Environmental Technology & Innovation 6 (2016) 132–140

Contents lists available at ScienceDirect

Environmental Technology & Innovation

journal homepage: www.elsevier.com/locate/eti

# Continuous removal of malachite green by calcium alginate immobilized *Bacillus cereus* $M_{16}^{1}$ in packed bed column

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## HIGHLIGHTS

- Biosorption of malachite green in continuous up-flow packed bed column reactor.
- Flow rate, bed height, influent dye concentration are important process parameters.
- Adam–Bohart, Yoon–Nelson and Thomas models employed for experimental data analysis.

## ARTICLE INFO

*Article history:*
Received 28 November 2015
Received in revised form 3 May 2016
Accepted 8 June 2016
Available online 14 June 2016

*Keywords:*
Biosorption
Packed bed column
Malachite green
Immobilization

## ABSTRACT

Continuous removal of malachite green from synthetic dye solution (pH 5.0) was studied with *Bacillus cereus* $M_{16}^{1}$ immobilized in calcium alginate (3%) using a packed bed column reactor operated at 30 °C. The experiments were conducted to study the effect of important parameters such as flow rate, bed height and influent dye concentration on the dye removal process. Maximum dye removal (74.7 ± 0.4%) was observed at 2 mL/min flow rate, 30 cm bed height and 100 mg/L influent dye concentration. The Adam–Bohart model, Yoon–Nelson model and Thomas model were employed to determine characteristic parameters such as saturation concentration ($N_0$), kinetic rate constant ($K_{AB}$, $K_{YN}$, $K_{Th}$), maximum biosorption capacity ($q_0$), and the time required for 50% adsorbate breakthrough ($\tau$), which are all useful for process design. Maximum biosorption capacity of 37.21 mg/g was obtained at optimum conditions. Experimental data fitted well with Adam–Bohart model at initial part of the breakthrough curve.



## 1. Introduction

Dyes are used in different industrial sectors, among which textile industries are one of the most significant users. In textile industries synthetic dyes are extensively used for dyeing process. The textile industrial processing is also very water-consuming (Spagni et al., 2010). Due to the wide application of dyes, these industrial pollutants are common contaminants in wastewater, which are extremely toxic for aquatic flora and fauna. The presence of dyes in wastewater is easily detectable even when released in small concentrations due to their characteristic strong color (Nath and Ray, 2015; Nigam et al., 2000). Moreover, majority of these dyes are non-biodegradable due to their complex aromatic structure and synthetic origin, which can remain in the environment for an extended period of time (Safa et al., 2011). Hence, removal of dyes from textile wastewater is a major environmental issue for consideration.

Packed bed column with continuous flow operations are more effective in large-scale industrial effluent treatment, as it makes best use of the concentration difference known to be a driving force for adsorption and thus allows more efficient

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http://dx.doi.org/10.1016/j.eti.2016.06.002
2352-1864/

utilization of the capacity of adsorbent (Adhikari et al., 2012, 2013). Passing the industrial effluent through packed bed column, results in a better quality of effluent for discharge into aquatic streams. Also large volume of wastewater can be continuously treated using a defined quantity of sorbent in a packed bed column (Aksu et al., 1998). However, only little effort has been focused on employing packed bed columns for adsorption of dyes by a few researchers (Chen et al., 2003; Fu and Viraraghavan, 2003; Padmesh et al., 2006; Saeed et al., 2009; Vijayaraghavan and Yun, 2008). A packed bed is also effective for cyclic sorption and desorption for maximum utilization of the biosorbent. Methods using dry cells suffer from washing out and cell damage during continuous flow operations whereas immobilized cells have several advantages such as minimal clogging, more stability during continuous flow and easier solid–liquid separation thus have higher possibility of reuse (Kathiravan et al., 2010). Aksu et al. (1998) have reported the use of calcium alginate immobilized *C. vulgaris* in packed bed column for biosorption of copper (II). Maity et al. (2009) have reported calcium alginate immobilized *Bacillus cereus* $M_{16}^{1}$ in packed bed column for biosorption of Cr (VI). Kathiravan et al. (2010) have also reported calcium alginate immobilized *Bacillus* sp. in packed bed column for reduction of Cr (VI).

In the present study, the treatment of malachite green solution using calcium alginate immobilized *Bacillus cereus* $M_{16}^{1}$ was investigated in an up-flow packed bed column reactor. Experiments were conducted as a function of bed height, flow rate and dye concentration. The performance of the packed bed column was described through a plot of the ratio of final and initial effluent dye concentration versus time, which is usually referred to as breakthrough curve (Adhikari et al., 2013; Cheng and Wang, 2000; Ekpete et al., 2011). The experimental data obtained were fitted to Adam–Bohart model, Yoon and Nelson model and the modified Thomas model in order to predict the breakthrough curve of the adsorption process.

## 2. Materials and methods

### 2.1. Microorganism and culture media

*Bacillus cereus* $M_{16}^{1}$ (MTCC 5521) used as biosorbent in the present study, was isolated, identified and mutated by Bera et al. (2007). The strain was maintained in modified nutrient agar medium having the composition (g $L^{-1}$): Beef extract, 1.0; Yeast extract, 2.0; Peptone, 5.0; Sodium chloride, 5.0; Agar, 25.0; pH 6.5, by monthly subculturing at 30 °C for 24 h and stored at 4 °C. Compositions of inoculum and growth medium were same as above except agar.

### 2.2. Chemicals

Malachite green used in the present study was obtained from Merck Specialities Private Ltd., India. Other chemicals including ingredients of the microbiological media, sodium alginate and calcium chloride were obtained from Himedia Laboratories and Merck Specialities Private Ltd., India.

### 2.3. Preparation of immobilized biomass

Inoculum was prepared by transferring one loop-full of cells from a slant culture of *Bacillus cereus* $M_{16}^{1}$ to 50 mL sterile inoculum medium in 250 mL Erlenmeyer flask followed by incubation at 30 °C, 120 rpm for 24 h. Growth medium (50 mL in 250 mL Erlenmeyer flask) was inoculated with 1 mL inoculum and incubated under the same conditions described above. Then, biomass was harvested by centrifugation (REMI R8C) at 5000 rpm for 10 min and washed twice with normal saline. A suspension of washed bacterial cells (concentration 100 mg/mL) was prepared in normal saline. Sodium alginate solution was mixed with bacterial cell suspension (100 mg/mL) in 2:1 ratio to get a final concentration of alginate 3% and the mixture was injected drop by drop into 0.5 M calcium chloride solution using a hypodermic syringe. Calcium alginate beads carrying bacterial cells were kept at 4 °C for 2 h and then washed repeatedly with de-ionized water. Similar technique of immobilization was also reported by other authors (Kathiravan et al., 2010; Bera et al., 2007; Gummadi et al., 2009; Nath et al., 2015).

### 2.4. Experimental setup

In this study the dye removal process was investigated on a laboratory scale packed bed up-flow column reactor. The experimental setup of the reactor is schematically shown in Fig. 1. Experiments were carried out in a glass column of height 35 cm and internal diameter ($d_{column}$) 1.4 cm. Bed was packed with biomass immobilized in calcium alginate beads. Glass wool was placed at the bottom of the bed. Dye solution was continuously fed into the reactor from bottom using variable speed peristaltic pump and silicone tubes of 1 mm tube bore. A second peristaltic pump was used to collect the effluent from top of the bed through latex tubes into a sample collection chamber.

### 2.5. Operation of packed bed column reactor

The packed bed column reactor was operated on a laboratory scale. Packed bed of variable height were prepared with calcium alginate immobilized *Bacillus cereus* $M_{16}^{1}$ biomass in a glass column and dye removal process was carried out by