

Alginate와 polysulfone으로 고정화된 *Corynebacterium glutamicum*을 이용한
Reactive black 5 염료의 흡착

K. VIJAYARAGHAVAN, 윤영상*
전북대학교 환경화학공학부
(ysyun@chonbuk.ac.kr*)

**Immobilization of *Corynebacterium glutamicum* in Alginate and Polysulfone Matrix for
Biosorption of Reactive Black 5**

K. VIJAYARAGHAVAN, YEOUNG-SANG YUN*

Division of Environmental and Chemical Engineering, Research Institute of Industrial Technology,
Chonbuk National University
(ysyun@chonbuk.ac.kr*)

1. Introduction

Industrial waters polluted with dyes, which are causing serious concerns, can be efficiently and possibly treated using biomaterials. Biosorption, a process which employs inactive/dead biomaterials to biosorb different organic and inorganic constituents, have been identified by several investigators [1,2] as a low cost and efficient technology. Because of the fragile nature of microbial biomass, its application is often limited in industries. Immobilization of microorganisms in a polymeric matrix can be a suitable solution. The polymeric matrix determines the mechanical strength and chemical resistance of the final biosorbent particle [3]. Therefore, in this study two polymeric matrices were investigated, which include sodium alginate and polysulfone.

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. Reactive black 5 (RB5) is employed in the present study as a model dye to elucidate the biosorption performance of immobilized *C. glutamicum*.

2. Materials and methods

2-1 Dye and preparation of biosorbent

RB5, C₂₆H₂₁N₅Na₄O₁₉S₆, was purchased from Sigma-Aldrich Korea Ltd. (Yongin, Korea); had a molecular weight of 991.82. The fermentation wastes (*C. glutamicum* biomass) were obtained in a dried powder form from a lysine fermentation industry (BASF-Korea, Kunsan, Korea).

Alginate solution of 2% (w/v) was prepared by dissolving 1 g of sodium alginate in 50 ml of hot distilled water (60°C). The slurry was then cooled to room temperature (25°C) and then biomass (4%) was added under stirring condition. The alginate-biomass slurry was then extruded into 0.1 M CaCl₂ solution and beads were formed.

A 9% (w/v) solution of polysulfone was prepared in *N, N*-dimethyl formamide (DMF) 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 are formed by a phase inversion process.

2-2 Biosorption studies

Biosorption experiments were conducted by contacting biomass/beads with 40 ml dye solution at desired pH in 50 ml plastic bottle (high-density polyethylene) kept on a rotary shaker at 160 rpm. The pH of the solution was initially adjusted and controlled during experiments using 0.1 M HCl or 0.1 M NaOH. After 16 h, the biosorbent was separated from dye solution by centrifugation at 3000 rpm for 5 min. The dye (RB5) concentration in the supernatant was determined using spectrophotometer (UV-2450, Shimadzu, Kyoto, Japan) at 597 nm.

2-3 Desorption studies

After biosorption, the dye-loaded biosorbent, which was exposed to 500 mg l⁻¹ of RB5 solution at pH 1 and temperature 25°C, was separated from solution by centrifugation. The biosorbent was then contacted with 20 ml of 0.01 M NaOH for 2 h on a rotary shaker at 160 rpm. The remaining procedure was the same as that employed in the biosorption equilibrium experiments. After desorption, the biosorbent was washed extensively with deionized water and used for next cycle.

3. Results and discussion

3-1 Effect of pH

The effect of pH on RB5 biosorption is presented in Fig. 1. The free, alginate-immobilized and polysulfone-immobilized biomass exhibited RB5 uptakes of 197.1, 99.5 and 85.3 mg g⁻¹ at pH 1, and further increase in pH resulted in decreased biosorption performance. Better performance at low pH conditions may be due to the protonation of amino groups. Our previous study identified amino groups of *C. glutamicum* are mainly responsible for reactive dye biosorption [4]. Most common amino acids have isoelectric points in the pH range 5-6. Thus, it is expected that amino groups in the biomass will be protonated at acidic pH values and thus the biomass will have a net positive charge. On the other hand, reactive dyes release colored negatively charged dye anions in solution, which will exhibit electrostatic attraction towards the positively charged cell surface. In general, hydrogen ion acts as a bridging ligand between the bacterial cell wall and the dye molecule. Polysulfone-immobilized biomass performed well as its removal efficiency was greater than 97% at pH 1.

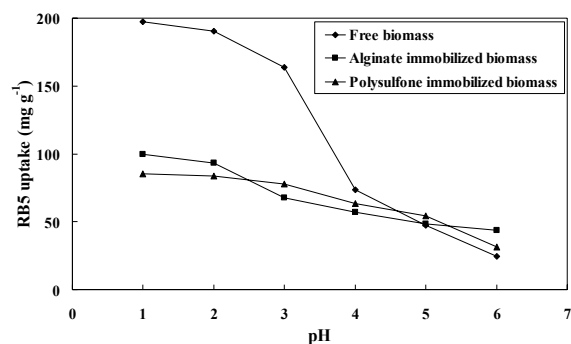


Fig. 1. Effect of pH on RB5 uptake by free and immobilized *C. glutamicum* biomass.

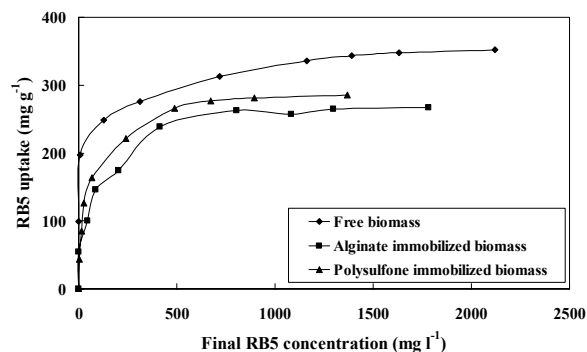


Fig. 2. Biosorption isotherms at pH 1 for free and immobilized *C. glutamicum*.

3-2 Biosorption isotherm

Experimental RB5 biosorption isotherms for free and immobilized *C. glutamicum* at pH 1 are presented in Fig. 2. All biosorption isotherms were hyperbolic as the RB5 uptake capacity reached complete saturation. Comparing the three forms of *C. glutamicum*, the isotherm of free biomass was the steepest and indicated highest affinity of RB5 to freely exposed binding sites. In general, the steepness of the isotherm generally indicates the degree of affinity of the sorbate towards sorbent [3]. Polysulfone immobilized biomass also performed very well and its RB5 uptake is comparable to that of free biomass. However, alginate beads exhibited relatively low RB5 uptake and its affinity towards the sorbate is the least.

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 Q_{max} values were in the order of 351.9, 282.1 and 290.7 mg g^{-1} ; whereas constant b was recorded as 0.033, 0.011 and 0.024 l mg^{-1} for free, alginate-immobilized and polysulfone-immobilized biomass, respectively. For favorable biosorption, high Q_{max} and a steep initial isotherm slope (i.e. high b) are desirable.

3-3 Desorption and regeneration

If the biosorption process is to be used as an alternative in wastewater treatment scheme, the regeneration of the biosorbent is crucial for keeping the process costs down. The elutant, 0.01 M NaOH, performed well in the case of free and immobilized *C. glutamicum*, exhibited elution efficiencies greater than 99%. In addition to the considerable weight loss (approximately 42%), the state of free biomass after desorption was very critical, as it become very difficult to separate the biomass from the final elution solution. In the case of alginate-immobilized biomass, the beads were collapsed during contact with the alkaline elutant. This may be due to the tendency of alginate to reswell in an alkaline environment. In contrary, polysulfone beads were stable at all conditions

examined. Considering the good stability and ease in dye desorption, polysulfone beads were employed in regeneration experiments.

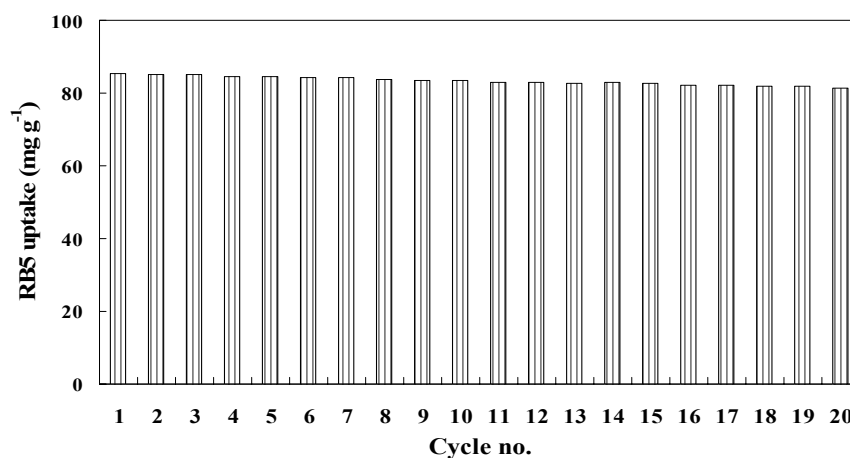


Fig. 3. RB5 uptake in successive cycles using 0.01 M NaOH as elutant

The results of RB5 biosorption by polysulfone-immobilized *C. glutamicum* in 20 consecutive cycles are presented in Fig. 3. Polysulfone beads exhibited good stability and constant RB5 uptake capacity up to 20 cycles examined. Weight loss was insignificant (< 3%) at the end of 20 consecutive cycles. The constant RB5 uptake capacity of polysulfone immobilized *C. glutamicum* is very interesting, since the binding sites were active enough to accommodate RB5 in multiple cycles and stability of polysulfone matrix in the entrapment of biomass. The decrease in RB5 biosorption capacity was insignificant as only 4.6% was observed at the end of twentieth cycle.

4. References

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