Ecological Engineering 37 (2011) 2076–2081

Contents lists available at ScienceDirect

Ecological Engineering

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

Short communication

Statistical modelling and optimization of substrate composition for bacterial growth and cadmium removal using response surface methodology

Divya Bhatia [a], Rajender Kumar [b,*], Rajesh Singh [b], Rout Chadetrik [b], Narsi R. Bishnoi [b]

[a] Université Paris-Est, Laboratoire Géomatériaux et Environnement, EA 4119, 5 bd Descartes, 77454 Marne la Vallée Cedex 2, France
[b] Department of Environmental Science & Engineering, Guru Jambheshwar University of Science and Technology, Hisar 125001, India

ARTICLE INFO

Article history:
Received 21 May 2011
Received in revised form 14 July 2011
Accepted 7 August 2011
Available online 6 September 2011

Keywords:
Substrate composition
Operating variables
A. xyloxidans
Sorption
Response surface methodology (RSM)
Cadmium

ABSTRACT

In this study, substrate composition was optimized for the growth of *Achromobacter xyloxidans* and biosorption of Cd(II) from aqueous solution. Response surface methodology (RSM) was used to investigate the function of three independent operating variables, namely, peptone (2.5–10 g/L), beef extract (2.5–5.0 g/L) and incubation time (24–96 h), on dependent variables, i.e. sorption of Cd(II) ions, protein content and biomass growth of *A. xyloxidans*. The maximum Cd(II) removal efficiency of 69.2%, protein content 1.9 mg/L and growth 0.354 optical density was found at optimal conditions of peptone 10 g/L, incubation time 60 h and beef extract 2.5 g/L. The significance of independent variables and interactions between variables were tested by means of the analysis of variance (ANOVA) with 95% confidence limits and values of "Prob > *F*" less than 0.0500 indicate that model terms are significant. Fourier transfer infrared (FTIR) analysis was used to investigate sorption mechanism and involved functional groups in Cd(II) binding.

1. Introduction

The increase of industrial activities has intensified environmental pollution problems and the deterioration of several ecosystems with the accumulation of many pollutants, such as toxic metals (Zouboulis et al., 2004). The retention of heavy metal at high concentrations in the environment exerts toxic effect on fauna and flora (Xue et al., 2010) such as loss of ecosystem and agriculture productivity, diminished food chain quality, and tainted water resources. Cadmium is among the heavy metals regarded as having high toxicity. On the basis of the toxicity, persistence and bioaccumulation, Cd(II) has been proposed in the red list of priority substances under the Department of Environment, UK (1991). Since microorganisms have developed survival strategies in heavy metal polluted habits, their different microbial detoxifying mechanisms such as bioaccumulation, biotransformation, biomineralization or sorption can be applied either ex situ or in situ to design economical bioremediation processes (Beveridge and Doyle, 1989; Lim et al., 2003; Umrania, 2006; Sasmaza et al., 2008; Kumar et al., 2008; Hanif et al., 2009; Lawal et al., 2010). Generally, the sorption mainly utilizes the various types of functional groups like carboxyl, phosphoryl, sulfhydryl, hydroxyl, amino, etc. (Sharma et al., in press) which are present on the surface of living or dead microorganisms to remove the heavy metals from aqueous solutions.

Response surface methodology is a combination of mathematical and statistical techniques used for developing, improving and optimizing the processes and used to evaluate the relative significance of several affecting factors even in the presence of complex interactions (Myers and Montgomery, 2002). Recently, this methodology is being used for the optimization of various biological processes such as enzyme production (Senthilkumar et al., 2005), hydrogen production (Ghosh and Hallenbeck, 2010), secondary metabolite production (Lofty et al., 2007) and dye decolorization by *Nostoc linckia* (Sharma et al., in press). Utilization of this tool for the optimization of process parameters for heavy metal removal from wastewater has also used to optimize the temperature, pH and metal concentration (Singh et al., 2010). In this study, three variables were optimized, i.e. peptone concentration, beef extract and incubation time, and used to evaluate the single and interactive effects of the variables on responses. FTIR study was carried out to understand surface properties and available functional groups involved in sorption mechanism.

2. Materials and methods

2.1. Experimental set up for batch study

The bacterial strain *Achromobacter xyloxidans* used for this study was isolated aerobically in 100 ml sterile nutrient broth medium at pH 7.0, temperature 35 ± 1 °C for 48 h on shaking incubator

* Corresponding author. Tel.: +91 1662263321.
E-mail address: rkumar.gju@gmail.com (R. Kumar).

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doi:10.1016/j.ecoleng.2011.08.014

Table 1
Experimental design and results of the responses.

Experiments run	Incubation time (h)	Peptone (g/L)	Beef extract (g/L)	% Removal	Protein (mg/L)	Growth (OD 620 nm)
1	96	2.5	3.75	24.07	1.69	0.226
2	96	6.25	2.5	48.2	1.62	0.115
3	60	2.5	2.5	26.93	1.39	0.098
4	60	6.25	3.75	65.95	1.74	0.265
5	60	2.5	5.0	23.87	1.43	0.116
6	24	2.5	3.75	19.67	1.23	0.074
7	24	6.25	5.0	37.65	1.37	0.094
8	96	6.25	5.0	45.33	1.45	0.101
9	60	10	2.5	69.17	1.87	0.354
10	24	6.25	2.5	42.85	1.31	0.093
11	60	6.25	3.75	65.65	1.74	0.265
12	24	10	3.75	52.98	1.4	0.098
13	60	6.25	3.75	64.73	1.76	0.267
14	60	6.25	3.75	64.35	1.87	0.336
15	96	10	3.75	68.12	1.81	0.323
16	60	6.25	3.75	64.89	1.87	0.336
17	60	10	5.0	61.45	1.73	0.243

ANOVA for RSM model: Cd(II) removal: $F_{model} = 5005.96$ ($P > 0.0001$, df = 9); $F_{Lack\ of\ Fit} = 6.97$ ($P > 0.0713$, df = 3); $R^2 = 0.9983$; protein content: $F_{model} = 0.68468$ ($P > 0.0093$, df = 9); $F_{Lack\ of\ Fit} = 0.05883$ ($P > 0.0985$, df = 3); $R^2 = 0.8985$; bacterial growth: $F_{model} = 0.144294$ ($P > 0.00497$, df = 9); $F_{Lack\ of\ Fit} = 0.024519$ ($P > 0.0665$, df = 3); $R^2 = 0.8257$.

at speed 120 rpm from electroplating industrial soil. For isolation, inoculated plates were incubated at 37 °C for 72 h. The pure colony was obtained and identified from microbial type culture collection (MTCC), Institute of Microbial Technology, Chandigarh.

The experiments were conducted to optimize pH, temperature, Cd(II) ions concentration and incubation time for sorption of Cd(II) ions and growth of bacterial sp. *A. xyloxidans* in batch process. For pH optimization, 50 ml of nutrient media with varying pH range from 1.0 to 9.0 at 100 mg/L concentration of Cd(II) ions were incubated at 34 °C for 48 h. The pH value of solution was adjusted using 0.1 M HCl or 0.1 M NaOH solution. Effect of temperature was studied from 25 to 45 °C at initial metal ions concentration 100 mg/L for 48 h. Similarly initial metal ions concentration was optimized with varying concentration from 20 to 120 mg/L, keeping the optimum pH and temperature from above experiments. For incubation time, bacterial growth (optical density OD) was monitored at regular interval time 8, 16, 24, 32, 40, 48, 56, 64 and 72 h at 620 nm using UV-spectrophotometer. After a fixed interval of time, 10 ml culture sample was withdrawn and centrifuged for 20 min. Supernatant and pellets were taken separate. The pellets were kept in oven at 70 °C till the constant weight was attained (Chubar et al., 2008). After centrifugation, supernatant was used for analysis of protein content. The total protein content in the solution was estimated by Lowry's method (Lowry et al., 1951). After acid digestion (HNO_3 + HCl), removal of Cd(II) ions was analysed using atomic absorption spectrophotometer (GBC-932 Plus). The experiments were performed in duplicate. Uptake capacity and % removal of metal ions were calculated according to equations given in previous study (Kumar et al., 2008).

2.2. RSM and statistical analysis

Design Expert software (Stat Ease, 7.13 trial version) was used for experimental design towards construction of a quadratic model (Table 1). Three independent variables i.e. peptone (2.5–10.0 g/L), incubation time (24–96 h), and beef extract (2.5–5.0 g/L) were taken to obtain responses removal of Cd(II) ions, growth of bacterial strains and protein content secreted by bacterial strains at pH 7.0, temperature 35 °C and Cd(II) ion concentration 100 mg/L. The quadratic equation model for predicting the optimal point was expressed according to equations, a three parameters were varied, 10 coefficients has to be estimated, i.e. coefficients for the three main effects, three quadratic effects, three interactions and one constant (Reddy et al., 2008):

$$Y_i = a_0 + \sum a_i X_i + \sum a_{ii} X_{ii}^2 + \sum a_{ij} X_i X_j + e \quad (1)$$

where Y_i ($i = 3$) is predicted response i.e. sorption of Cd(II) ions with bacterial strain, a_0 is the constant coefficient, a_i is ith linear coefficient, a_{ii} is ith quadratic coefficient and a_{ij} is different interaction coefficients of the model; X_i X_j are coded independent variables related to factors, and e is error of model. However, in this study, the independent variables were coded as A, B and C. Thus, the second order polynomial equation can be presented as follows:

$$Y = a_0 + a_i A + a_i B + a_i C + a_i A^2 + a_i B^2 + a_i C^2 + a_{ij} A * B + a_{ij} A * C + a_{ij} B * C \quad (2)$$

In this study, Cd(II) removal, protein content and growth was preceded for Eq. (2) including ANOVA to obtain interaction between process variables and the responses. The quality of fit of polynomial model was expressed by the coefficient of determination (r^2) and statistically significance by F-test in the programme.

2.3. Fourier transform infrared spectroscopy (FTIR)

FTIR spectrum study was carried out to explain sorption mechanism for identifying the presence of functionalities of the bacterial biomass. The sample preparation, technical detail of the instrument has already been mention in our earlier study (Singh et al., 2010).

3. Results and discussion

3.1. Effect of operating parameters on bacterial growth and Cd(II) removal

Growth curves of the bacterial isolate in different pH (1.0–9.0) were plotted and indicate that the cells were grown well at pH 7.0 and decrease at lower and higher pH range (Fig. 1(A)). Maximum removal of Cd(II) was observed 68.5% during the incubation time 48 h at pH 7.0. The medium pH affects the solubility of metals and the ionisation state of the functional groups (carboxylate, phosphate and amino groups) of the microbial cell (Bishnoi and Garima, 2005). The optimum temperature is 35 °C at which maximum bacterial growth was noticed at pH 7.0, incubation time 48 h