Spectrophotometric Determination of Some Functional Groups on *Chlorella* for the Evaluation of Their Contribution to Metal Uptake

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Introduction

Biosorption is a process that utilizes inexpensive dead biomass to collect toxic heavy metals. It is accepted as a particularly useful method for removing contaminants from industrial effluents.^{1,2} Compared with the conventional metal removal methods such as coprecipitation or ion-exchange, biosorption provides the advantages of low cost, minimization of chemical sludge, and high efficiency in detoxifying very dilute waste waters.³ However, since the removal of heavy metals in biosorption is often incomplete, numerous kinds of biomass have been tested in order to obtain sufficient recoveries of toxic heavy metals under various conditions.⁴⁻⁶ Thus, the factors for metal uptaking abilities of biosorbents need to be studied in detail.

The contribution of functional groups on the biomass which includes *chlorella* to metal uptake has been examined by some researchers.⁷⁻⁹ Good agreement in the results obtained by potentiometric titration with pH-dependent metal adsorption strongly suggests that carboxyl groups play an important role in metal biosorption. However, few studies have clarified the contribution of minor functional groups including amino or thiol moieties on biosorbents to metal uptake.^{7,10} In this study, we determined amino and thiol groups as well as carboxyl groups on a biosorbent and correlated these functional groups to their contribution to metal uptake. For this purpose, some spectrophotometric agents and blocking agents for these functional groups were examined. *Chlorella* has been most extensively investigated as a biosorbent and was used for clarifying the feasibility of such an approach to evaluate the contribution of the respective functional groups to metal uptake.

Experimental

A *chlorella* suspension (1 mg (dry)/ml) was prepared by diluting concentrated suspension (*vulgaris*-12, Chlorella Industrial Co. Ltd., Chikugo, Japan) after autoclaving at 121°C for 20 min and subsequent washing with Milli-Q water. It was standardized gravimetrically by drying at 110°C for 72 h. The chemicals

used were of analytical grade.

Functionally specific spectrophotometric agents: dithiobis(5-nitropyridine) (DNP, for thiol)¹¹ and fluoresceinisothiocyanate (FITC, for amine)¹² were used for the spectrophotometric determination of thiol and amino groups. The reaction was performed in 10 ml or 50 ml *chlorella* suspension for 20 min or 24 h, respectively. On the other hand, carboxylic acid was determined by acid titration method.¹³

In order to block functional groups on *chlorella* surfaces, to 10 ml of *chlorella* suspension was added 0.97 g (0.01 mol) of *N*-maleimide (for thiol), 0.4 ml of 25% glutaraldehyde (for amine), or the mixture of 1.92 g (0.01 mol) of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (WSC) and 1.5 ml of 30% aqueous ethylamine (pH 7.0) (for carboxylic acid) was added. After the solution was mixed at room temperature for 72 h, excess reagents were removed by repeated washing with Milli-Q water.

For metal sorption, each 10-ml *chlorella* suspension containing 1.00×10^{-5} M of metal ion and buffer components was incubated at room temperature for 24 h. The buffer components used were 2-morpholinoethanesulfonic acid (MES) and 2-[4-(2-hydroxyethyl)-1-piperadyl]ethane sulfonic acid (HEPES). After centrifuging at 3500 rpm for 10 min, the metal ion which remained in the supernatant was determined with a Seiko SPQ610 ICP-atomic emission spectrometer.

Results and Discussion

When the respective spectrophotometric agent was added to *chlorella* suspension, the absorbance in the supernatant gradually increased (or decreased) and then became constant and maximum (minimum) after 10 – 40 min for DNP (or 6 – 48 h for FITC). On the other hand, negligible absorption change was observed when the agent was mixed with the *chlorella* so that the functional groups were specifically blocked. These facts strongly suggest that the chromogenic agents used in this study react with the functional groups but seldom adsorb on *chlorella* surfaces unselectively.

The increase of the absorbance at 386 nm in DNP system is attributed to the formation of 5-nitro-2-thiopyridone, while the decrease in FITC system is a decrease of its concentration due to the stoichiometric binding to amino groups (Fig. 1). Thus,

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