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U(VI) biosorption by bi-functionalized *Pseudomonas putida* @ chitosan bead: Modeling and optimization using RSM

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ABSTRACT

In this work, *Pseudomonas putida* cells immobilized into chitosan beads (PICB) were synthesized to investigate the impact of microorganism entrapment on biosorption capacity of prepared biosorbent for U(VI) biosorption from aqueous solutions. Response Surface Methodology (RSM) based on Central Composite Design (CCD) was utilized to evaluate the performance of the PICB in comparison with chitosan beads (CB) under batch mode. Performing experiments under optimal condition sets viz. pH 5, initial U(VI) concentration 500 mg/L, biosorbent dosage 0.4 g/L and 20 wt.% bacterial cells showed that the observed biosorption capacity enhanced by 1.27 times from 398 mg/g (CB) to 504 mg/g (PICB) that confirmed the effectiveness of cells immobilization process. FTIR and potentiometric titration were then utilized to characterize the prepared biosorbents. While the dominant functional group in the binding process was $-NH_3^+$ (4.78 meq/g) in the CB, the functional groups of $-NH_3^+$, $-NH_2$, $-OH$, $-COOH$ (6.00 meq/g) were responsible for the PICB. The equilibrium and kinetic studies revealed that the Langmuir isotherm model and the pseudo–second-order kinetic model were in better fitness with the CB and PICB experimental data. In conclusion, the present study indicated that the PICB could be a suitable biosorbent for uranium (VI) biosorption from aqueous solutions.

1. Introduction

A surge in anthropogenic activity has unfortunately generated a large amount of effluent containing heavy metals. Heavy metal pollution has posed a serious challenge related to the ecosystem and human health due to their toxicity, non-degradability, persistence and accumulation nature [1]. Among the most frequent heavy metals, uranium is well known to pollute the environment through nuclear activities such as mining, processing, waste disposal and etc. which also possess radiochemical and toxicological effects. Excessive amounts of uranium can cause nephritis in human beings and due to its carcinogenicity causes bone cancer [2]. Therefore, the removal of uranium from waste is a hot topic in the environmental research field.

In the last few years, the application of biotechnology for cleaning-up heavy metal-bearing solution is rapidly growing due to environmentally-benign nature, no secondary pollution and low-cost. Therefore, treatment of industrial wastewater has received significant attention [3]. Particularly, biosorption which utilizes various certain biological materials has been heralded as a promising passive process for use in metal removal and/or recovery from aqueous solution [4,5]. Various types of bio-based material derived from bacteria, fungi, yeast and algae can be applied to sequester metal species out of dilute complex solutions quickly with high efficiency [6].

As the most broadly distributed microorganisms in nature, bacterial biomass can be applied as biosorbent for the heavy metals removal from aqueous solutions. Bacteria depict one of the most common naturally taking place biotic lives from worldwide. Their cell membranes contain numerous functional groups such as phosphate, carboxyl and amide that may have the potential to bind to metal with various degrees of affinity. In addition, bacteria have high surface-to-volume ratio thanks to their small size. They can tender a significant fraction of the reactive surface. [2,4]. Similar to crustacean waste, bacterial biomass of the common bacteria including *Agrobacterium*, *Arthrobacter*, *Bacillus*, *Micromonospora*, *Pseudomonas* and *Streptomyces* can be considered as economical biosorbents due to their easiness of their culture and minimal nutritional requirements [4].

Unfortunately, application of microbial biomass in its free form for industrial usage as a commercial biosorbent has been obstructed owing to several technical drawbacks such as the small particle size and poor mechanical strength. These may create difficulties in solid-liquid post-separation, high pressure drop in column

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Table 1
The CCD design matrix of four factors along with experimental and predicted U(VI) biosorption capacity.

Variables	Units	Symbols	Levels				
			− ∝	−1	0	+1	∝
pH	–	A	2	3	4	5	6
Initial concentration	mg/L	B	100	200	300	400	500
Biosorbent dosage	g/L	C	0.40	1.05	1.70	2.35	3.00
Bacteria wt.%	–	D	0.00	7.50	15.00	22.5	30.00

Run	Standard order	Coded levels of variables				U(VI) biosorption capacity (mg/g)		
		A	B	C	D	Observed	Predicted	Residual
1	22	0	0		0	98.81	85.04	13.77
2	29	0	0	0	0	175.02	175.72	−0.71
3	12	1	1	−1	1	377.85	349.83	28.02
4	20	0		0	0	222.12	230.69	−8.57
5	10	1	−1	−1	1	188.49	172.27	16.23
6	28	0	0	0	0	170.58	175.72	−5.15
7	2	1	−1	−1	−1	160.27	166.45	-----6.18
8	17		0	0	0	104.54	80.73	23.81
9	8	1	1	1	−1	141.40	136.71	4.69
10	15	−1	1	1	1	137.70	124.16	13.54
11	6	1	−1	1	−1	78.71	63.91	14.80
12	18	2	0	0	0	165.36	200.75	−35.39
13	26	0	0	0	0	174.15	175.72	−1.58
14	3	−1	1	−1	−1	163.94	164.13	−0.18
15	1	−1	−1	−1	−1	103.94	97.85	6.10
16	9	−1	−1	−1	1	109.83	103.66	6.17
17	14	1	−1	1	1	83.27	69.73	13.54
18	25	0	0	0	0	173.97	175.72	−1.76
19	7	−1	1	1	−1	73.25	85.29	−12.04
20	30	0	0	0	0	173.78	175.72	−1.95
21	19	0		0	0	55.54	58.55	−3.01
22	13	−1	−1	1	1	74.62	96.53	−21.91
23	4	1	1	−1	−1	327.55	310.95	16.60
24	5	−1	−1	1	−1	79.54	90.71	−11.17
25	21	0	0		0	253.13	266.41	−13.29
26	16	1	1	1	1	170.18	175.59	−5.41
27	27	0	0	0	0	174.74	175.72	−0.98
28	23	0	0	0		118.71	119.26	−0.55
29	24	0	0	0		152.92	163.95	−11.03
30	11	−1	1	−1	−1	186.40	203.00	−16.60

mode and significant loss of mass after regeneration [5]. Therefore, researches focusing on new technologies for heavy metals removal are required. However, cell immobilization into a carrier has gained increasing attention for its advantages including enhanced mechanical strength, rigidity, porosity and reusability. It also facilitates separation of the biomass from treated solutions. In addition, immobilization allows a higher biomass loading, minimal clogging under continuous-flow mode and high resistance to harsh media [7].

The selection of proper carrier is very important for use in immobilization. Ideally, the carrier material could be biodegradable, available in large quantities, low cost, and have appropriate physical properties to allow sufficient aggregation of specific microbial cells [8].

The carrier materials that have been employed comprise calcium alginate, sawdust, orange peel cellulose, polyurethanes, chitosan etc. [9]. Compared to various carrier materials used in adsorption studies, the natural biopolymer chitosan that is obtained from chitin, the second most abundant polymer next to cellulose, is attractive to be utilized industrially. Thanks to presence of amino ($-NH_2$) and hydroxyl ($-OH$) functional groups in its structures, chitosan: (i) is a cationic polyelectrolyte (pK_α 6.3–7.0), (ii) is soluble in aqueous acidic media, (iii) forms water insoluble complexes with anionic polyelectrolytes and (iv) chelates metal ions [10]. Furthermore, due to the physical and chemical properties of chitosan, it can be easily formed into the membrane, bead, and fiber forms, all of which could have widespread application as effective biosorbent in sorption processes [5]. Therefore, it can play dual-function as an ideal candidate for cells immobilization and adsorbent simultaneously.

Generally, the biosoprtion process is affected by various factors including, pH value, initial concentration of metal ions (uranium in the present study), contact time, sorbent dosage etc. So, utilization of inexpensive biosorbent material which is accompanied by identification of optimal combination of influencing factors is essential to design an efficient adsorption system [9,11]. Therefore, in recent years, statistical-based design and experiments have been used to specify the process modeling, characterization, and optimization of process. For example, biosorptions of Cu(II) [11], Cr(VI) [12], Cd [13] and Pb [14] by flax meal, spent *cyanobacteria*, *Saccharomyces cerevisiae*, *Aspergillus niger*, respectively, were optimized using RSM.

In this present research, the cells of *Pseudomonas putida*, a highly solvent-tolerant and metal-binding bacterium, were immobilized into chitosan, a dual-function biopolymer, to construct a bi-functional biosorbent for biosorption of U(VI) from aquatic solutions. Then, the biosoprtion performance of the PICBs (with various weights of bacterial cells loaded) was evaluated by batch experiments and compared to the CB. The effects of pH value, initial U(VI) concentration, biosorbent dosage and bacteria wt.% into biosorbent on biosorption were assessed using RSM based on the CCD. After determining the process kinetics, the biosoprtion results were analyzed using isotherm models. Moreover, the biosorbents were characterized by Fourier transform infrared spectroscopy (FTIR) and potentiometric titration.