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Toxicity and uptake of Iron ions by *Synechocystis* sp. E35 isolated from Kucukcekmece Lagoon, Istanbul

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ABSTRACT

The study demonstrates the potential of using unicellular cyanobacteria species, isolated from Kucukcekmece Lake, Turkey, for biological Iron removal from aqueous solutions. EC_{50} at 96 h was estimated to be 13.92 mg/L for *Synechocystis* sp. E35. The optimum pH value and incubation temperature for the resistant isolate were 7.0 and 23 °C, respectively. The Iron biosorption/bioaccumulation by *Synechocystis* sp. E35 was evaluated by fractionating the Fe content as the remaining metal in supernatant, the adsorbed metal on the cell surface and the intracellular accumulation. *Synechocystis* sp. E35 adsorbed appreciable quantities of Iron ions on the cell surface within 5 min. Metal ions were adsorbed onto the surface of cells followed by active uptake resulting in the transportation of the metal ions across the cell membrane and into the cytoplasm. Intracellular metal uptake increased with increasing metal concentrations from 10 to 15 mg/L. Extracellular polysaccharides (EPSs) were clearly seen in SEM images of *Synechocystis* sp. E35 grown at a 10 mg/L metal concentration. EPS region was analyzed with Energy Dispersive X-Ray Analysis (EDXA) and the SEM images further confirmed our experimental observations about the Iron biosorption/bioaccumulation mechanism.

1. Introduction

Heavy metal pollution of water through the discharge of industrial wastewater has become one of the most serious environmental concerns threatening the ecosystem of water bodies. Many industries including metal plating, mining, battery, metallurgy, ceramic and chemical industries have seriously contributed to the release of toxic heavy metals to water streams (by runoff). These heavy metals pose a serious threat to the environment, animals and humans because of their toxicity. Conventional treatment technologies, such as ion exchange, chemical precipitation, and reverse osmosis are often ineffective and/or expensive particularly for the removal of heavy metal ions at low concentrations [1,2]. Most of these processes further compound the problem by generating toxic sludge. Therefore economically viable and eco-friendly technologies such as biosorption and/or bioaccumulation are required to reduce heavy metal concentrations to acceptable environmental levels. The bioprocess of metal removal is classified into two categories: biosorption by non-living or non-growing biomass and bioaccumulation by living cells [3].

Some heavy metals are important co-factors of enzymes and critical components of electron transport reactions in biological systems, but they can be toxic if taken in excess quantities [4]. Hence, toxicity gains importance in biosorption/bioaccumulation studies with living cells. In biosorption/bioaccumulation studies, microorganisms (algae, bacteria, yeast and fungi) have been traditionally used for remediation of wastewaters or soils contaminated with heavy metals [5]. Küçükçekmece Lake is one of the natural Lagoon lakes in Turkey and is a habitat of various endemic species [6]. Iron, copper and zinc were found in the water and the sediment structure of Küçükçekmece Lake at toxic levels [7].

Waste waters from automobile manufacture, metal cleaning, plating and metal processing industries contain Fe concentrations ranging over 10–120 mg/L [8,9,10]. Iron biosorption has been studied by various investigators using a variety of different biomass types including bacteria and fungi as biosorbents [11,12,13]. However, algae and cyanobacteria (blue-green algae) are the dominant group of microorganisms in the lakes due to carbon limitation and high nutrient concentrations. Hence, biosorption/bioaccumulation potential of algae and cyanobacteria grown in the lakes should be known to estimate the fate of metal pollutants in lakes.

Cyanobacteria (blue-green algae) are a diverse group of prokaryotes widespread in different habitats [14]. These prokaryotic organisms quickly respond and adapt to stress conditions in general and heavy metals in particular [15]. Cyanobacteria also produce

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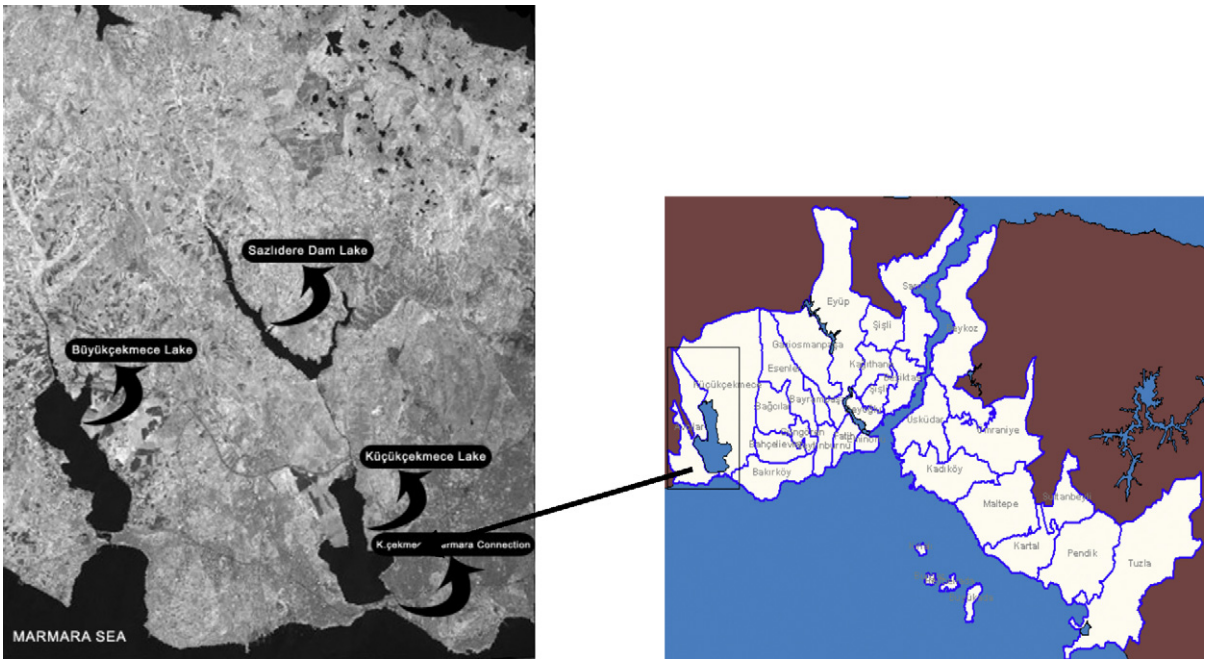

Fig. 1. Location of Kucukcekmece Lagoon.

metal-binding proteins which internally sequester and detoxify high concentrations of metals within the cell [16]. Therefore, this study aims at demonstrating the potential of unicellular cyanobacteria species isolated from Küçükçekmece Lake in removing Iron ions from aqueous solutions.

2. Materials and methods

2.1. Kucukcekmece Lagoon

Kucukcekmece Lagoon, located in the European part of Istanbul in Turkey, has typical spoon shaped topography (Fig. 1). The surface area of the lake is approximately 17 km^2, and the water volume is 145 million m^3 at sea level. Untreated wastewaters, both domestic and industrial (metal, textile, plastic etc.), are routinely being discharged into the creeks of Kucukcekmece Lagoon [6].

2.2. Isolation, identification and culture conditions of microorganisms

E1, E4, E8, E35 and E37 were isolated from Kucukcekmece Lake (İstanbul), Turkey. Samples were isolated by the method of Rippka et al. [17]. Isolates were identified according to cell division morphology [18] and were grown in a BG-11 medium. Composition of BG-11 medium used is: $NaNO_3$·(15 g/L), K_2HPO_4·(0.4 g/L), $MgSO_4·7H_2O$ (0.75 g/L), $CaCl_2·2H_2O$ (0.36 g/L), citric acid (0.06 g/L), Iron (III) ammonium citrate (0.06 g/L), Na_2-EDTA (0.01 g/L), Na_2CO_3 (0.2 g/L) and trace elements solution (1 mL) containing H_3BO_3 (61 mg/L), $MnSO_4·H_2O$ (169 mg/L), $ZnSO_4·7H_2O$ (287 mg/L), $(NH_4)6Mo_7O_{24}·4H_2O$ (12.5 mg/L), $CuSO_4·5H_2O$ (2.5 mg/L). pH of BG-11 medium was 6.8. Cultures were incubated at 25 °C with light/dark cycle of 12/12 h by using an incubator shaker (MINITRON) for 20 days, which is suitable for photosynthesis. The intensity of light during the light period was 3000 lx at 25 °C Isolates were

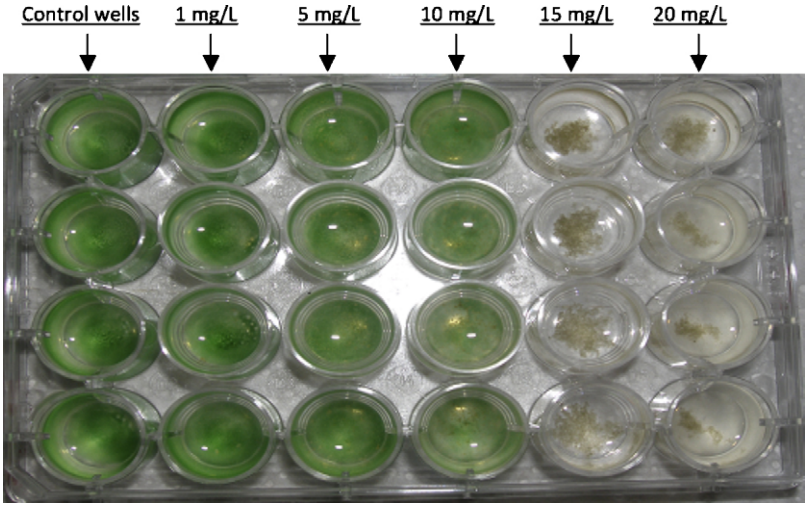

Fig. 2. E35 Cyanobacterial isolate after an incubation period of 144 h.

Table 1
EC_{10} and EC_{50} values of Fe at 96 h.

Cyanobacterial Isolates	EC_{10} value (mg/L)	EC_{50} value(mg/L)
Synechocystis sp.(E_1)	3.01	5.41
Synechocystis sp. (E_4)	3.61	6.01
Microcystis sp.(E_8)	4.82	8.93
Synechocystis sp.(E_{35})	11.52	13.92
Synechocystis sp. (E_{37})	6.59	10.40

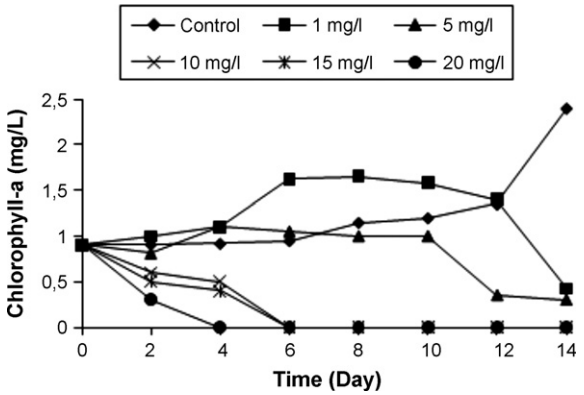

Fig. 3. Growth of Synechocystis sp. E35 in solutions containing different concentrations of Iron.

also identified according to 16S rRNA by the method of Nubel et al. [19]. Isolation, identification, and growth of the isolates were held in the Biotechnology Laboratory of Gazi University, and the isolates were stored at the Culture Collection of Microalgae of Gazi University.

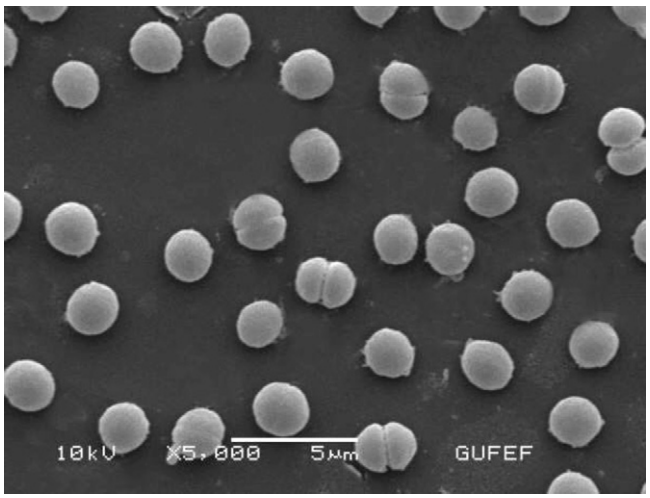

Fig. 5. SEM image of control culture of *Synechocystis* sp. E35.

2.3. Screening of Fe resistant cyanobacterial isolates

In the preliminary experiments, the resistance of 5 cyanobacterial isolates to Fe, added as $FeSO_4{\cdot}7H_2O$, (0, 1, 5, 10, 15 and 20 mg/L Fe) were screened in 24-well micro plates (Fig. 2). After an incubation period of 144 h, cell growth for each metal concentration was measured using a spectrophotometer at 664 nm. Then, EC_{10} and EC_{50} values were determined according to the probit analyze method [20]

2.4. Effect of Fe ions on chlorophyll content

Resistant cyanobacterial isolates were incubated in Iron containing a BG11 medium for 14 days, under the growth conditions

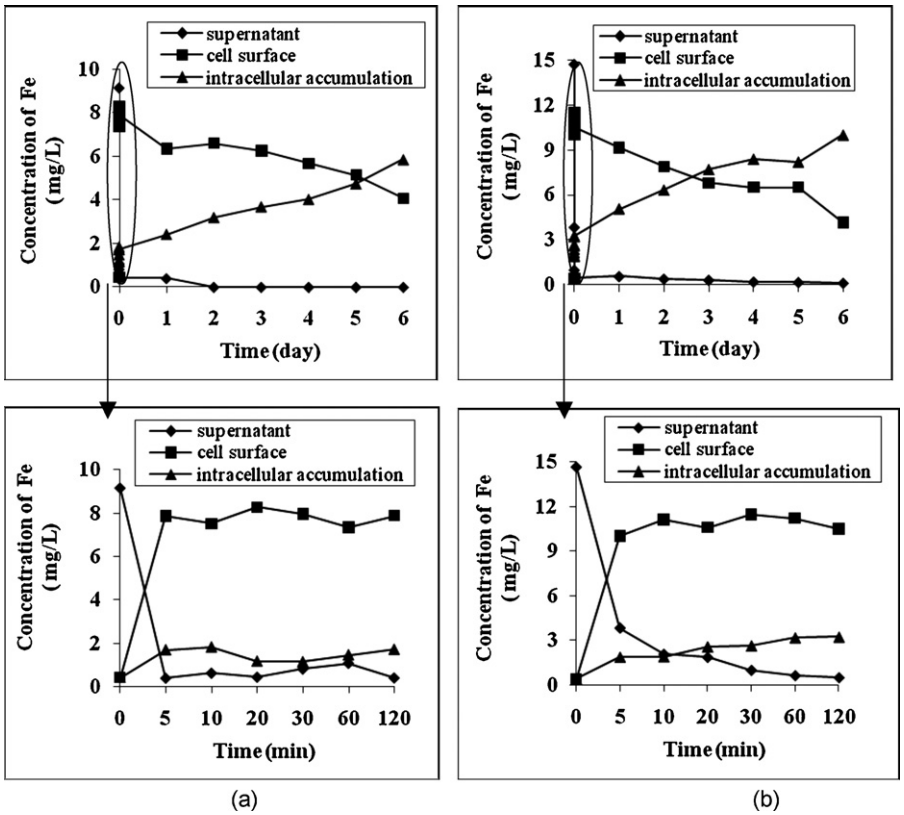

Fig. 4. Time course study of Iron biosorption/bioaccumulation in Synechocystis sp. E35 (a) 10 mg/L Fe, (b) 15 mg/L Fe. The cultures were maintained at 23 °C, pH 7, agitation speed 100 rpm and for 144 h.

Table 2
EDXA values of control cultures and cultures grown in a medium supplemented with 10 mg/L Fe.

Spectrum	C	O	Na	Mg	P	Ca	Fe	Cu	Zn	Total
Control	38.77	42.22	9.17	2.28	1.56	5.67	0.33	0.00	0.00	100.00
High mpr	53.22	20.78	4.58	0.34	10.94	2.32	7.81	0.00	0.00	100.00
Low mpr	46.32	39.77	5.65	0.73	3.87	1.71	1.96	0.00	0.00	100.00

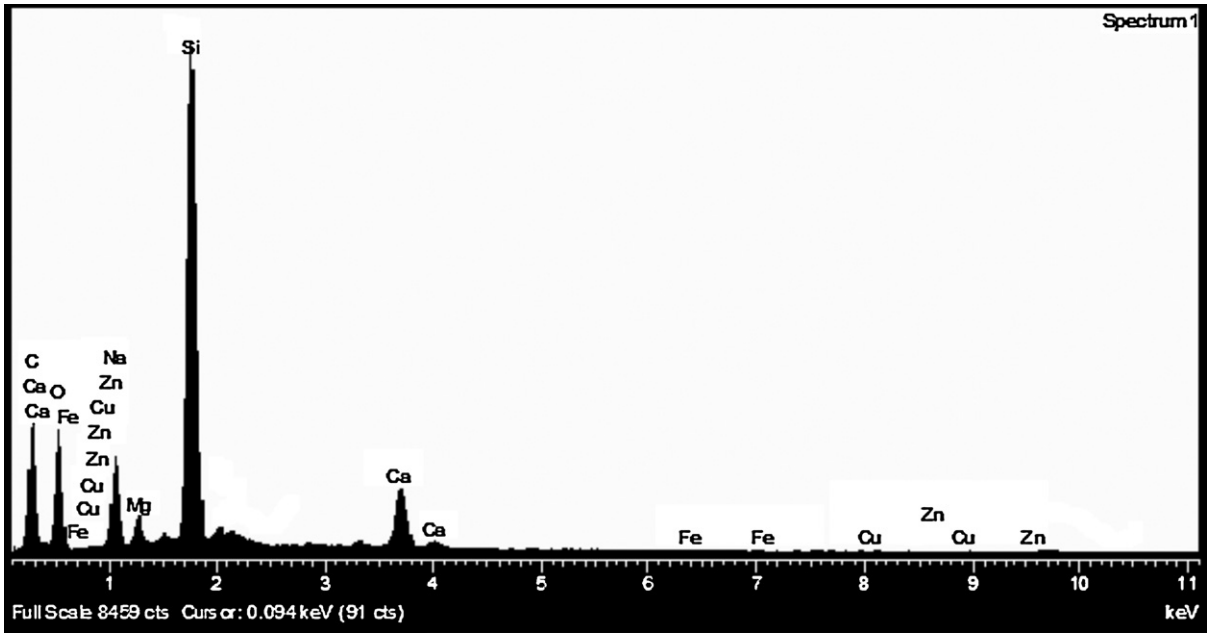

Fig. 6. . EDXA spectra obtained from control cultures of *Synechocystis* sp. E35.

described above. Chlorophyll-a was extracted by the cold acetone extraction method [21] and quantified in the resistant isolate.

2.5. Optimization of pH and incubation temperature

pH and incubation temperature are important environmental factors affecting microbial growth and enzyme reactions. Since in an earlier study conducted on Kucukcekmece Lagoon, pH values ranging from 5.5 to 8.0 were found to be suitable for the growth of cyanobacterial isolates [6], we also used a similar pH range for the present study. Additionally, three different incubation temperatures (20, 23 and 25 °C) were used to test the growth of Iron resistant cyanobacterial isolates. In the optimization step, the medium contained only trace amounts of metal ions. The optimum pH, incubation temperature and resistant isolate were determined for the further stages of the study.

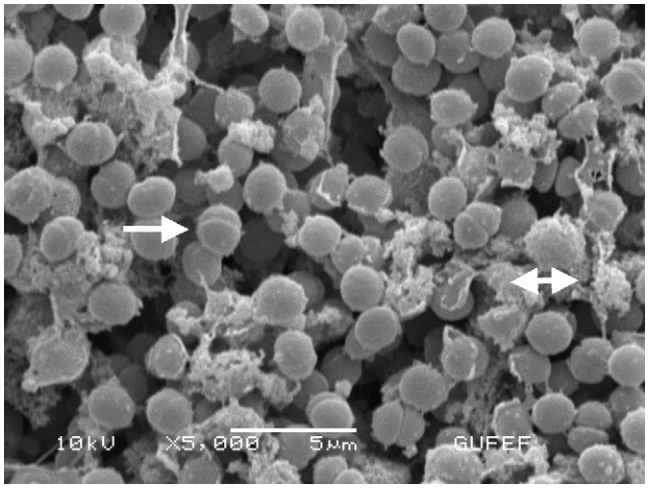

Fig. 7. SEM image of *Synechocystis* sp. E35, grown in a medium supplemented with 10 mg/L Fe, showing binary fusion of cells during reproduction (one sided arrow), and EPS (double sided arrow).

2.6. Biosorption/bioaccumulation of Iron ions

Metabolically active cells which have been equalized in optical density were used in the biosorption/bioaccumulation experiments. Isolated species were incubated in sterilized 100 ml erlenmeyer flasks containing 40 ml of metal bearing BG11 medium. The pH of the metal solutions was adjusted to the desired level using 1 N NaOH/HCl prior to the addition of the cyanobacterial isolate. The bacterial isolates were incubated for 144 h. Biosorption/bioaccumulation tests were performed under the optimal incubation conditions (light intensity = 3000 lx; T = 25 ± 2 °C) in an incubator shaker at 100 rpm.

2.7. Cell fractionation

Cells were fractionated depending on (a) remaining metal in the supernatant, (b) adsorbed metal on the cell surface, and (c) intracellular accumulation as described earlier [22]. To determine the residual Fe ion content in an aqueous solution, samples (1 ml) were withdrawn at different time intervals (0, 5, 10, 20, 30, 60, 120 min and 1, 2, 3, 4, 5, 6 days), centrifuged (7000 × *g*) and the residual metal in the medium was quantified from the supernatant. Thereafter, the pellet was washed with 1 ml of 10 mM EDTA solution for desorption of test metals from the cell surface, centrifuged (7000 × *g*),

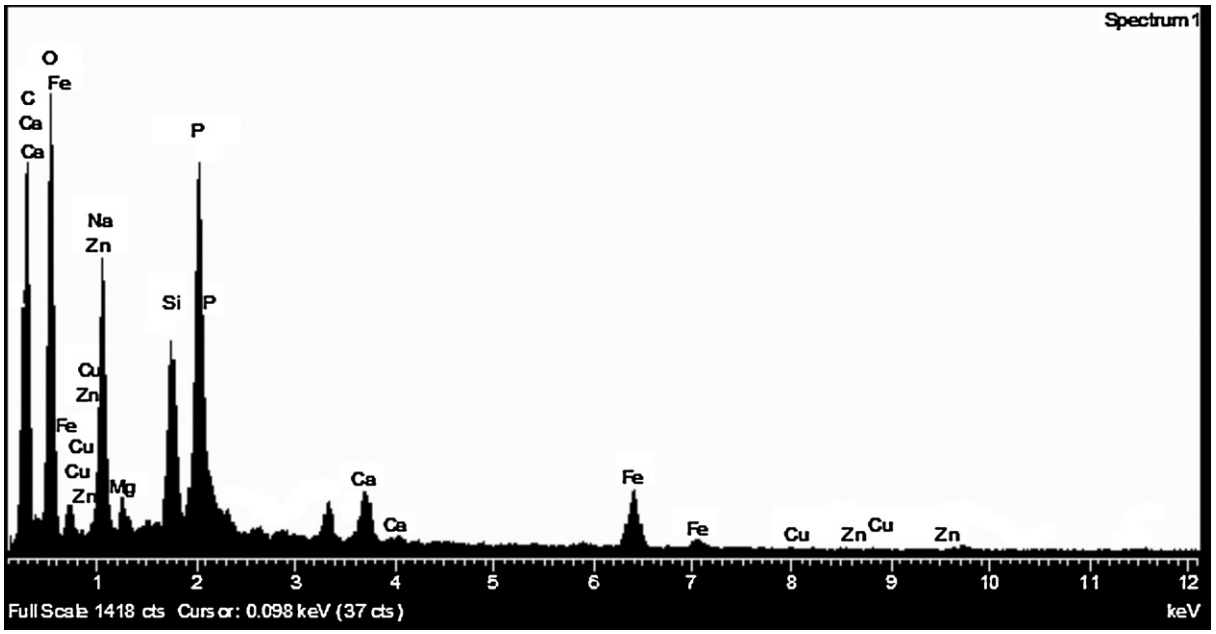

Fig. 8. EDXA spectra obtained from *Synechocystis* sp. E35 grown in a medium supplemented with 10 mg/L Fe (in high mpr, EPS).

and the supernatant was used for the quantification of adsorbed metal on the cell surface. The amount of intracellular accumulation of metal was determined by resuspending the pellet in 1 ml of 1 N HNO_3 (pH < 2), stored at 4 °C in the dark, and an Inductively Coupled Plasma-Mass Spectrometry (ICP-MS Agilent 7500) was used for the elemental analysis.

2.8. SEM and EDX analysis

The Fe tolerant cyanobacterial isolate was incubated in the presence of 10 mg/L Fe. At the end of the incubation period (144 h), control and treated cells were collected by centrifugation at 4 000 × *g* for 15 min at 4 °C., the cells were washed thrice in phosphate buffered saline (PBS, pH 7.2) and then centrifuged again. For the fixation step, 2.5% glutaraldehyde buffered with 0.2 M PBS (pH 7.2) was added on the cyanobacterial pellet. After 2 h of incubation at +4 °C, centrifugation was carried out. Supernatant was removed and the pellet was resuspended in PBS. The cells were washed two more times with PBS. This was followed by dehydration of cells in a graded series of ethanol. Cells were then passed to amyl acetate. The sample was next critical-point dried with CO_2 (Polaron, CPD 7501) and coated with gold (Polaron SC 502 sputter coater) for SEM examination. Scanning electron micrographs of the microbial culture were taken at 10–20 kV with JEOL JSM 6060 at Gazi University, Faculty of Arts and Sciences. EDX analyses were performed with a JEOL JSM-5910LV scanning electron microscope equipped with an EDXA-detector (Oxford, INCA 300) at Marmara University, Faculty of Engineering.

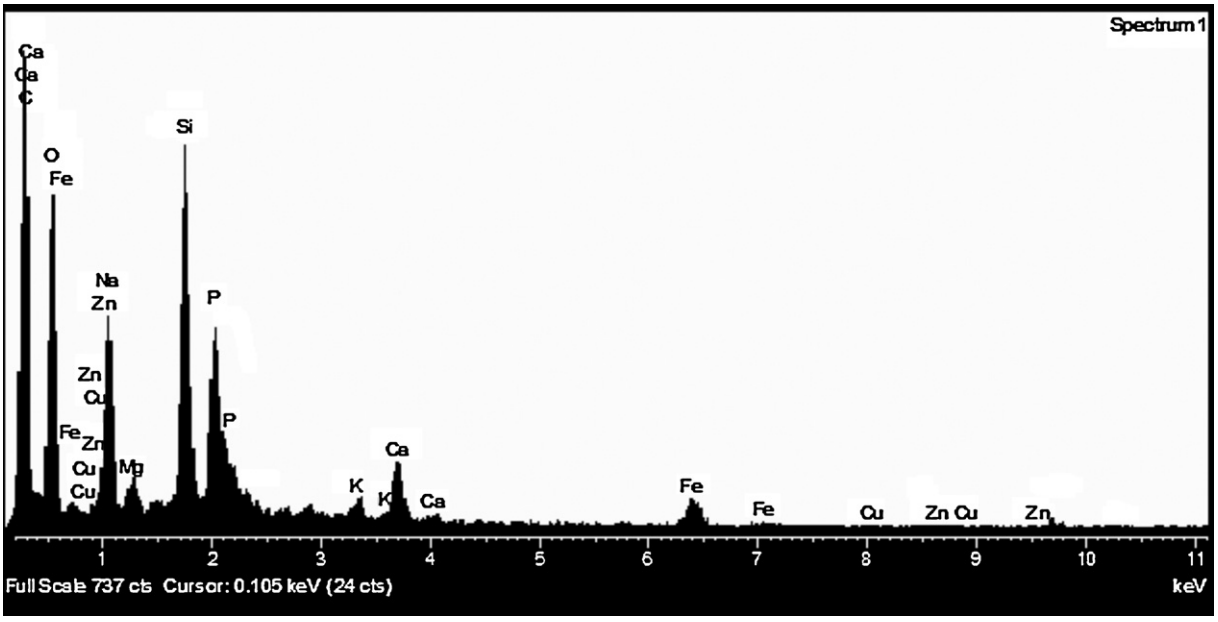

Fig. 9. EDXA spectra obtained from *Synechocystis* sp. E35 grown in a medium supplemented with 10 mg/L Fe (in low mpr).

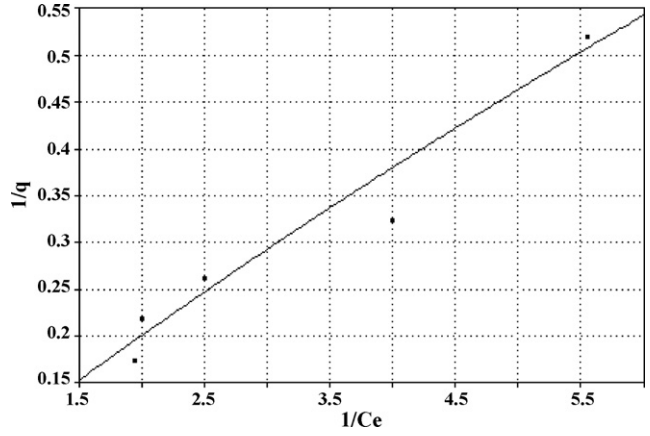

Fig. 10. Langmuir biosorption isotherm.

2.9. Biosorption isotherms

The distribution of metal ions between the liquid phase and the solid phase can be described by several mathematical model equations such as the Langmuir isotherm model and the Freundlich isotherm model [23].

The Langmuir model assumes that the uptake of metal ions occur on a homogenous surface by monolayer biosorption without any interaction between adsorbed ions. Linearized Langmuir equation [24] was given below:

$$\frac{c\theta}{q} = \frac{1}{Q_{\max} b} + \frac{1}{Q_{\max}} C_e \quad (1)$$

C_e (mg/L) represents the solute concentration in the solution at equilibrium; q (mg/g) represents the concentration of solute adsorbed per unit weight of biosorbent at equilibrium. $Q_{\max}$ and b are the Langmuir constants related to maximum biosorption capacity and bonding energy of biosorption, respectively.

The Freundlich model is described by the following equation:

$$q = k_F \cdot C_e \cdot 1/n \quad (2)$$

$$\log q = \log k_F + \frac{1}{n} / \log C_e \quad (3)$$

K_F and n are Freundlich constant. K_F (mg/g) is an indicator of the extent or degree of biosorption and n is a measure of the biosorption intensity.

Biosorption by cyanobacterium (*Synechocystis* sp.) was carried out at five initial Iron concentrations (5, 8, 10, 12, 15 mg/L) at 23 °C for 24 h.

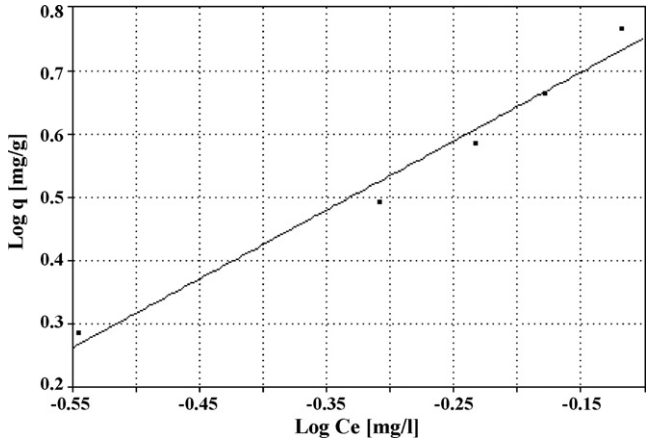

Fig. 11. Freundlich biosorption isotherm.

3. Results and discussion

3.1. Toxicity of Iron on cyanobacterial isolates

The bacterial cells were cultivated in microplates containing growth medium with different concentrations of Fe ions (1, 5, 10, 15, 20 mg/L) to investigate the toxic effect of Iron (Fe) on cyanobacterial isolates (Fig. 2). The effect of Fe toxicity on bacterial isolates was determined after an incubation period of 96 h by evaluating the EC_{10} and EC_{50} values by probit analysis (Table 1). These values were used as estimates of Fe concentrations where statistically significant toxic effects started to appear. EC_{50} at 96 h was 13.92 mg/L for *Synechocystis* sp. E35 suggesting that this isolate is the most tolerant to Iron within the studied species. Furthermore, the influence of different concentrations of Iron on the growth of *Synechocystis* sp. E35 was evaluated according to chlorophyll content (Fig. 3). Compared to the control culture (not receiving Iron), the resistant isolate revealed higher chlorophyll content in the presence of 1 and 5 mg/L Fe concentrations. This suggested that availability of low concentrations of Fe is essential for the growth of the *Synechocystis* sp. E35. However, the effects of higher concentrations of Fe were detrimental and inhibited the growth of the microorganism irreversibly.

Biosorption of heavy metals is affected by many environmental factors such as pH, incubation temperature and ionic strength [25]. To test the responses to these environmental factors, the resistant isolate was incubated at different temperatures (20, 23 and 25 °C). The maximum optical density was observed at 23 °C for the resistant isolate. Furthermore, to find the pH of the medium facilitating optimal growth for the resistant isolate, the cultures were grown in media with pH values ranging from 5.5 to 8.0. An earlier study had shown this pH range to be ideal for the growth of cyanobacterial isolates from Kucukcekmece Lagoon [6]. The resistant isolate achieved optimal growth in the medium with pH 7.

3.2. Biosorption/bioaccumulation of Iron

The uptake of metal ions by microorganisms occurs in two stages: rapid (passive) and slow (active) uptake [2]. During the passive uptake, metal ions are adsorbed onto the surface of cells followed by active uptake resulting in the transportation of the metal ions across the cell membrane and into the cytoplasm.

Taking into account the EC_{50} values and Kucukcekmece Lagoon's metal concentration, we evaluated the time taken for Iron biosorption/bioaccumulation by *Synechocystis* sp. E35 experiments at two concentrations of Fe (10 and 15 mg/L) (Fig. 4). This figure also verifies that metal removal takes place in two stages: very rapid surface biosorption and a slow intracellular accumulation. Similar results were reported by Sing and Yu [26], Volesky and Holan [27], while in some other studies a single-step uptake was suggested for different biosorbents [28].

The bacterial isolate adsorbed appreciable quantities of Iron ions from the aqueous solution to the cell surface in the initial 5 min. Intracellular metal uptake increased with increasing metal concentration. Such rapid uptake of metal ions by living cells is very significant, particularly when the cells are being employed for bioremediation of contaminated sites.

3.3. SEM and EDX analysis

Synechocystis sp. E35 isolate was incubated in BG11 medium without any metal supplement and used as the control culture for SEM analysis (Fig. 5). The elemental composition of the selected areas in SEM images of the control culture was determined by Energy Dispersive X-Ray Analysis (EDXA) (Table 2 and Fig. 6). Analysis revealed that Fe covers 0.33% of the spectrum.

Table 3
Langmuir and Freundlich constants and correlation coefficients of isotherm models for the biosorption of Iron ions from aqueous solutions.

Langmuir constants			Freundlich constants		
$\frac{C_e}{q} = \frac{1}{Q_{max}b} + \frac{1}{Q_{max}} C_e$			$q = k_F \cdot C_e \cdot 1/n$		
Q_{max} (mg/g)	b (L/mg)	R^2	K_F	n	R^2
37.03	0.32	0.954	0.85	0.92	0.978

Most bacteria and cyanobacteria produce extracellular polysaccharides (EPSs). EPS play an important role in the detoxification of heavy metals and radionuclides in contaminated waters and the removal of solid matter from water reservoirs [29,30].

SEM of *Synechocystis* sp. E35 revealed white encrustations on the cell surface possessing a net negative charge like most other bacterial EPSs and indicated the availability of a large number of binding sites for metal ions (Fig. 7). In SEM images, the binding of metal ions on the surface of EPS is clearly visible as bridges connecting two cells. Furthermore, *Synechocystis* sp. E35 isolate incubated in BG11 medium supplemented with Fe was analyzed for EDXA of SEM images in two different zones: a high metal predicted region (high mpr, EPS) and a low metal predicted region (low mpr) (Fig. 8 and Fig. 9). When the high mpr and the low mpr were analyzed with EDX, Fe covered 7.81 and 1.96% of the spectrum, respectively (Table 2). This observation further confirmed the role of EPS in biosorption process.

3.4. Biosorption studies

Isotherms for the biosorption of Iron by live biomass of *Synechocystis* sp. after 24 h of exposure are shown in Figs. 10 and 11. The Freundlich isotherm provides good information ($R^2 = 0.978$) about the Iron uptake by Synechocystis sp. The values of K_F and n obtained from this model were 0.85 and 0.92, respectively. The experimental data also were well correlated to the Langmuir isotherm model with $R^2 = 0.954$. The maximum uptake as derived from Langmuir was 37.03 mg Fe g^{-1} (Table 3).

4. Conclusions

Iron is an essential nutrient for living organism and is required as a cofactor in enzymatic reactions involving the transfer of electrons. However, Fe could be toxic to living organism when supplied in excess. *Synechocystis* sp. E35, isolated from Kucukcekmece Lagoon, was evaluated for its efficacy for accumulating toxic levels of Iron in the growth medium. Our results clearly indicated the potentiality of the isolated cyanobacterial isolates in biosorption/bioaccumulation of Fe. The SEM images further corroborated our results. Since Synechocystis sp. E35 has a high metal-chelating ability, the culture itself or its product (exopolysaccaharide) could be potentially used in situ as a suitable candidate for metal scavenging by biosorption or/and of bioaccumulation for the remediation of Kucukcekmece Lagoon. Further studies involving preference of heavy metal uptake by this cyanobacterium in the presence of other heavy metals are also warranted.

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