Optimization of Malachite Green Biosorption by Green Microalgae from Aqueous Solutions

Masoud Kousha[1], Omidvar Farhadian[2*], Salar Dorafshan[3], Nasrollah Mahboobi Soofiani[4]

1. M.Sc., Department of Natural Resources, Isfahan University of Technology, Isfahan, Iran. (masoudkoush76@gmail.com)
2. Assistance Professor, Department of Natural Resources, Isfahan University of Technology, Isfahan, Iran.
3. Assistance Professor, Department of Natural Resources, Isfahan University of Technology, Isfahan, Iran. (sdorafshan@cc.iut.ac.ir)
4. Professor, Department of Natural Resources, Isfahan University of Technology, Isfahan, Iran. (soofiani@cc.iut.ac.ir)

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Introduction

Malachite green (MG) has been used as a food coloring agent, food additive, medical disinfectant as well as a dye in silk, wool, jute, leather, cotton, paper, and acrylic industries. It is also extensively used as a topical fungicide and ectoparasiticide in aquaculture industries throughout the world. The findings reveal that this dye has now become one of the most controversial compounds used in aquaculture due to the risks it poses on the consumers, including its effects on the immune and reproductive systems as well as its genotoxic and carcinogenic potentials. Despite being banned in several countries, the dye is still being used in many parts of the world due to lack of a proper alternative. Thus, we must also focus our attention on ways for reducing malachite green from aquaculture and industrial wastewaters. Different methods are available for the remediation of dye wastewaters. These include physicochemical methods such as chemical oxidation, precipitation, coagulation, filtration, electrolysis, and photodegradation. The major disadvantage of the physicochemical methods are high operational cost, limited versatility, interference by other wastewater constituents, and less adaptability to a wide range of dye wastewaters. Sorption on activated carbon is an effective method for the removal of color, but it is an expensive method. The development of efficient and environmentally friendly technologies to decrease dye content in industrial wastewater to acceptable levels at an affordable cost is of great importance. In recent years, a number of studies have been focused on some bacteria, fungi, yeasts, and micro/macroalga. Biosorption of MG in the presence of algae such as *Caulerpa racemosa* cylindracea, *Cosmarium* sp., *Pithophora* sp., *Hydrilla verticillata,* and *Chara* sp. has been reported previously. The aim of this study was to investigate the biosorption characteristics of green microalgae *Scenedesmus quadricauda and Chlorella vulgaris* for the removal of MG dye. The effects of such process parameters as initial MG dye concentration (mg/L), initial solution pH, algae amount (mg/L), and contact time (min) on the dye biosorption were analyzed using Box–Behnken design in this work.

Materials and methods

MG stock solution was prepared by dissolving 100 mg MG (Serva, United States) in 1 L deionized water. All the other MG solutions were prepared by diluting the MG stock to obtain different concentrations ranged from 0.1 to 10 mg/L. The pH was adjusted using diluted NaOH and HCI solutions. The algal species were acquired from natural fresh water, Zayandeh-Rood River, Isfahan province, Iran, in November 2010. Algae were grown in several 2 L conical flasks containing Bold's basal medium (BBM) in order to obtain algal stock. The cells were cultured at 25±2^{o}C temperature, 12h L: 12h D photoperiod and a light intensity of 80 µmol photons/m^2/s for a maximum 10 day exposure period. The algal biomass was centrifuged and the remaining biomass dried at 50^{0}C for 48 h. The dried cells, which were ground, produced a uniform material which was stored in the desiccator for further use. Batch sorption experiments were carried out at 26 ± 2^{o}C on a rotary shaker (Dragon LAB, sk-330-pro, Germany) at 135 rpm using 100 ml conical flasks. Malachite green sorption studies were conducted by taking different amounts 2, 4 and 6 mg of algal biomass in 50 ml conical flasks containing 2, 6 and 10 mg/L of MG at different initial solutions, pH 3, 4.5, and 6, and at different contact times of 10, 50, and 90 min. After the end of predetermined time, the resulted solutions were filtered through a 0.2 µm membrane filter (Orange

∗ Corresponding Author: Tel:03113913564 E-mail: omfarhad@cc.iut.ac.ir

Scientific, GyroDisc CA-PC, Belgium) and analyzed quantitatively. The remaining MG was determined with a spectrophotometer (Jenwey, 6400 spectrophotometer, England) at the maximum sorption wavelength, (λ_{max} = 618 nm). Box-Behnken Design, which is well suited for fitting a quadratic surface and usually works well for the process optimization, was used for the experimental design. Total 29 experiments were designed by Design Expert software (Version 8.0.4, Stat-Ease, Inc., Minneapolis, United States) statistical package.

Results and Discussion

The second-order polynomial analysis and quadratic model were employed to find out the relationship between variables and responses. The regression equation obtained after the ANOVA showed that the correlation coefficient (R^2) was 0.9918 and 0.9936 for the MG removal percentage of *S. quadricauda* and *C. vulgaris*, respectively. However, in this case, R^2 values of 0.9918 and 0.9936 implied a sample variation of 99.18% and 99.36% were attributed to the variable and only 0.82% and 0.64% of the total variance could not be explained by the model.

Therefore the high R^2 value, significant *F*-value, and insignificant lack-of-fit *P*-value indicate high adequacy and validity of the models in predicting the MG removal by *S. quadricauda* and *C. vulgaris*. Therefore, these models were used for further analysis. The MG removal efficiency of algae decreased with an increase in initial MG concentration. The reason for the decrease in the MG removal efficiency can be attributed to the fact that all the algal biomass had a limited number of active sites, which would have become saturated above a certain initial MG dye concentration. In other words, as dye molecules are adsorbed on all active sites of algal biomass, the higher concentration of MG dye would have no impact on the removal efficiency of dye.

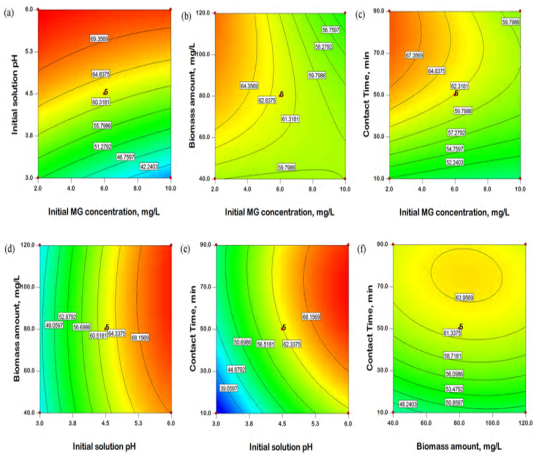

Figure 1. Contour plots for MG removal efficiency onto *S. quadricauda* biomass: a) pH versus MG conc.; b) biomass versus MG conc.; c) time versus MG conc.; d) biomass versus pH; e) time versus pH; and f) time versus biomass.

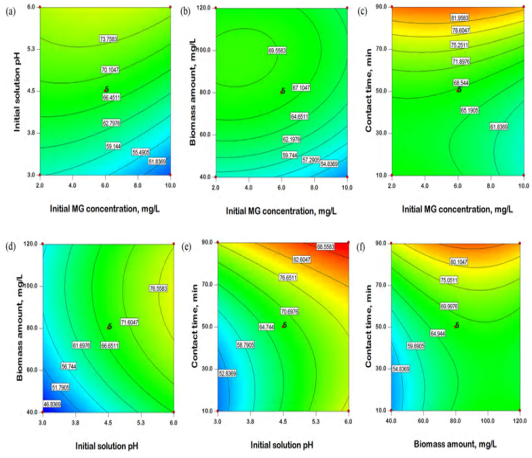

Figure 2. Contour plots for MG removal efficiency onto *C. vulgaris* biomass: a) pH versus MG conc.; b) biomass versus MG conc.; c) time versus MG conc.; d) biomass versus pH; e) time versus pH; and f) time versus biomass.

The removal efficiency of MG rapidly increases with an increase in initial pH of solution from 3.0 to 6.0. The pH of the solution plays an important role in the whole dye sorption process and particularly on the dye sorption efficiency. With increasing pH, the OH^- ion concentration in the system increases and the surface of algae achieves a negative charge by adsorbing OH^- ions. Negatively charged surface sites onto algal biomass favored the sorption of the cationic dye molecules such as MG due to electrostatic attraction. Furthermore, the initial pH of dye solution affected not only the surface charge of the biosorbent, but also may also have increased the degree of ionization of the dye present in the solution and the dissociation of functional groups on the active sites of the biosorbent. Thus, these effects facilitate electrostatic interaction between algal biomass and the positively charged cationic dyes leading to maximum MG removal.

Also, the removal of MG dye increased with increasing biomass amount of algae. After dye removal reached the optimum sorption points, the dye sorption decreased slowly. The reason for this observation is thought to be the fact that the increase in algal biomass up to optimum points gives more surface area and more binding sites for sorption of the MG ion on the surface of algae; thereafter, the biomass particle aggregation in higher biomass results in increase of diffusional path length and a decrease in total surface area of the biosorbent and the removal efficiency of dye.

These results revealed that biosorption of MG was strictly time dependent. In this condition, a large quantity of dye has been adsorbed onto biomass after a relatively short contact time, where the main sorption of molecules was noticed within the first 20 min of the experiments; thereafter, the sorption rate decreased gradually and the sorption reached equilibrium in about 90 min. The reason for this observation can be the fact that at the beginning, the dye molecules were adsorbed externally and the biosorption rate increased rapidly. When the external surface became saturated, the dye molecules adsorbed into the porous structure of the biomass and finally, at some point in time, reached a constant value where no more dye was adsorbed from the solution. At that time, the amount of dye being adsorbed onto the biomaterial was in a state of dynamic equilibrium with the amount of dye desorbed from the biosorbents.

Keywords: biosorption, *Chlorella vulgaris*, malachite green, response surface methodology, *Scenedesmus quadricauda*.