EFFECT OF INITIAL REACTIVE RED 120 CONCENTRATIONS ON THE BIOMASS PRODUCTION AND DYE UPTAKE BY *Spirulina platensis*

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ABSTRACT

Uptake of Reactive Red (RR) 120 and biomass production by *Spirulina platensis* in the Schlösser medium was followed at different initial dye concentrations (0, 15, 25, 50, 75 and 100 mg L^{-1}) under 2.0 klux continuous illuminations. Initial dye concentrations and cultivation time significantly affected ($p<0.01$) biomass productions. It was observed that increment rate of biomass was especially achieved between 77 and 144 h at dye concentration range between 0.0-75 mg L^{-1}. However, remarkable increment rate was not observed at 100 mg L^{-1} dye concentration. Change in initial dye concentrations from 15 to 100 mg L^{-1} strongly affected ($p<0.01$) amount of the dye uptake (q_t). Increasing initial dye concentrations from 15 to 100 mg L^{-1} increased ($p<0.01$) the equilibrium dye uptake (q_{eq}) values from 8.08 to 18.31 mg g^{-1}. Tukey HSD test revealed that there was no significant difference ($p>0.05$) among q_{eq} values at initial dye concentrations of 25, 50, and 75 mg L^{-1}. This cyanobacterium is able to withstand high concentrations of RR 120, which is to be important for wastewater treatment systems.

KEYWORDS:
Biomass; Reactive Red 120; *Spirulina*; dye uptake.

INTRODUCTION

Photosynthetic filamentous cyanobacterium, *Spirulina* species (Oscillatoriales) is identified by the main morphological feature of the genus, i.e. the arrangement of multicellular cylindrical trichomes in a helix along the entire length of the filaments [1,2]. Biomass of *Spirulina* from Chad Lake (Africa) and Texcoco Lake (Mexico) has been harvested by native people as a source of food for centuries [2]. However, *Spirulina platensis* has been commercially cultivated due to its biotechnological importance since 1970s [3]. *Spirulina* have been used as an additive and food supplier for human and the feeding of animals from fishes to pets due to its high nutritional values [4-6]. Recently, biomass of *Spirulina* has been performed to remove pollutants such as excess fertilizer, heavy metals, textile dyes and pesticides from wastewaters [7-9].

Various industries such as textile, plastic, leather, paper industries etc., have extensively used dyes and pigments to colour their final products [10,11]. The effluents of these industries are discharged into receiving waters cause damages to the ecological balance, affecting photosynthetic activity in aquatic food web due to curtained light penetration [12,13]. Besides, wastewaters may be toxic and even carcinogenic, which poses a serious hazardous effect to aquatic life due to the presence of dye with metals, salts etc. [14]. Many studies about removal of dye have been performed to find a natural, cheap and alternative biological adsorbent instead of physical and chemical treatment to reduce the cost and hazardous effect on aquatic life [11,15]. Many microorganisms such as fungi, algae and bacteria etc., either in their living or inactivated (dead) form, have been studied to remove dyes from wastewaters [16-21]. Many investigations focused on the usage of inactivated biosorbents for removal of dye from wastewaters. However, there are few studies concerning the bioaccumulation of unwanted materials from wastewaters by living microalgae [8,22,23].

Previously microalgal species have been proved to be effective microorganism for bioaccumulation of cadmium by *S. platensis* [8,22], removal of dye-rich wastewaters by *Phormidium* sp. [23] and bioaccumulation of reactive dyes by thermophilic cyanobacteria [24].

The objective of this research was to study reactive red 120 uptake potential of *S. platensis* and its biomass production at different initial dye concentrations. Besides, behavior of the cyanobacterium during cultivation time was described by statistical analysis.

MATERIALS AND METHODS

Microorganism and growth conditions

The cyanobacterium used in this study, *S. platensis* obtained from University of Ege, EBILTEM Culture Collection, was inoculated on the Schlösser's medium [25]. Cells were maintained in the culture medium of Schlösser, having the following composition (per liter): 13.61 g $NaHCO_3$, 4.03 g Na_2CO_3, 0.50 g K_2HPO_4 , 2.50 g $NaNO_3$, 1.00 g K_2SO_4, 1.00 g NaCl, 0.20 g $MgSO_4 \cdot 7\ H_2O$, 0.04 g $CaCl_2 \cdot 2\ H_2O$. All nutrients were dissolved in distilled water containing (per liter): 6 mL of metal solution (97 mg $FeCl_3 \cdot 6\ H_2O$, 41 mg $MnCl_2 \cdot 4\ H_2O$, 5 mg $ZnCl_2$, 2 mg $CoCl_2 \cdot 6\ H_2O$, 4 mg $Na_2MoO_4 \cdot 2\ H_2O$), 1 mL of micronutrient solution (50.0 mg Na_2EDTA, 618 mg H_3BO_3, 19.6 mg $CuSO_4 \cdot 5\ H_2O$, 44.0 mg $ZnSO_4 \cdot 7\ H_2O$, 20.0 mg $CoCl_2 \cdot 6\ H_2O$, 12.6 mg $MnCl_2 \cdot 4\ H_2O$, 12.6 mg $Na_2MoO_4 \cdot 2\ H_2O$) and 0.15 mg of B12 vitamin. The culture was incubated under 2.0 klux with measuring light meter (Lutron Lx-130 model) continuous illumination using cool, white fluorescent lamps.

Batch cultivations were carried out in 250 mL Erlenmeyer flasks containing 100 mL of the medium, placed on an orbital shaker at 90 rpm for 168 hours. Inoculated culture was carried out with an initial *Spirulina* biomass concentration of 0.460 g L^{-1}(dry weight). Biomass concentration was calculated through Optical Density (OD) measurements at 670 nm using a spectrophotometer (UV/VIS Jenway 6305) from standard calibration curve of OD against a known concentration of *Spirulina* biomass. During cultivation, biomass values were measured at 0, 1, 2, 3, 24, 48, 53, 72, 77, 96, 120, 144, 149 and 168 h. In our previous study [26], biomass of *Spirulina* was performed at three pH regimes (pH 9.5, 10.0 and 10.5). These results showed that optimum pH for biomass production was 10.0. After addition of dye, pH of the medium was adjusted by dilute (0.1 M) HCI and concentrated (2 M) NaOH solutions to predetermined optimum growth pH of *Spirulina*, determined by pH meter (Hanna, pH 211 microprocessor).

Reactive Red (RR) 120 (Procion Red HE-3B; $C_{44}H_{24}Cl_2N_{14}O_{20}S_6Na_6$) was taken from Sigma (Sigma-Aldrich Chemical Co., St. Louis, USA). Stock solution of this dye was prepared as 5.0 g L^{-1} in the distilled water. Appropriate volumes of the stock dye solution were added into culture medium. Initial dye concentrations of the batch culture were adjusted as 15, 25, 50, 75, and 100 mg L^{-1} during cultivation in order to evaluate the effects of initial dye concentrations on biomass production and the dye uptake of RR 120 by the species. The required dye concentrations were freshly prepared. Samples obtained from the culture solution were filtered by Sartorious filtration system with 0.45 µm mesh size acetate filter. Amount of remaining dye in solution was determined using a spectrophotometer (UV/VIS Jenway 6305) by monitoring the absorbance at wavelength of 515 nm and also 750 nm for turbidity. Residual dye concentration was measured at 0, 1, 2, 3, 24, 48, 53, 72, 77, 96, 120, 144, 149 and 168 h. Experiments were carried out in duplicate with control cyanobacterium-medium without RR 120.

Dye uptake

The amount of RR 120 uptake per unit weight of biosorbent (q_t, mg g^{-1}); were obtained by the following Eq. (1).

$$q_t = \frac{(C_o - C_t) * V}{M} \quad (1)$$

where, C_o and C_t (mg L^{-1}) represent at initial and at t time concentrations of reactive red in the solution, respectively. V (L) is the volume of solution, M (g) is the mass of adsorbent.

The amount of RR 120 uptake per unit weight of biomass at equilibrium (q_{eq}, mg g^{-1}); were calculated by using Eq. (2).

$$q_{eq} = \frac{(C_o - C_{eq}) * V}{M} \quad (2)$$

where, C_o and C_{eq} (mg L^{-1}) represent initial and at equilibrium concentrations of RR 120 in the solution, function of contact time respectively. V (L) is the volume of solution, M (g) is the mass of adsorbent.

Statistical Analysis

Analysis of Variance (ANOVA) was performed for biomass value and dye uptake at initial dye concentrations to determine significant differences using the SPSS version 15.0 (SPSS Inc., Chicago, IL, USA). Tukey's Honestly Significant Difference (HSD) multiple range test was also carried out to distinguish examined groups.

RESULTS AND DISCUSSION

The cyanobacterium, *S. platensis* showed slightly spiral, left direction of helix, 7-10 µm width of cylindrical trichome, 33-48 µm diameter of spiral and pH tolerance range was 9-11, also reported by Vonshak [1] and Komárek and Anagnostidis [27].

Effects of initial RR 120 concentrations and cultivation time on biomass productions by S. *platensis* are shown in Figure 1. Statistical analysis (two-way ANOVA) indicated that S. *platensis* had a short lag time. After this phase, the cultivation course of biomass production showed a linear trend ($p<0.01$). It was observed that the increment rate of biomass was especially achieved between 77 and 144 h at dye concentration range 0.0-75 mg L^{-1} (Figure 1). Similar results were found by Soletto et al. [28], whereas Colla et al. [29] observed this rate was continued up to 500 h. However, in the present study, initial dye concentrations higher than 75 mg L^{-1} did not show ($p>0.05$) remarkable increment rate of biomass. Increasing initial dye concentration decreased ($p<0.01$) biomass value during cultivation.

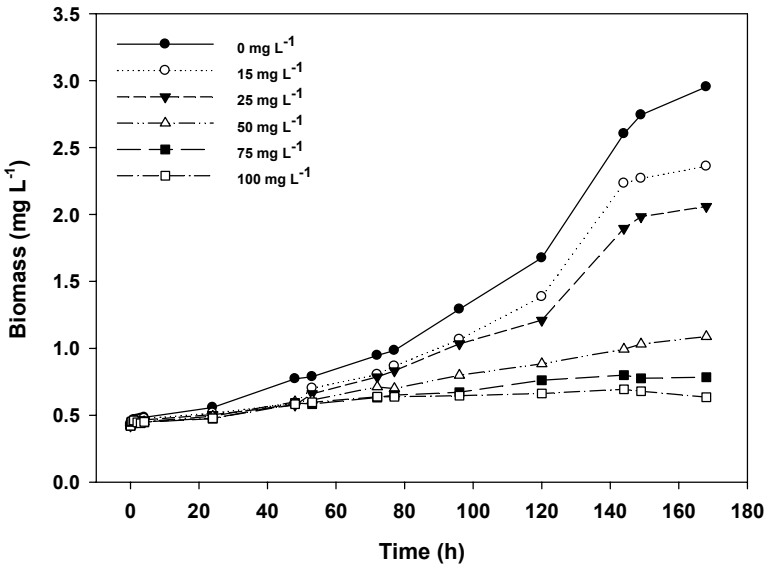

FIGURE 1 - Effect of different initial reactive red 120 concentrations on biomass production by *Spirulina platensis*.

It could be concluded that the high initial dye concentrations might significantly affect photosynthetic activity due to reduction of light penetration, in agreements with Zollinger [12] and Robinson et al. [14] for aquatic ecosystem. Besides, it may be also toxic and even carcinogenic, which poses a serious hazard to aquatic life.

Dye uptake by *S. platensis* at various initial RR 120 concentrations (15, 25, 50, 75 and 100 mg L^{-1}) is shown in Figure 2 during the cultivation. It was found that initial dye concentrations and cultivation time significantly affected ($p<0.01$) the uptake of RR 120 by the cyanobacterium. Increasing initial dye concentration from 15 to 150 mg L^{-1} caused to increase amount of the dye uptake (q_t) along cultivation time (Figure 2). The removal yields of RR 120 by S. *platensis* varied from 50.68 % to 17.95 % at 15 and 100 mg L^{-1}, respectively. With decreasing the initial dye concentration resulted to significantly increase ($p<0.01$) in bioaccumulation percentage of the dye. This result was in agreement with findings of Ertuğrul et al. [23] and Sadettin and Dönmez [24] for removal of remazol blue by *Phormidium* sp. and bioaccumulation reactive dyes by thermophilic cyanobacteria, respectively.

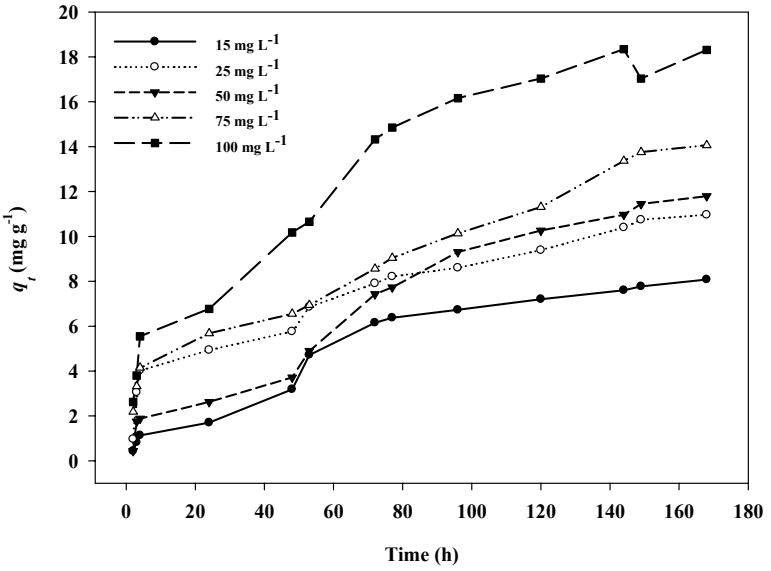

FIGURE 2 - Effect of different initial dye concentrations and contact time on reactive red 120 uptake by *Spirulina platensis*. Where q_t represent dye uptake by the unit weight of the alga at any time *t*.

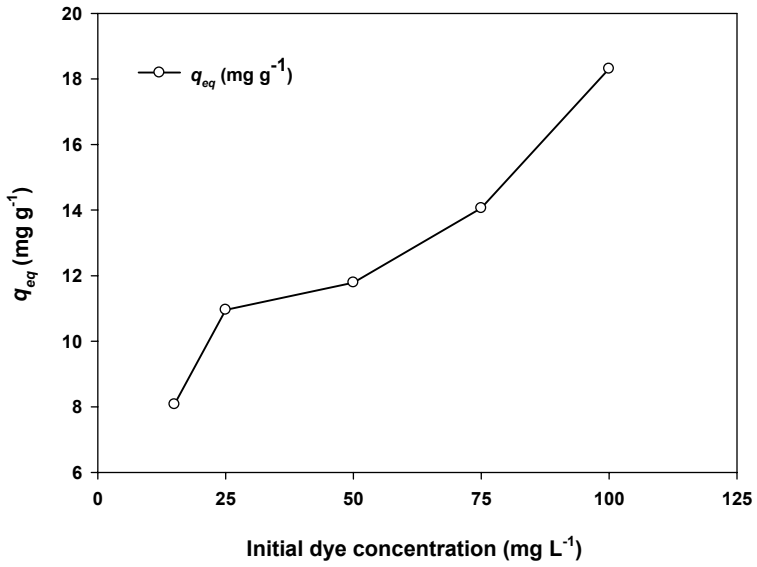

FIGURE 3 - The variation in dye uptake at equilibrium by *Spirulina platensis* at different initial dye concentrations.

Effects of different initial RR 120 concentrations on equilibrium dye uptake by S. *platensis* are shown in Figure 3. The equilibrium dye uptake values increased ($p<0.01$) from 8.08 to 18.31 mg g^{-1} with increasing initial dye concentration from 15 to 100 mg L^{-1}. This could be due to increasing dye driving force on biomass [30]. Besides, Tukey HSD test did not significantly distinguished ($p>0.05$) q_{eq} values among 25, 50, and 75 mg L^{-1} initial RR 120 concentrations (Figure 3).

The present study showed that S. *platensis* had a potential to removal RR 120 from culture medium at different initial dye concentrations. Initial reactive red 120 concentrations and cultivation time significantly affected ($p< 0.01$) biomass productions by S. *platensis*. This species produced remarkable biomass value, performed to treatment of unwanted pollutants from artificial wastewaters [7-9]. The equilibrium dye uptake by the species increased with increasing in the initial dye value up to 100 mg L^{-1} dye value. This cyanobacterium is able to withstand high concentrations of RR 120, which is to be important for wastewater treatment systems.

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