International Biodeterioration & Biodegradation 119 (2017) 111–117

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

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

Cu(II) removal by *Nostoc muscorum* and its effect on biomass growth and nitrate uptake: A photobioreactor study

S. Arun [a], N. Arul Manikandan [a, b], Kannan Pakshirajan [a], G. Pugazhenthi [b, *], Mayashree B. Syiem [c]

[a] Department of Biosciences and Bioengineering, Indian Institute of Technology Guwahati, Guwahati 781039, Assam, India
[b] Department of Chemical Engineering, Indian Institute of Technology Guwahati, Guwahati 781039, Assam, India
[c] Department of Biochemistry, North Eastern Hill University, Shillong 793022, Meghalaya, India

ARTICLE INFO

Article history:
Received 27 June 2016
Received in revised form
20 September 2016
Accepted 20 September 2016
Available online 28 September 2016

Keywords:
Nostoc muscorum
Nitrate uptake
Cu(II) removal
Photobioreactor
Cyanobacterium
Han-levenspiel model

ABSTRACT

This study investigated the Cu(II) removal by *Nostoc muscorum*, a cyanobacterium isolated from a toxic metal polluted site in Meghalaya, using a batch photobioreactor with an aim to elucidate the removal mechanism and its effect on nitrate uptake by the cyanobacterium. Experiments were carried out using a batch photobioreactor by varying the initial Cu(II) concentration in the range of 5–30 mg L^{-1}. Results obtained in this study showed that a maximum of 5.628 ± 0.05 g L^{-1} of biomass concentration could be obtained in the absence of Cu(II), whereas this value of biomass reduces to 4.3 ± 0.0057 g L^{-1} in presence of 5 mg L^{-1} of initial Cu(II) concentration, which further reduces to 2.51 ± 0.01 g L^{-1} for an initial Cu(II) concentration of 30 mg L^{-1}. Besides, an increase in the initial Cu(II) concentration delayed the uptake of nitrate by the cyanobacterium. However, complete removal of Cu(II) was observed for all the four different initial Cu(II) concentration. The estimated value of nitrate uptake rate in the absence of Cu(II) was found to be 0.115 mg L^{-1} d^{-1}, which was reduced to 0.029 mg L^{-1} d^{-1} due to 30 mg L^{-1} initial Cu(II) concentration in the medium. The experimental nitrate uptake rate values were fitted to three unstructured kinetic models reported in the literature for estimating the biokinetic constants. Among the three models, Han-levenspiel and Andrew models fitted the experimental data closely with a determination coefficient (R^2) value of 0.942 and 0.921, respectively. The critical Cu(II) concentration obtained as per the Han-levenspiel model was found to be 0.0325 g L^{-1}.

1. Introduction

Cyanobacteria or blue-green algae predominantly occur in diverse environmental conditions including freshwater, marine water, toxic heavy metal contaminated sites, bare rocks and soil (Hazarika et al., 2015; Ozturk et al., 2014; De la Noüe and Basseres, 1989). Cyanobacteria possess highly attractive advantages over the other organisms, which include simple nutrient requirement, rapid growth rate, large surface area, high mucilage volume and high metal binding affinity (Gupta and Rastogi, 2008). Therefore, the use of cyanobacteria for the treatment of toxic metal ions from wastewater is one of the recent research interests (Bulgariu and Bulgariu, 2013). Copper is toxic metal commonly found in contaminated water, which adversely impacts the environment and human health (Bilal et al., 2013; Yu et al., 2014). Copper occurs in three oxidation states out of which Cu(II) is more common and hard to remove. Cu(II) is more toxic and carcinogenic than the other form (Yan and Pan, 2002). Different industrial activities, such as galvanizing, petroleum refining, metal finishing, paints and pigments production, coal mining, smelting and electro-plating, discharge Cu(II) ions into their wastewater (Akbari et al., 2015). Conventional techniques to remove such toxic metals from wastewater, which are mainly ion exchange, membrane filtration, chemical precipitation, adsorption, coagulation/flocculation and reverse osmosis, still suffer from certain disadvantages, such as high treatment cost and/or inefficient removal of metals, particularly at low initial metal concentration below 100 mg L^{-1} (Won et al., 2014). According to the World Health Organization (WHO) and United State Environmental Protection Agency (USEPA) the allowable limit of Cu(II) in water is 1.3 mg L^{-1} and 1.5 mg L^{-1}, respectively (Jain et al., 2008). In view of these strict regulations,

* Corresponding author.
E-mail address: pugal@iitg.ernet.in (G. Pugazhenthi).

http://dx.doi.org/10.1016/j.ibiod.2016.09.022
0964-8305/

Cu(II) removal by bioaccumulation using cyanobacteria is proving to be an attractive alternative (Yang et al., 2015).

Previous studies have demonstrated a very good potential of the cyanobacterium *Nostoc muscorum* to remove heavy metals from wastewater (Hazarika et al., 2015; Manikandan et al., 2014; Roy et al., 2015). However, all these studies were limited to only batch shake flask level. This is very important because all the biological system responds contrarily in different set up. At the shake flask level, organisms do not suffer from aeration and light limitations, but in a reactor mass transfer and light conditions limit the biological process, thereby posing a serious problem with the scale up of such bioprocesses (De Morais and Costa, 2007; Jacome-Pilco et al., 2009; Kim et al., 2016; Kumar et al., 2007; Luo et al., 2003). Hence, results obtained at the shake flask level need to be validated at the reactor level in order to determine the bioprocess mechanism and kinetics. But, no studies have been reported so far on heavy metal removal by cyanobacterium at the bioreactor level. Moreover, a detailed understanding of the kinetics of the heavy metal removal, biomass growth and nitrate uptake by cyanobacteria has largely limited their application for heavy metal removal from wastewater. Therefore, this study was aimed at investigating the removal of Cu(II) by *N. muscorum* in a batch operated photobioreactor and to study the effect of Cu(II) on biomass growth and nitrate uptake by the cyanobacterium. Moreover, the effect of Cu(II) on *N. muscorum* biomass growth and nitrate uptake was studied to understand its inhibitory effect on the organism. The experimental nitrate uptake by the cyanobacterium in presence of Cu(II) was further modeled using unstructured kinetic models in order to determine the critical Cu(II) concentration.

2. Methods

2.1. Chemical and reagents

All reagents and chemicals used in this study were of analytical grade and were obtained from either Merck India Ltd or Himedia India Ltd.

2.2. N. muscorum and culture conditions

The cyanobacterium *N. muscorum* used in this study was previously isolated from a toxic metal contaminated site in Meghalaya, India (Hazarika et al., 2015). Details of biochemical and molecular characterization of the strain was earlier reported in Goswami et al. (2014) and Hazarika et al. (2015). This strain was grown in liquid blue-green BG11 media using Erlenmeyer flasks with alternate light and dark periods of 16 h and 8 h, respectively. The growth conditions of *N. muscorum* are as follows: temperature, 25–30 °C; light intensity, 3000–3500 lux (cool white light). The BG11 medium was composed of 1.500 g L^{-1} sodium nitrate; 0.0314 g L^{-1} dipotassium hydrogen phosphate; 0.036 g L^{-1} magnesium sulphate; 0.0367 g L^{-1} calcium chloride dehydrate; 0.020 g L^{-1} sodium carbonate; 0.001 g L^{-1} disodium magnesium EDTA; 0.0056 g L^{-1} citric acid and 0.006 g L^{-1} ferric ammonium citrate. The cyanobacterium *N. muscorum* was routinely cultured and maintained under its metabolically active state by sub-culturing and washing with BG11 medium after every 20 days.

2.3. Photobioreactor and batch cultivation of N. muscorum

Batch cultivation of *N. muscorum* was carried out using a bioreactor with a working volume of 2 L and fitted with a stirrer, pH, Dissolved Oxygen (DO) probes and fluorescent lamps (3 nos.). The schematic of the photobioreactor setup used in the present study is shown in Fig. 1. All experiments with the photobioreactor were performed using axenic cultures of the cyanobacterium *N. muscorum* phototropically grown using BG11 medium. The reactor operating conditions were 25–30 °C temperature; 3000–3500 lux light intensity; 1200 ml min^{-1} airflow rate. For illumination, three 15 W (natural white) fluorescent lamps were fixed outside to the reactor, and the light supply was provided so as to simulate a 16:8 (light: dark) photo period. All batch experiments in this study were conducted at different initial Cu(II) concentration of 5, 10, 20 and 30 mg L^{-1} in the BG11 medium. The Cu(II) stock solution (1 g/L) was prepared using $CuSO_4 \cdot 5H_2O$ in deionized water and a suitable amount of the stock was added to BG11 media to

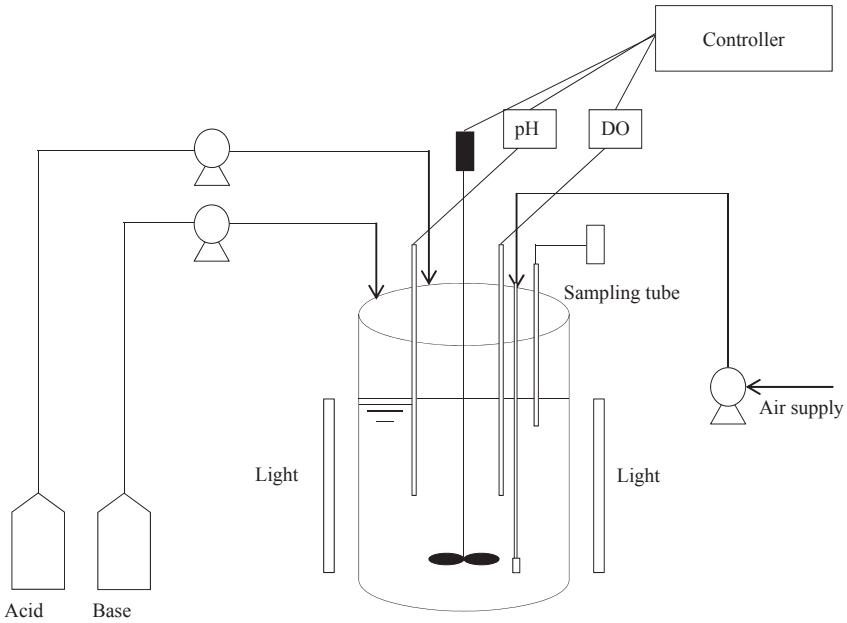

Fig. 1. Schematic of the photobioreactor system used in this study.