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Integrated system of multiple batches to evaluate the continuous performance of microbial cells in decolourization processes

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ABSTRACT

Azo dye degradation in wastewater treatment is a subject which has garnered the attention of many research studies. In this study, an innovative approach, namely, an integrated system of five batches (ISFB), was developed to investigate the capability of *Saccharomyces cerevisiae* ATCC 9763 for continuous degradation of methyl red as a representative azo dye. Toward this end, an expanded immobilized microbial bed (EIMB) reactor was established with a bed of encapsulated yeast cells in sodium alginate. EIMB reactor was run in two modes, single batch and ISFB. Moreover, durability of the microbial cells was evaluated by repeating the continuous decolourization eight sequential times in EIMB at ISFB mode with the same cells. ISFB set-up can simulate a continuous process while avoiding the complexity of utilizing continuous reactors. Spectrophotometry results show that 6 ± 0.17 h was required for complete decolourization in EIMB reactor at single batch mode which estimates a decolourization rate (R_{Dec}) of 8.9 ± 0.3 g h^{-1}in this set-up. Subsequently, decolourization rate incrementally increased ≃2.8 times while ISFB mode was applied to the EIMB system and this rate was approximately maintained during eight consecutive runs. The changes in morphology and bioavailability of the immobilized *S. cerevisiae* were monitored through scanning electron microscopy.

1. Introduction

Azo dyes are the largest class of synthetic dyes including one or more chromophoric azo groups (−N═N−). They are used extensively in a wide range of industries including foodstuff, confectionery and, in particular, candy manufacturing as well as in soft drink, pharmaceutical, paper, textile and leather industries. Since synthesized dyes typically maintain their sustainability qualities in the face of destructive factors including pH and ionic strength changes, microbial attacks, chemical reactions and thermal fluctuations, they tend to remain stable over time and therefore attempting to eliminate them from wastewater proves challenging. To date, different processes and techniques, including various physical, chemical and biological methods, have been suggested for removal of azo dyes in wastewater treatment systems. Typically, biological treatment methods have proved most effective due to their high efficiency, cost effectiveness, environmental sustainability, ease of use and flexibility [1–3]. Therefore, a large number of studies have been devoted to the evaluation of different microorganisms in synthetic and/or real wastewater samples for microbial degradation of a wide range of azo dyes used primarily in textile industries [4–7]. They assert that anaerobic decolourization occurred due to the reductive cleavage of azo bond and the generation of corresponding aromatic metabolites. Various anaerobic systems such as up-flow anaerobic sludge blanket (UASB) [8]; anaerobic sequencing batch reactor (ASBR) [9,10]; anaerobic baffled reactor (ABR) [11]; membrane bioreactor (MBR) [12]; up-flow packed-bed reactor (UPBR) [13]; and up-flow anaerobic bio-filter (UAF) [14] have been studied for decolourization of dye-containing wastewater prepared synthetically or obtained from wastewater treatment plants.

Since wastewater treatment plants are typically programmed to operate continuously for extended periods of time, generally, anaerobic bioreactors require that microorganisms be able to survive and remain active during the decolourization processes in order to maintain acceptable levels of efficiency. Two techniques have been employed for lab-scale measurement of the performance of microorganisms during a continuous process. First, the technique of sequencing batches was used

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in which biomass was collected at the end of each batch and used in the subsequent batch [9,15]. This method requires the emptying and filling of fresh medium for each batch, therefore microorganisms are not exposed to toxic metabolites generated during the decolourization degradation. Hence, this controlled environment may not adequately simulate the continuous state and thus the results may not be a good estimation of continuous conditions. The second technique entailed the establishment of a lab-scale continuous set-up which proved to be an overly-complicated system. This study, however, aims to establish an integrated system of multiple batches to investigate continuous performance of *Saccharomyces cerevisiae* ATCC 9763 in decolourization processes. Through this approach, rather than running a set-up of individual consecutive batches, separate batches are integrated and operate as a single set-up.

According to our previous study [16], *S. cerevisiae* ATCC 9763 exhibited an efficient microorganism in degradation of methyl red. The present study attempts to assess this yeast for continuous degradation of methyl red in an integrated system of five batches (ISFB). To accomplish this, an expanded immobilized microbial bed (EIMB) reactor was designed and conducted under anaerobic conditions while mixing and/or bed expansion was supplied by recycling a fraction of medium to the reactor entrance. Sodium alginate capsulated *S. cerevisiae* was employed to decolourize the methyl red containing synthetic wastewater (SWW) which filled the EIMB reactor up to the recirculation line defined as one batch working volume (BWV) and the process was carried out in single batch mode (Fig. 1a). In order to evaluate the performance of *S. cerevisiae* in continuous implementations, the EIMB reactor began at a working volume of five BWVs divided into a 2:3 ratio between the reactor column and feedstock container (Fig. 1b). Through this set-up, processed medium is discharged into the feedstock tank and mixed with the fresh feedstock then the mixture returns to the reactor and circulates in the system within a closed cycle. In this way, microbial cells are exposed to dye and degradation metabolites and their performance can be subsequently evaluated in terms of their survival rate and decolourization ability.

This approach, using an integrated system of multiple batches proposed in the present study, may be used for lab-scale assessment of microbial cell capability for use in continuous decolourization applications.

2. Experimental

2.1. Chemicals

Azo dye methyl red (MR) was purchased from Sigma-Aldrich Chemical Company (USA). Alginic acid sodium salt B25266 was obtained from Alfa Aesar. All other chemicals and reagents were of analytical grade and prepared by Merck Co., Germany.

2.2. Microorganism and immobilization

Saccharomyces cerevisiae ATCC 9763 purchased from the Iranian Research Organization for Science and Technology (IROST) was grown in YPD medium containing yeast extract, 1% (w/v); peptone, 2% (w/v) and dextrose, 2% (w/v) at 30 °C and 180 rpm shaking. Cell pellets were harvested from broth culture ($OD_{600\,nm} \simeq 3.9 \pm 0.4$) by centrifugation at 4000 rpm (1520 × *g*) for 10 min under cold conditions (4 °C) and resuspended in sterile distilled water. This thick yeast suspension was mixed with four times the volume of sterilized sodium alginate gel (3%).

The cell-alginate mixture was then placed in a 50 ml plastic syringe and dripped into a sterile 0.2 M $CaCl_2$ solution by using a syringe pump (Zist Rahe Danesh, SP93-1, Iran) at a rate of 4 ml/min. The spherical beads formed by contact of the gel droplets with Ca^{2+} cations were collected by decanting the solution, washed three times with saline water (0.9% NaCl solution), weighted and stored in saline solution at 4 °C for use in reactor experiments [17].

2.3. Feedstock composition

Feedstock was a dye-containing solution used as the synthetic wastewater (SWW) which included methyl red (100 mg/L), glucose (500 mg/L), lactose (500 mg/L), urea (40 mg/L), KH_2PO_4 (8 mg/L), K_2HPO_4 (10 mg/L) and $NaHCO_3$ (250 mg/L). The SWW was prepared in tap water according to Koupaie et al. [15].

2.4. Design of the expanded immobilized microbial bed reactor

The EIMB reactor used for the decolourization experiments in the

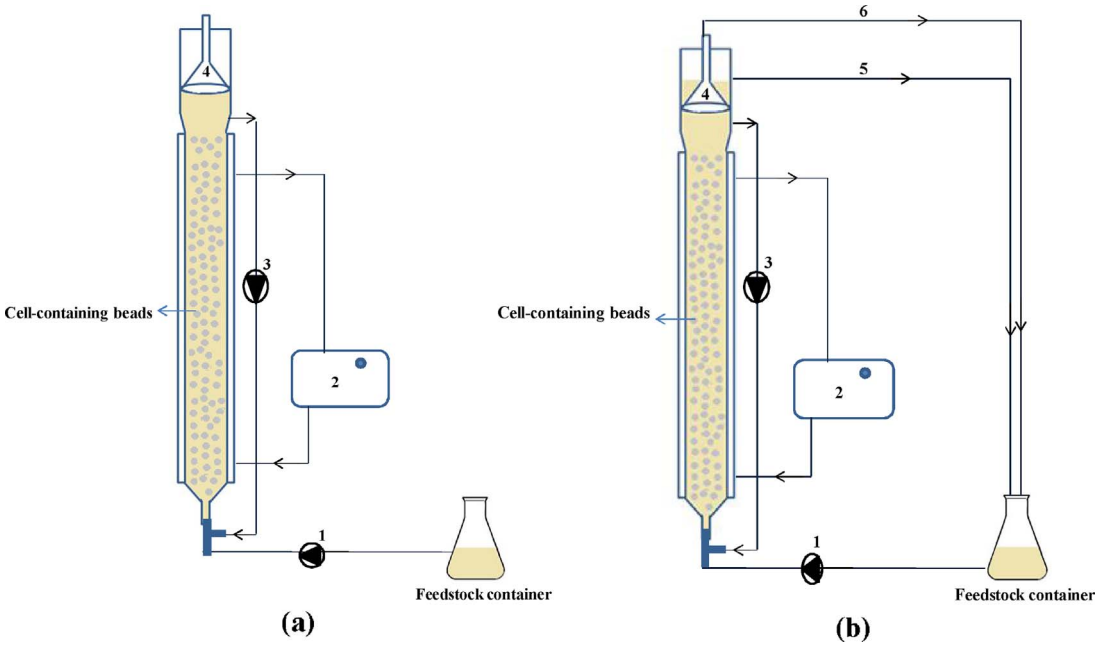

Fig. 1. Schematic diagrams of EIMB reactor at the modes of (a) single batch, and (b) ISFB. 1, influent pump; 2, thermostatic bath circulator; 3, effluent recirculation pump; 4, gas-liquid separator; 5, effluent discharging line; 6, biogas exhaust.