Journal of Industrial and Engineering Chemistry 37 (2016) 295–305

Contents lists available at ScienceDirect

Journal of Industrial and Engineering Chemistry

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

Interaction of environmental factors on simultaneous biosorption of lead and manganese ions by locally isolated *Bacillus cereus*

Hassimi Abu Hasan [a,b,*], Siti Rozaimah Sheikh Abdullah [a,b], Noorhisham Tan Kofli [a,b], Shy Jay Yeoh [a,b]

[a] *Department of Chemical and Process Engineering, Faculty of Engineering and Built Environment, Universiti Kebangsaan Malaysia, 43600 UKM, Bangi Selangor, Malaysia*

[b] *Research Centre for Sustainable Process Technology (CESPRO), Faculty of Engineering & Built Environment, Universiti Kebangsaan Malaysia, 43600 UKM, Bangi, Selangor Darul Ehsan, Malaysia*

ARTICLE INFO

Article history:
Received 29 June 2015
Received in revised form 18 March 2016
Accepted 25 March 2016
Available online 1 April 2016

Keywords:
Simultaneous biosorption
Pb and Mn removals
Box–Behnken design
OVAT design
Bacillus cereus

ABSTRACT

In this study, the interaction of pH, biomass and initial concentrations of lead (Pb) and manganese (Mn) ions in simultaneous removal of Pb and Mn were investigated using the Box–Behnken design. The results showed that there was a significant interaction between pH-Pb:Mn concentrations, pH-biomass concentration and Pb:Mn-biomass concentrations. The maximum uptake of Pb and Mn was achieved at pH 7. By increasing the Pb and Mn concentrations from 10 to 50 mg/L, the uptake capacities also increased. By increasing the biomass concentration from 0.3 to 1.5 g/L, the uptakes (g/g) of both metal ions by *Bacillus cereus* I6 was depleted. The precipitation of Pb and Mn on the surface of *B. cereus* I6 cells was confirmed by energy dispersive X-ray (EDX) transmission electron microscopy (TEM), and Fourier transform infrared (FTIR). Thus, simultaneous biosorption of Pb and Mn by isolated I6 strain can be efficiently performed under different interactions of environmental factors.

Introduction

Recently, the usage of heavy metals such as iron (Fe), manganese (Mn), zinc (Zn), lead (Pb), nickel (Ni), chromium (Cr), and arsenic (Ar) has increased dramatically. Without considering the natural geochemical processes, Pb and Mn contaminations are mainly caused by human industrial activities such as mining, electroplating, power industry, fertiliser, petrochemical and waste dumping sites. Pb and Mn can contaminate air, water and soil when released into the environment. These metals are a main problem for producing safe drinking water if their discharged concentration in the river is high. Exposure to lead at low concentration but continuously, can cause a failure of the kidney and liver, Alzheimer's disease, brain and the nervous system deterioration [1], reduction of men and women fertility, and various forms of blood disorders and anaemia [2]. Meanwhile, Mn is highly toxic to living things and the environment, and causes low haemoglobin levels [3], gastrointestinal accumulation [4], and neurotoxicity [5]. Moreover, Mn also causes problems to the water distribution and treatment systems due to the oxidation process which is precipitate Mn [6,7]. With the negative impacts of Pb and Mn on humans and the environment, the contaminants must, therefore, be effectively treated before being released into the environment.

Nowadays, there are several techniques that have been implemented for the removal of Pb and Mn, such as chemical precipitation and electrocoagulation [8], adsorption [9], ion exchange [10,11] and membrane filtration [12]. However, there are a few disadvantages of these technologies such as the high cost for energy and maintenance, and also production of toxic by-products. These drawbacks have led to the studies aimed to develop more effective processes for heavy metals removal. In this study, the biosorption approach using locally isolated *Bacillus cereus* was adopted as an alternative method in removing Pb and Mn.

Biosorption has become a valuable technology for metal removal from wastewater because of its lack of chemical usage [13], high efficiency in detoxifying metal from effluents, and low

* Corresponding author at: Department of Chemical and Process Engineering, Faculty of Engineering and Built Environment, Universiti Kebangsaan Malaysia, 43600 UKM, Bangi Selangor, Malaysia. Tel.: +6 03 89216402; fax: +6 03 89118035.
E-mail addresses: simiabuhasan@gmail.com, hassimi@ukm.edu.my (H. Abu Hasan), rozaimah@ukm.edu.my (S.R. Sheikh Abdullah).

http://dx.doi.org/10.1016/j.jiec.2016.03.038
1226-086X/

operation and maintenance cost. Many bio-based materials have been investigated as biosorbents such as algae, bacteria, yeast and fungi, which are have been found to biosorp Pb and Mn efficiently. An oligosporogenic *B. cereus* is a facultative strain, motile, aerobic spore-former and rod shape. It can grow in either acidic (>pH 4.5) or base (<9.5) conditions, and optimum pH is within pH 6–7. The temperature limits for its growth is 10–48 °C [14]. Due to the unique characteristics of growth conditions, *B. cereus* would be a better option as a biosorbent in treating wastewater, which significantly varies in pH and temperature conditions. This strain is previously studied to have the ability to biosorp Ar [15], silver [16], cadmium [17] and Zn [18]. However, based on our best knowledge, there is still no investigation on simultaneous biosorption of Pb and Mn by *B. cereus*.

Thus for this reason, the main objective of this study was to determine the maximum inhibition concentration of Pb and Mn on locally isolated *B. cereus* growth, to investigate the simultaneous biosorption of Pb and Mn affected by several factors including pH, Pb and Mn concentrations, as well as biomass concentrations, which were tested by the method of one variable at one time (OVAT). The selectivity of Pb and Mn for this study was based on the concentration, where these heavy metals were among the highest in surface water. Based on the conditions studied, the interaction of the factors affecting simultaneous biosorption was simulated using Box–Behnken (BBD) by aiming to maximise the removal of Pb and Mn.

Experimental

Isolation and identification of Bacillus cereus *I6*

The *Bacillus cereus* I6 species was isolated from sewage activated sludge obtained from a mixed culture of a sewage treatment plant located in Putrajaya, Malaysia. The strain was identified previously as one of the species communities that is responsible in the simultaneous removal of ammonia and manganese from drinking water [6]. Approximately 50 mL of sampled was agitated to obtain homogeneous suspensions. One millilitre of the suspensions was transferred into 9 mL of sterile saline water to make a total of 10 mL mixture solution, and was gradually diluted from 10^{-1} to 10^{-5}. Then, 0.1 mL of each dilution sample was spread on nutrient agar (NA) (Oxoid, Hampshire, England) and incubated in a growth chamber (GC 1050, Protech, Malaysia) at 37 °C for 24 to 48 h.

The DNA of *B. cereus* I6 was extracted from a bacterial suspension in nutrient broth that was cultivated at 37 °C for 24 h. The extraction was conducted using Wizard® Genomic DNA Purification Kit (Promega, USA) protocol for isolation of genomic DNA from Gram positive and negative bacteria. Universal primers 27F (AGAGTTTGATCCTGGCTCAG) and 1492R (GGTTACCTTGTTACGACTT) were used to amplify 16S RNA gene according to the polymerase chain reaction (PCR) amplification protocol provided by Promega Manufacture (USA). The PCR was performed using Mastercycler (Epgradient S, Eppendorf, Version 3.608). The PCR-amplified product was purified by Wizard® Plus SV Minipreps DNA Purification System (Promega, USA). The PCR product was sent to First BASE Laboratories Sdn. Bhd (Kuala Lumpur, Malaysia) for the 16S rRNA sequencing. Finally, the result of 16S rRNA sequence of the *B. cereus* I6 was compared with those of other microorganisms by way of BLAST through the National Centre for Biotechnology Information homepage (http://www.ncbi.nlm.nih.gov).

Phylogenetic tree

The 16S rRNA gene sequence of the *B. cereus* I6 strain was aligned with all the sequences available from the GenBank by BLAST. All sequences were retrieved from GenBank individually and aligned using ClustalW packaging in MEGA software (MEGA version 5.2, USA). The phylogenetic analysis was later performed by MEGA version 5 software using the neighbour-joining tree method, which was tested by the Bootstrap method (1000 repetitions).

Screening test on maximum inhibition concentration

The test to determine the maximum inhibition concentration (MIC) of Pb and Mn that inhibited *B. cereus* I6 growth was conducted by exposing the strain in (1) NA containing Pb, (2) NA containing Mn, and (3) NA containing both Pb and Mn. The Pb and Mn concentrations in the NA were varied from 5 to 200 mg/L. After exposing *B. cereus* I6 on the plates, the strain was incubated at 37 °C for 24 h and the growth was observed after incubation was completed.

Harvesting of biosorbent

The isolated *B. cereus* I6 was grown in 250-mL conical flasks containing 150 mL of nutrient broth, and it was cultivated in a shaking incubator (AMBI-100, Protech, Malaysia) at 150 rpm and 37 °C for 24 h. The cells were harvested using a centrifuge (Eppendorf 5804, Germany) at 5000 rpm for 10 min. After three rinses with sterilised distilled water, the biomass was suspended in sterilised distilled water to prepare as the biomass stock solution. The dry weight of the biomass stock solution was determined after drying at 105 °C for 24 h through gravimetric method.

Simultaneous biosorption of Pb and Mn

Simultaneous Pb and Mn biosorption studies were conducted using 250 mL conical flasks with incubation temperature at 37 °C for 24 h and were shaken at 150 rpm. The studies were divided into three parts: (I) the effect of initial pH in range of 3–11 by fixing Pb and Mn concentration at 50 mg/L and biomass concentration at 1.5 mg/L, (II) the effect of Pb and Mn concentrations in range of 10–50 mg/L by fixing pH at 7 and biomass concentration at 1.5 mg/L, and (III) the effect of biomass concentrations in range of 0.3–1.5 mg/L by fixing Pb and Mn concentration at 50 mg/L and pH at 7. The experiments were conducted according to the OVAT method. For part I, the initial pH was adjusted using either 0.1 M NaOH or 0.1 M HNO_3 and measured using a pH meter (CyberScan 510, Singapore). All of the experimental runs were conducted in triplicates. The stock solution of Pb and Mn were separately prepared using lead nitrate, $Pb(NO_3)_2$ (Systerm, ChemAR, Poland) and manganese chloride, $MnCl_2.4H_2O$ (Systerm, ChemAR, Poland), respectively. Samples were taken at periodic intervals and centrifuged (Eppendorf 5804, Germany) at 5000 rpm for 10 min. The supernatant and biosorbent pellet were used for analysis purpose.

Desorption of Pb and Mn

A capability of metals to be desorped from the biosorbent could be a benchmark for the successful biosorption process. In a real application, the biosorption of metal can be desorped and reused for a further cycle. Its can reduce the cost of the process and the dependency on a continuous supply of biosorbent [19]. In this study, desorption of Pb and Mn was performed through biomass digestion. Samples were centrifuged at 5000 rpm for 10 min and the supernatant was collected for analysis purposes. Then, the biomass pellet was rinsed with ammonium acetate ($NH_4C_2H_3O_2$: Systerm, Malaysia) three times. After rinsing, hydrogen peroxide (H_2O_2: R&M, United Kingdom), nitric acid (HNO_3: R&M, United