*Corynebacterium glutamicum*을 이용한 Nickel(II)의 흡착 연구

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Investigation of nickel(II) biosorption onto *Corynebacterium glutamicum*: batch and column studies

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1. Introduction

Waste biomass production from fermentation industries increases every year and its disposal has been a central issue in Korea for many years. Ocean dumping, land fill and incineration have been the most widely used methods for the disposal of these waste biomasses [1]. Alternatively, the fermentation wastes can be used for several applications, thereby increasing its value. *Corynebacterium glutamicum*, a gram positive bacterium used in the present study, is widely used for the biotechnological production of amino acids. Hence, it can be obtained in huge quantities for no/less cost from fermentation industries. Thus, this study explores the possible utilization of fermentation waste (*C. glutamicum*) for the removal of nickel(II) ions from aqueous solutions in batch and up-flow column mode of operations.

2. Materials and methods

The fermentation wastes (*C. glutamicum*) were obtained in the form of a fine powder from Daesang Corporation (Kunsan, Korea). For immobilization of the biomass, a 9% (w/v) solution of polysulfone was prepared in *N, N*-dimethyl formamide solution. After stirring the mixture for 10 h, biomass (14%) was mixed with the polysulfone slurry and the resulted slurry was dripped in deionized water, where beads (PIC) are formed by a phase inversion process.

Titration of biosorbent was carried out at constant temperature (25^0C) using 50 mL plastic bottles (high-density polyethylene) comprises of 30 mL CO_2 stripped water and 0.3 g biomass. A certain amount of 1 N HNO_3 or NaOH was added to each of the biomass suspensions. The air-tightened bottles were then agitated at 160 rpm and allowed to equilibrate for 24 h. Thereafter, the equilibrium pH was measured using an electrode (Ingold). Infrared spectrum of *C. glutamicum* sample was analyzed using Fourier transform infrared spectrometer (FT/IR-Nicolet NEXUS-470).

Free biomass (0.1 g) or wet PIC beads (1 g) were contacted with 40 mL of desired Ni(II) concentrations in a 50 mL plastic bottle (high-density polyethylene). The pH of the solution was initially adjusted and controlled using 0.1 M HCl or NaOH. The bottles were then kept in an incubated rotary shaker at 160 rpm and 25^0C. After equilibrium attainment, the supernatant was separated and analyzed for nickel concentrations [2], after appropriate dilution.

A glass column (1.5 cm ID and 25 cm height) was packed with 19.7 g (wet weight)/4.4 g (dry weight) of PIC to yield a bed height of 20 cm. The column was then fed with 100 mg/L (pH 6) of Ni^{2+} solution in an up-flow mode, at a flow rate of 1 mL/min using a peristaltic pump. The samples were collected at the exit of the column, and the Ni(II) concentrations then analyzed.

3. Results and discussion

3.1. Potentiometric titration and FT-IR

The biomass titration data of *C. glutamicum* is reported in Fig. 1. To describe the titration curve, a proton-binding model as proposed by Yun et al. [3] was used in this study. The model assumes the presence of four binding sites. To find the number (b_j) and nature of binding sites, the model was fitted to the titration cure (Fig. 1) using the Marquardt-Levenberg non-linear regression algorithm in Sigma Plot (version 4.0, SPSS, USA) software. Three negative (pK_H = 2.47±0.09, 4.90±0.09 and 6.82±0.05) and one positive (pK_H = 10.35±0.08) functional groups were predicted by the proton-binding model with high correlation coefficient of 0.992. The first functional group (pK_H = 2.47±0.09) was relatively unknown; however nearly 2.57±0.34 mmol/g was present in *C. glutamicum* biomass. The number of carboxyl groups (pK_H = 4.90±0.09) was found to be 0.41±0.02 mmol/g according to the proton-binding model. The third group, whose pK_H and b_j values were 6.82±0.05 and 0.63±0.02 mmol/g, respectively, can be assigned as phosphonate groups ($B\text{-}HPO_4^-$) or dicarboxylic groups. The last functional group (pK_H = 10.35±0.08) seemed to be amine groups ($B\text{-}NH_3^+$) that generally shows pK_H values between 8.5 and 11 depending on the biomaterial. Also, the amine groups were found to be the abundant in *C. glutamicum* (b_j = 3.85±0.35 mmol/g).

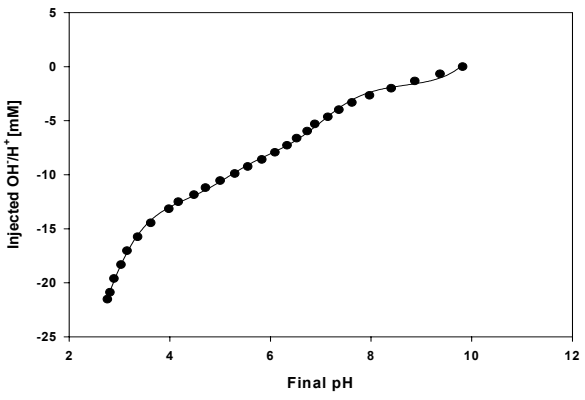

Fig. 1. Potentiometric titration curve

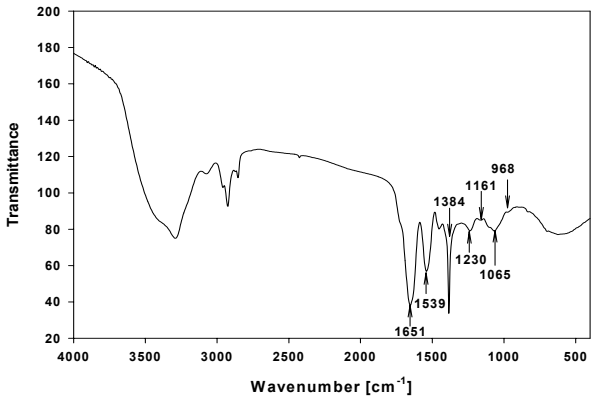

Fig. 2. FT-IR spectra of *C. glutamicum*

To better understand the nature of the functional groups present in the *C. glutamicum*

biomass, the FT-IR spectrum was obtained (Fig. 2). The broad absorption band in the range of 3600 ~ 3000 cm^{-1} indicated the existence of the amine groups and –OH in the carboxyl group. A medium strength absorption peak at 1384 cm^{-1} can be assigned to the symmetrical stretching of the carboxylic acid. The phosphonate groups show some characteristic absorption peaks around 1161 cm^{-1} (P=O stretching), 1065 cm^{-1} (P-O-C stretching), and 968 cm^{-1} (P-OH stretching). The absorption peaks around 1651 (N-H bending band) and 1539 cm^{-1} (H-N-C stretching) are indicative of the existence of amine groups. Thus, the FT-IR spectrum supports the presence of carboxyl, phosphonate and amine groups in *C. glutamicum*.

3.2. pH edge

In the present study, pH edge experiments (Fig. 3) revealed that pH > 4 favored nickel(II) biosorption. The negatively charged functional groups such as carboxyl and phosphonate can attract and bind positively charged nickel ions. For instance, the *pKa* value of carboxylic group was earlier identified as 4.9. Therefore, they have negative charges at pHs approximately higher than 4.9; and thereby attract positively charged Ni^{2+} ions. The reason for low Ni(II) uptake at strong acidic conditions was due to the competition between protons and nickel ions in occupying the binding sites. The nickel(II) uptake increases with increase in pH; however control experiments revealed that nickel tend to precipitate at pH ≥ 7. Therefore, pH 6 was identified as the optimum pH condition for nickel biosorption onto *C. glutamicum*. The polysulfone-immobilized *C. glutamicum* (PIC) also exhibited a similar trend as that of the free biomass, with maximum uptakes at pH ≥ 6.

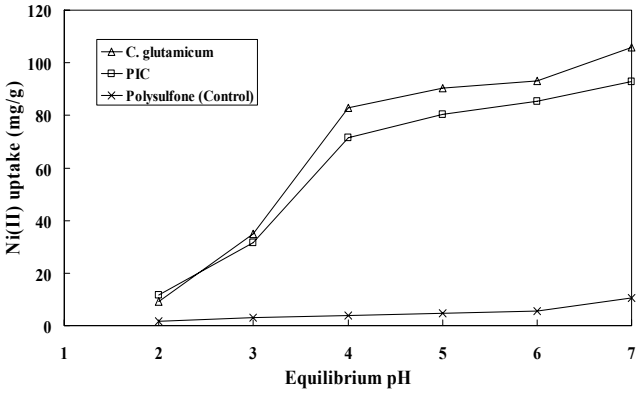

Fig. 3. Effect of solution pH on Ni(II) biosorption

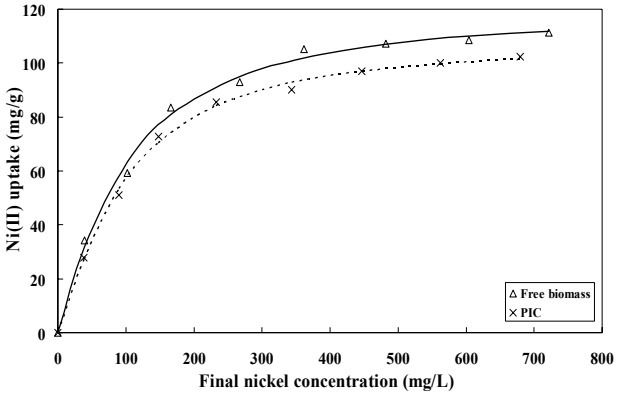

Fig. 4. Ni(II) biosorption isotherms

3.3. Biosorption isotherms and modeling

To evaluate the maximum biosorption potential of *C. glutamicum*, isotherm experiments were conducted at pH 6. Typical L-shaped biosorption isotherms were observed for both the free and immobilized forms of *C. glutamicum* (Fig. 4). The ratio between the Ni^{2+} concentration remaining in solution and biosorbed on the solid decreases when Ni^{2+}

concentration increases, providing a concave curve with strict plateau. As expected, high Ni^{2+} uptake was observed in the case of free *C. glutamicum*.

In order to investigate the biosorption isotherms, the Langmuir model was used in the present study. The Langmuir model served to estimate the maximum dye uptake (Q_{max}) values where they could not be reached in the experiments. The constant *b* represents affinity between the sorbent and sorbate. The maximum nickel uptake values were recorded as 130.4 and 120.9 mg/g for free biomass and PIC, respectively; whereas *b* values were determined as 0.0094 and 0.0089 L/mg in the case of free biomass and PIC, respectively.

3.4. Biosorption in an up-flow packed column

Continuous biosorption studies are utmost important to evaluate the technical feasibility of the process for real applications. Considering this, a PIC-loaded up-flow packed column was devised and employed in this study for the biosorption of nickel(II) ions. The PIC column bed performed well in Ni^{2+} biosorption, with the breakthrough (1 mg/L in the effluent) appeared only after 8 h of column operation. Thereafter, the PIC bed still performed well, and resulted in a smooth breakthrough curve, which finally became exhausted (99.2 mg/L in the effluent) after around 32 h of column operation. The column uptake and percentage removal were calculated to be 26.1 mg/g beads (48.1 mg/g biomass) and 60.4%, respectively.

4. Conclusions

The current study has demonstrated the feasibility of utilizing fermentation wastes for the removal of nickel(II) ions from aqueous solutions. Potentiometric titration and FT-IR studies revealed that the cell wall of *C. glutamicum* comprises of carboxyl, phosphonate and amine groups. With the aid of pH edge data, the negatively charged groups were confirmed to be responsible for binding Ni^{2+} ions. The performance of PIC-loaded up-flow packed column was effective with high Ni(II) uptake (48.1 mg/g) and % Ni(II) removal (60.4%) observed at 1 mL/min. Thus, polysulfone showed competitive properties for bacterial biomass immobilization and may have potential in process applications due to its good physical and chemical stabilities.

5. References

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