Journal of Water Process Engineering 12 (2016) 89–104

Contents lists available at ScienceDirect

Journal of Water Process Engineering

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

Sequestration of Zn^{2+} from aqueous solution using *Klebsiella pneumoniae*: Batch kinetics and continuous studies

Subhajit Majumder, Smita Raghuvanshi, Suresh Gupta *

Department of Chemical Engineering, Birla Institute of Technology and Science (BITS), Pilani, Rajasthan 333031, India

ARTICLE INFO

Article history:
Received 17 December 2015
Received in revised form 23 June 2016
Accepted 25 June 2016

Keywords:
Zinc (Zn^{2+})
K. pneumoniae
Bioremediation
Biofiltration
Shock loading

ABSTRACT

The present study was aimed to isolate, *Klebsiella pneumoniae* from activated sludge. It was used to remove Zn^{2+} from aqueous solution in batch and continuous modes. Batch study was conducted for 20–200 mg L^{-1} of initial Zn^{2+} concentration to determine the optimum value of initial Zn^{2+} concentration. Batch bioremediation studies were also performed to determine the optimum values of solution pH, minimal salt medium (MSM) dosage, macronutrients amount, aerobic & facultative anaerobic conditions, temperature and inoculum volume. The optimum solution pH for the growth of *K. pneumoniae* was found as 7 for the Zn^{2+} bioremediation. The growth of *K. pneumoniae* was found optimum in aerobic condition and in the temperature range of 37–40 °C. In continuous mode, the removal of Zn^{2+} from aqueous solution was investigated in a hybrid biofilter column packed with coal and compost as medium over a period of 60 days. *K. pneumoniae* was used as a potential metal removing agent. The maximum removal efficiency was obtained as 91.5% during phase II for 10 mL min^{-1} flow rate of Zn^{2+} and 20 mg L^{-1} of Zn^{2+} inlet concentration. Biofilter column was run under shock loading conditions for 14 days immediately after 60 days of operation which revealed the stable nature of the column with fluctuating inputs. Field emission scanning electron microscopy with electron dispersive X-ray (FESEM-EDX) analysis confirmed the presence of Zn^{2+} and change in surface morphology after Zn^{2+} sorption on the outer surface of *K. pneumoniae*.

1. Introduction

Zinc (Zn) is considered as heavy metal and acts as a micro nutrient for human body. The most stable ionic state of Zn is divalent Zn^{2+}. It plays an important role in maintaining the functions of human beings such as wound healing, cell growth, immune systems etc. Zn^{2+} has antioxidant properties which protects skin from aging. It finds applications in various industrial processes such as manufacturing, paint, rubber, cosmetics etc. However, it is reported that high dosage of Zn^{2+} (80 mg daily) causes malfunctioning of kidney in human beings [1]. Inhalation of freshly formed zinc oxide during galvanization process may induce “zinc chills” in human beings which causes fever, nausea, headache, vomiting and joint pains. At higher concentration level, Zn^{2+} can change enzyme activity, imbalance cellular functions and destroy cell membranes and the structure of DNA [2]. This possess a significant threat to human beings, other living organisms and the environment due to the toxicity and non-biodegradability of Zn^{2+} [3,4]. Since, this metal is used as valuable resources in various industries, its removal; recovery and recycling have greater significance. For the last few decades, adsorption technique have been extensively used for the removal of various heavy metals from wastewater. Mushtaq et al. demonstrated the viability of a biocomposite prepared from sodium (Na)-bentonite and *Eriobotrya japonica* seed biomass for the removal of copper (Cu^{2+}) from aqueous media. Effects of different process variables such as pH, initial (Cu^{2+}) concentration, adsorbent dose, contact time and temperature were studied for the adsorption of Cu^{2+} from aqueous media. Results revealed that the maximum Cu^{2+} adsorption could be achieved at pH 5, initial Cu^{2+} concentration 75 mg L^{-1}, adsorbent dose 0.1 g, contact time 45 min and temperature 45 °C [5]. Desorption studies showed the recyclable nature of the biocomposite. In a separate study, Rashid et al. evaluated the efficiency of fungal dead biomass with bentonite (FBC) for the adsorption of Ni^{2+} and Zn^{2+} from aqueous media. The different process variables were optimized. The maximum adsorption was obtained at pH 6 and 5 for Ni^{2+} and Zn^{2+}, respectively. The optimum values of adsorbent dose, contact time, initial metal ion concentration and temperature were obtained as 0.05 g, 30 min,

* Corresponding author.
E-mail addresses: subhajit@pilani.bits-pilani.ac.in (S. Majumder), smita@pilani.bits-pilani.ac.in (S. Raghuvanshi), sureshg@pilani.bits-pilani.ac.in, sureshg25@gmail.com (S. Gupta).

http://dx.doi.org/10.1016/j.jwpe.2016.06.010
2214-7144/

200 mg L^{-1} and 51 °C, respectively [6]. A comparative study on the biosorption of Pb^{2+}, Cd^{2+} and Zn^{2+} was conducted using lemon grass (*Cymbopogon citratus*) at different physicochemical parameters [7]. Results revealed the fact that the biosorption of each metal ions was dependent on solution pH, contact time, biosorbent dose, initial metal ion concentration and temperature. The biosorption process was suitably described by pseudo-second-order model. In a separate biosorption study, the equilibrium, kinetics, and thermodynamics of Pb^{2+}, Cd^{2+} and Zn^{2+} onto groundnut (*Arachis hypogaea*) shell were investigated at different physicochemical parameters [8]. It was found that the metal biosorption increased with increase in solution pH, biosorbent dose, contact time and initial metal ion concentration. However, extent of biosorption decreased with increase in temperature of the system. Kinetic study showed that the biosorption of metal ions was best described by pseudo-second-order model. Iqbal and Khera investigated Pb^{2+} and Cu^{2+} removal ability of *Fumaria indica* biomass under different initial metal ion concentration. Both metal removal study was carried out in single and binary system. Results revealed that the copper adsorption decreased in binary system while lead adsorption did not change in binary system [9]. The adsorption study for the removal of Pb^{2+} and Cu^{2+} from aqueous solution was conducted using *Rosa bourbonia* waste phyto-biomass (RBWPB) pretreated with organic acids at different operating conditions [10]. It was observed that the bisorption of metal ions depends on biosorbent dose, initial metal ions concentration and contact time. Kinetic study showed that the biosorption of both metal ions was best described by pseudo-second-order model. The metal (Pb^{2+}) removal ability of native and immobilized *Mangifera indica* biomass (MIB) was investigated under different physicochemical parameters such as solution pH, initial concentration, biosorbent dose and contact time [11]. Maximum uptake of Pb^{2+} was obtained at pH 5, biosorbent dose 0.1 g, contact time 240 min for initial Pb^{2+} concentration of 100 mg mL^{-1}. The biosorption of Pb^{2+} was best described by pseudo-second-order kinetic model. It was also observed that the immobilized biomass did not have much impact on the efficiency of Pb^{2+} biosorption.

Bioremediation is an eco-friendly technique which employs living microorganisms for the treatment of Zn^{2+} contaminated environment in contrast to conventional physico-chemical techniques [12]. Microorganisms are ubiquitous and they have different metal tolerance capacity. The advantages associated with the usage of living microorganisms in metal removal include easy availability to treat voluminous amount of contaminated wastewater, self replenishment and natural occurrence [13]. Microorganisms can transform the non-biodegradable Zn^{2+} into non-hazardous or less hazardous biochemical species which can be safely disposed to the environment [14]. Various studies were conducted for the removal of Zn^{2+} using living microorganisms [12,15,16]. However, the selection of microorganisms (bacteria, fungi, yeast etc.) for the remediation of particular heavy metal plays an important role in the successful operation of bioremediation process [13]. The effects of operating conditions for bioremediation of Zn^{2+} such as initial metal concentration, initial pH of the solution, minimal salt medium (MSM) dosage, micronutrient [nitrogen (N)-phosphorus (P)-potassium (K)] quantity in MSM, temperature, aerobic & facultative anaerobic environments and inoculum volume were not reported in earlier studies. The parameters for rate and growth kinetics are important in understanding the microbial mechanism for Zn^{2+} removal which were ignored in earlier studies.

The successful application of bioremediation process lies in its commercial implications. In in-situ applications, microorganisms fixed to a porous medium can be used as a potential candidate for treating the voluminous amount of wastewater contaminated with Zn^{2+}. This technique is known as biofiltration which employs bioremediation process in continuous mode. In this technique, microorganisms are attached with the inert packing materials to form biofilm layer. Toxic metal contaminants which are transported through the wet biofilm layer are taken up by the microorganisms and eventually transformed to non-hazardous compounds. The literature on biofiltration experimental studies revealed that very limited studies have been carried for the removal of Zn^{2+} using bacterial strains in continuous mode. One of the studies reported 97.5% removal of Zn^{2+} (initial concentration: 10 mg L^{-1}) from a multiple metal system (Cu, Zn, Ni, As, Fe, Mg and Al) in a bench scale bioreactor using sand as filter medium and sulfate reducing bacteria (SRB) [17]. The selection of packing material plays an important role in biofilter operation. Sand used in earlier study may cause several operation problems in the biofilter column including back pressure development and poor attachment of biofilm on the surface of sand. In an earlier study, it was reported that matured compost provides good support to the biofilm [18]. Matured compost was amended with coal to provide more compactness in the biofilter bed. A hybrid biofilter system consisting of coal and matured compost as packing materials may be utilized for the removal of Zn^{2+}. Limited literature are available on kinetic modeling of biolfiltration for Zn^{2+} removal. Kinetic modeling of the biofilter column is necessary in designing and understanding the hydrodynamic behavior of the column during the continuous mode of operation. For the successful application of biofilter column in industrial level, the stability of the biofilter operation under fluctuating operating conditions (shock loading) needs to be assessed. Limited studies have been carried out under shock loading conditions.

The present study was aimed to isolate a bacterial strain, *Klebsiella pneumoniae* from activated sludge which was utilized for the bioremediation of Zn^{2+}. The sludge was collected from Pilani Sewage Treatment Plant, Pilani, India. Batch bioremediation studies for Zn^{2+} removal were conducted using the isolated bacterial strain to determine the optimum values of different operating parameters such as initial Zn^{2+} concentration, biomass concentration, nutrient dosage, micronutrient [nitrogen(N)-phosphorus(P)-potassium(K)] amounts in nutrient, pH, temperature and inoculum volume. A hybrid biofilter column was fabricated and used to treat aqueous solution contaminated with Zn^{2+}. The isolated bacterial strain was used as potential metal removing agent. The performance of the column was assessed in terms of removal efficiency and elimination capacity for a period of 60 days. After 60 days, the stable nature of the column was checked by operating the column under fluctuating input conditions for a period of 14 days. The kinetic parameters for the biofiltration of Zn^{2+} were determined using Michaelis-Menten kinetic model. Ottengraf-van-den Oever model with zero-order diffusion limitation was also validated using the biofiltration experimental data.

2. Materials and methods

2.1. Media preparation

Pure and analytical grade chemicals were used throughout the experiments. Stock solution of Zn^{2+} (1000 mg L^{-1}) was prepared by weighing 4.4 g of zinc sulfate ($ZnSO_4 . 7H_2O$) and dissolving in distilled water. Different concentrations of aqueous Zn^{2+} solution (20, 40, 60, 80, 100, 150 and 200 mg L^{-1}) were prepared using the stock solution. One liter of nutrient minimal salt medium (MSM) solution was prepared using specific amount of mineral salts in distilled water [19]. In all the experimental studies, this composition of MSM was taken as standard composition unless otherwise specified. A stock glucose solution (10,000 mg L^{-1}) was prepared by dissolving specific amount of D-glucose (10 g) in 100 mL of distilled water. The nutrient agar (NA) media used for the growth of