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Utilisation of native, heat and acid-treated microalgae *Chlamydomonas reinhardtii* preparations for biosorption of Cr(VI) ions

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Abstract

The hexavalent chromium biosorption onto native, heat- and acid-treated *Chlamydomonas reinhardtii* was studied from aqueous solutions. Biosorption equilibrium was established in about 120 min. The surface properties of the microalgae preparations varied with pH, and the maximum absorption of chromium ions on the microalgae preparations was obtained at pH 2.0. The biosorption of chromium ions by the microalgae preparations increased as the initial concentration of chromium ions increased in the medium. The maximum biosorption capacities of the native, heat- and acid-treated algal preparations were 18.2, 25.6 and 21.2 mg Cr(VI) per g of dry biosorbents, respectively. Equilibrium concentration (q_m) and dissociation constant (K_d) were calculated by fitting the experimental data with the Langmuir isotherms. The chromium adsorption data were analysed using the first- and the second-order kinetic models. The experimental results suggest that the second-order equation is the most appropriate equation to predict the biosorption capacities of all the biosorbents. All the tested algal preparations could be regenerated using 0.1 M NaOH solution, with up to 96% recovery.

Keywords: *Chlamydomonas reinhardtii*; Heat-treatment; Acid-treatment; Biosorption; Cr(VI); Kinetic characterization

1. Introduction

Heavy metals are toxic because they are present as ions in aqueous systems and can be readily absorbed into the human body. Even a very small amount can cause severe physiological or neurological damage. One of the most dangerous metal ions for human life is Cr(VI), which is often encountered in industrial wastewaters because of the extensive use of chromate and dichromate in electroplating, leather tanning, metal finishing, nuclear power plant, textile industries and chromate preparation [1,2]. Chromium, Cr(VI), is a powerful carcinogenic agent that modifies the DNA transcription process causing important chromosomic aberrations. The Cr(VI) may also cause epigastric pain, nausea, vomiting, severe diarrhea, and hemorrhage [3].

The prevention of heavy metal contamination in aquatic environments is often performed by conventional methods, which include the addition of chemicals for metal precipitation or exchange resins to bind them. The use of microbial biomass to remove metal ions from aqueous environment has gained considerable interest as a potential alternative method to chemical treatments [4]. Many types of biomass in living and non-living form have been studied for their heavy metal uptake capacities and suitability to be used as bases for biosorbent development. These include bacteria [5], fungi [6], yeast [7], fresh water algae [8], marine algae [9] and others [10,11]. A wide variety of microalgal species such as *Chlorella* sp., *Ankistrodesmus* sp., *Eremosphaera* sp. has been used for heavy metal removal from aqueous medium [12–14]. The microalgae *Chlamydomonas reinhardtii* is a common species inhabiting a wide range of environments, and it has recently gained considerable interest in bioremediation of heavy metal pollution.

Of two main types of mechanisms for heavy metal removal processes in microorganisms, passive uptake, also called biosorption is defined as the accumulation and

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concentration of pollutants from aqueous solutions by the use of biological materials, thus allowing the recovery and/or environmentally acceptable disposal of the pollutants. This mechanism is metabolically independent. The amount of metal accumulation per unit of biomass is proportional to the concentration of metal ion in the solution. In addition, biosorption can be affected by pH and the presence of other ions in the medium that may precipitate heavy metals as insoluble salts, but it is unaffected by metabolic inhibitors or light/dark cycles. Second, active uptake is metabolically dependent and more effective than biosorption for low concentrations of heavy metals (below 1 ppm). It may involve metal ion consumption for algal growth and/or intracellular accumulation of heavy metals. In addition, heavy metals may be precipitated by excreted secondary metabolites [15,16].

Algal cell walls, mainly consisting of polysaccharides, proteins and lipids, offer many functional groups, which can bind metal ions such as carboxyl, hydroxyl, sulphate, phosphate and amino groups [17]. The use of native and treated biological materials for heavy metal removal has gained importance. Non-living (i.e., heat, acid, base and/or chemically treated) cells accumulate heavy metal ions to the same or to a greater extent as growing or resting cells [18]. The use of non-living microbial cells in industrial application may offer some advantages over living cells, such as lower sensitivity to toxic metal ions concentrations and adverse operating conditions [18–20].

In this study, a wild type of *C. reinhardtii* was isolated from a polluted site of Kızılırmak river and was cultured to achieve the most probable removal efficiency because the species grown in polluted areas were known to be more resistant and thus have more capability for accumulating heavy metals. Native, heat- and acid-treated forms of *C. reinhardtii* were utilised for the removal of Cr(VI) ions from aqueous solution. The effects of contact time, solid/liquid ratio, initial concentration, and pH on the biosorption of Cr(VI) ions have been investigated. The biosorption of Cr(VI) ions from aqueous solutions on the algal preparations under different kinetic and equilibrium conditions are scrutinized in some details. Finally, elution-reuse of native and treated algal preparations was evaluated. The information gained from these studies was expected to indicate whether the native, heat- and acid-treated forms might have the potential to be used for the removal and recovery of Cr(VI) ions from wastewaters.

2. Materials and methods

2.1. Microorganism and media

C. reinhardtii was isolated from the fresh water samples obtained from Kızılırmak river in Turkey. The sampling site selected on the Kızılırmak river was located 1 km away downstream to the discharge point of an oil refinery. Cell culture was grown in modified Sager and Granick medium [21] adjusted to pH 7.0, maintained at 22 ± 1 °C with 16:8 h of light–dark cycle using 4000 lux light intensity of cool-white fluorescent. Algal cells at the middle of the logarithmic phase, which was reached on the 15th day, were harvested by centrifugation at 2000 rpm for 10 min.

The wet weights of algal cells were derived from volume, assuming a cell specific gravity of 1. The volume of each cell was determined by approximating its shape to the nearest geometric configuration. The total biomass was calculated by multiplying the average number of cells with the average volume on each day during the growth of culture according to the method of Wetzel and Likens [22].

2.2. Heat- and acid-treatment of Chlamydomonas reinhardtii

Heat-treated form of *C. reinhardtii* was prepared in physiological saline solution by heating at 65 °C for 30 min and after treatment referred as heat-treated algal biomass. The native algal biomass was transferred into 0.1 M HCl solutions, and the mixture was stirred at 200 rpm for 6.0 h at ambient temperature and, hereafter they called acid-treated algal biomass. Each treated algal biomass was centrifuged at 5000 rpm for 10 min, washed with same saline solution and dried in a vacuum oven at 50 °C.

2.3. FT-IR Spectroscopy

FT-IR spectra of native, acid and heat-treated *C. reinhardtii* were obtained by using a FT-IR spectrophotometer (Mattson 1000 FT-IR, England). The dry algal biomass (about 0.1 g) mixed with KBr (0.1 g) and pressed into a tablet form. The FT-IR spectrum was then recorded.

2.4. Biosorption studies

The biosorption of Cr(VI) ions, on the native, heat- and acid-treated algal biomass was investigated in a batch system. Solutions containing Cr(VI) ions were prepared from the analytical grade potassium dichromate. A stock solution (1000 mg/L) of Cr(VI) was obtained by dissolving dried potassium dichromate in Milli-Q water. A range of concentrations of Cr(VI) ions was prepared from stock solution. To determine the effect of initial concentrations of Cr(VI) ions on the biosorption rate and capacity on the algal preparations, the initial concentration of Cr(VI) ions was varied between 20 and 400 mg/L in the medium.

The effects of medium pH and temperature on the biosorption capacity of the algal biomass were investigated in the pH range 1.0–8.0 (which was adjusted with H_2SO_4 or NaOH at the beginning of the experiment and not controlled afterwards) at 25 °C and at four different temperatures (i.e., 5, 15, 25 and 40). Cr(VI) ions concentration in each solution (100 mg/L) was prepared in saline solution (25 mL), and 15.0 mg of algal preparations was transferred into the medium