treatment technologies with a proposed alternative. *Bioresour Technol* 77:247–255
- Sharma RK, Arora DS (2010) Changes in biochemical constituents of paddy straw during degradation by white rot fungi and its impact on in vitro digestibility. *J Appl Microbiol* 109:679–686
- Si J, Yuan TQ, Cui BK (2015) Exploring strategies for adsorption of azo dye Congo Red using free and immobilized biomasses of *Trametes pubescens*. *Ann Microbiol* 65:411–421
- Vantamuri AB, Kaliwal BB (2017) Decolourization and biodegradation of Navy blue HER (Reactive Blue 171) dye from *Marasmius* sp. BBKAV79. *3 Biotech* 7:48
- Zille A, Tzanov T, Gubitz GM, Cavaco-Paulo A (2003) Immobilized laccase for decolourization of reactive black 5 dyeing effluent. *Biotechnol Lett* 25:1473–1477