Detection of boron removal capacities of different microorganisms in wastewater and effective removal process

Bengü Laçin, Burcu Ertit Taştan and Gönül Dönmez

ABSTRACT

In this study boron removal capacities of different microorganisms were tested. *Candida tropicalis*, *Rhodotorula mucilaginosa*, *Micrococcus luteus*, *Bacillus thuringiensis*, *Bacillus cereus*, *Bacillus megaterium*, *Bacillus pumilus*, *Pseudomonas aeruginosa* and *Aspergillus versicolor* were examined for their boron bioaccumulation capacities in simulated municipal wastewater. *A. versicolor* and *B. cereus* were found as the most boron-tolerant microorganisms in the experiments. Also boron bioaccumulation yield of *A. versicolor* was 49.25% at 15 mg/L boron concentration. On the other hand biosorption experiments revealed that *A. versicolor* was more capable of boron removal in inactive form at the highest boron concentrations. In this paper maximum boron bioaccumulation yield was detected as 39.08% at 24.17 mg/L and the maximum boron biosorption yield was detected as 41.36% at 24.01 mg/L boron concentrations.

Key words | *Aspergillus versicolor*, *Bacillus cereus*, bioaccumulation, biosorption, boron removal

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INTRODUCTION

Boron is an essential element for growth and development of plants. The amount of boron in irrigation water and soil plays an important role in the crop yield and the production quality. Boron participates in the structure of the primary cell wall (Goldbach & Wimmer 2007) and is also associated with membrane transport systems, enzyme interactions, pollen germination, nucleic acid synthesis, phenol and carbohydrate metabolism (Blevins & Lukaszewski 1998; Brown *et al.* 2002; Reid 2007; Wang *et al.* 2014).

Although it is an essential element for plants, high concentrations of boron cause toxic effects. Boron toxicity on growth, stomatal resistance, lipid peroxidation and membrane permeability was demonstrated in grapevine (*Vitis vinifera* L. cv. Kalecik Karasi) at high boron treatments at 20 and 30 mg/kg (Gunes *et al.* 2006). Also it has been demonstrated that higher concentrations of boron (640 mg/L) could damage the organs and even produce toxic effects in rats (Hu *et al.* 2014).

The production of boron additive materials is of increasing interest nowadays. Several industries such as ceramics, glass, frit, detergents and soaps use boron in their processes. Rising boron consumption causes pollution and irreversible environmental problems due to the discharge of untreated boron-polluted waters. Boron compounds are widely available in ground and surface waters. An average boron concentration in soil is 30 mg/kg, with variation of concentration from 2 to 100 mg/kg (Nable *et al.* 1997) and in wastewater is from 1 to 2,000 mg/L depending on the nature of the industry (Türker *et al.* 2014). According to the US Department of Health and Human Services (ATSDR) it is also used in agriculture as a micronutrient and there are 189 pesticide products registered in the United States that contain boric acid or one of its salts as an active ingredient (ATSDR 2010).

Boron deposits are mostly located in Turkey, Argentina, Russia, Kazakhstan, China, Bolivia, Peru and Chile. The major manufacturers are Turkey (Eti Holding Company) and the USA (Rio Tinto Borax). These reserves are managed by MTA (Mineral Research & Exploration General Directorate) and Eti Holding Company in Turkey. Turkey has the richest boron reserves in the world with about 3 billion tonnes of capacity and therefore toxicity of boron has come into more prominence.

When boron concentration is higher than required it needs to be removed in order to decrease the effects of boron toxicity. According to the literature, some different chemical and physical methods have been tried to reduce

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boron pollution in the environment, including electrocoagulation (Yılmaz *et al.* 2008), ultrafiltration (Palencia *et al.* 2014), reverse osmosis (Sassi & Mujtaba 2013) and electron beam injection (Tan *et al.* 2014). But biological boron treatment studies are very limited (Del-Campo Marín & Oron 2007; Bursali *et al.* 2009; Sasmaz & Obek 2009; Taştan *et al.* 2012a). The biological removal process can involve bioaccumulation and biosorption. As reviewed by Chojnacka (2010) biosorption is a passive process and no cellular growth is observed, whereas bioaccumulation is an active process and cellular growth occurs. Biosorption is also not controlled by metabolism and the rate is quick. On the other hand bioaccumulation is controlled by this mechanism and the rate is slow.

The main goals of this study were: (i) to investigate the most boron-tolerant microorganism and use it as an effective biomaterial in boron removal process, (ii) to examine which mechanism, bioaccumulation or biosorption, was the most effective way in this process, and (iii) to increase the boron removal capacity of the most boron-tolerant microorganism via optimizing experimental parameters. Factors affecting the boron bioaccumulation process were comprehensively evaluated by using different microorganisms.

MATERIALS AND METHODS

Microorganisms and culture conditions

Candida tropicalis, Rhodotorula mucilaginosa, Micrococcus luteus, Bacillus thuringiensis, Bacillus cereus, Bacillus megaterium, Bacillus pumilus, Pseudomonas aeruginosa and *Aspergillus versicolor* were obtained from Culture Collection of Biotechnology Research Laboratory, Department of Biology, University of Ankara, Turkey. The strains were grown in synthetic wastewater composed of (Bracklow *et al.* 2007): (g/L) peptone 0.025; sunflower oil 0.035; yeast 0.080; whey powder 0.160; glucose 2; starch 0.2; NH_4COOH 0.15; K_2HPO_4 0.026; KH_2PO_4 0.026; $MgSO_4.7H_2O$ 0.006; urea 0.05; $FeSO_47H_2O$ 0.008. The pH of the growth medium was adjusted to 5 for bacteria (*B. pumilus, B. thuringiensis, P. aeruginosa, B. megaterium, M. luteus, B. cereus*) and 6 for fungi (*R. mucilaginosa, C. tropicalis, A. versicolor*) by dilute and concentrated H_2SO_4 or NaOH, which are the optimum pH values for incubation of these microorganisms. Experiments were performed in 250 mL Erlenmeyer flasks containing 100 mL of sterile growth medium (at 121 °C, for at least 15 min) at known boron concentrations, and 0.1 mL of exponentially growing microorganisms were inoculated into the flasks and incubated on a rotary shaker at 100 rpm at a temperature of 25 ± 2 °C.

Stock solutions

A stock solution of boron was prepared by dilution of boric acid (H_3BO_3) (Carlo Erba) (99%) to a final concentration of 10 g/L of boron. Appropriate volumes of the stock solution were added to the media.

Bioaccumulation experiments

Selection of microorganisms

In order to select the most boron-tolerant microorganisms, nine different strains were cultivated in 250 mL Erlenmayer flasks containing 100 mL of culture media and 20 mg/L of boron for 4 days of incubation. The reported highest boron concentrations in surface waters ranged from 15 to 360 mg/L (ATSDR 2010); therefore we chose 20 mg/L boron concentration. Cell free medium at 20 mg/L boron concentrations was used as the blank. The pH of the growth medium was adjusted to 5 for bacteria and 6 for fungi. Biomass was determined by measuring the maximum dried cell mass for any set of growth conditions. All of the experiments were performed in triplicate.

Effect of pH

The selected microorganisms *A. versicolor* and *B. cereus* were cultivated in 100 mL of culture media at 20 mg/L boron concentration in order to detect the effect of pH on boron bioaccumulation. The pH of the culture media was adjusted to 4, 5, 6 and 7 for *A. versicolor* and 6, 7, 8 and 9 for *B. cereus*. All of the experiments were performed in triplicate.

Effect of increasing boron concentrations

In order to highlight the effect of increasing boron concentrations on boron removal process, the experiments were conducted at 16.20, 19.20 and 24.17 mg/L boron concentrations. *A. versicolor* was cultivated in 100 mL of culture media at selected boron concentrations in 250 mL Erlenmeyer flasks. All of the experiments were performed in triplicate.

Biosorption experiments

Biomass preparation

A. versicolor was harvested after the incubation period. Harvested biomass was washed twice with distilled water and then dried overnight at 80 °C. The same amount of dried cells as for the bioaccumulation experiments was used for the biosorption experiments.

Boron biosorption

The biosorption experiments were performed in 250 mL Erlenmeyer flasks containing 100 mL of sterile distilled water (at 121 °C, for at least 15 min). Fungal cells were incubated on a rotary shaker at 100 rpm at 25 ± 2 °C. During the incubation period, samples were taken at 0, 5, 15, 30, 60, 240 min and 24 h for boron analysis.

Analytical methods

During the incubation period, 3 mL samples were taken daily from each of the flasks. The boron concentration was determined by measuring the absorbance at 585 nm with a Shimadzu UV 2001 model spectrophotometer using carmine as the complexing reagent (Adams 1990). The percentage removal of boron and q_m (the maximum specific boron uptake) was calculated from Equation (1) and Equation (2), respectively:

$$Y = (C_0 - C_t)/C_0 \times 100 \quad (1)$$

$$q_m = (C_0 - C_t)/X_m \quad (2)$$

In the study, Y is the percentage boron removal, q_m represents the maximum amount of boron removal per unit dry weight of fungal cells (mg/g), X_m maximum dried cell mass (g/L), C_0 the initial concentration of boron (mg/L) and C_t the final concentration of boron (mg/L).

Biomass was determined by measuring the maximum dried cell mass for any set of growth conditions. The maximum dried cell mass was determined by the measurement of the pellets, which were dried at 80 °C for overnight (Nüve FN 400 model sterilizer) after filtration of the cells with filter papers. Cell free medium at different boron concentrations was used as the blank. All of the experiments were performed in triplicate.

RESULTS AND DISCUSSION

Microorganisms and boron tolerance

As seen in Table 1 bacterial boron removal yield was a bit lower than fungal removal yield. *B. pumilus* showed the minimum boron tolerance with a yield of 9.11%. The highest boron removal yield was obtained as 12.86% by *B. cereus* and this bacterial strain was selected as the most boron-tolerant bacteria in this study and also it has a fast growth rate. The results given in Table 1 show that *A. versicolor* was the most boron-tolerant microorganism in the fungal group. Fungus reached 24.39% removal yield at the fourth day of incubation period. As seen in Table 1 boron removal yield of *A. versicolor* was 2.5 times higher than that of *R. mucilaginosa*. Also there was no important change in the blank medium. The selected microorganisms (*A. versicolor* and *B. cereus*) are very important biomaterials in biotechnological studies (Romero *et al.* 2002; Wu *et al.* 2006; Taştan *et al.* 2010, 2012b). Among them *A. versicolor* showed a good boron uptake efficiency due to its fast growth rate.

pH experiments

One of the most important parameters in the boron removal process is pH because it is not only a factor affecting the culture media but also is important for the fungal boron removal capacity. Thus pH of the culture media was adjusted to 6, 7, 8 and 9 for *B. cereus* and 4, 5, 6 and 7 for *A. versicolor* (Figure 1(a) and (b)). As seen in Figure 1(a) the maximum boron removal yield was obtained at pH 6 as 12.86% by *B. cereus*. The boron removal yield decreased when pH increased from 6 to 8. On the other hand 10.31%

Table 1 | Boron bioaccumulation yields of different microorganisms in synthetic wastewater

	Microorganisms	Y %
Bacteria	*B. pumilus*	9.11 ± 0.59
	B. thuringiensis	9.82 ± 1.29
	P. aeruginosa	10.81 ± 1.27
	B. megaterium	11.44 ± 1.90
	M. luteus	11.53 ± 1.23
	B. cereus	12.86 ± 1.33
Fungi	*R. mucilaginosa*	10.72 ± 0.17
	C. tropicalis	19.03 ± 2.38
	A. versicolor	24.39 ± 0.52

T: 25 ± 2 °C.
Stirring rate: 100 rpm.
C_0, initial boron concentration: 20 mg/L.
Y%: percentage of boron bioaccumulation.
Incubation time: 4 days.

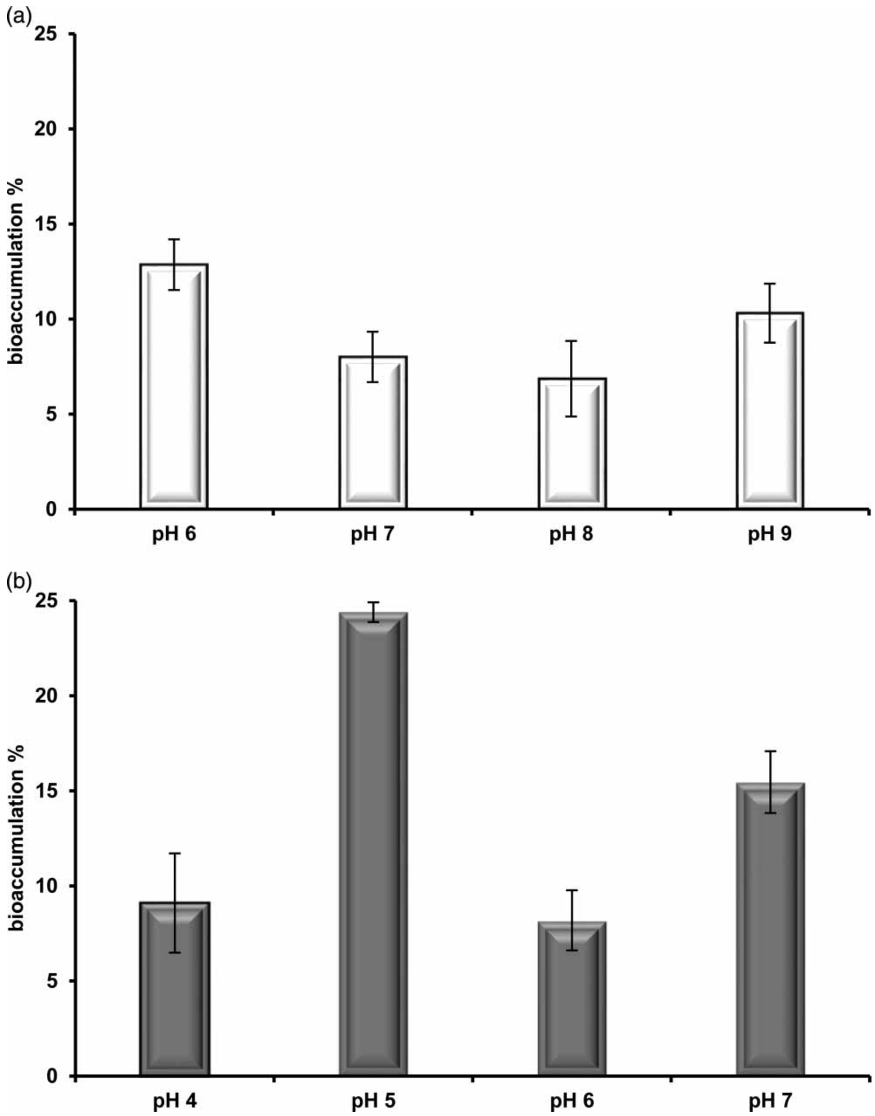

Figure 1 | The effect of increasing pH values on the boron bioaccumulation yields (Y%) of B. cereus (a) and *A. versicolor* (b) in synthetic wastewater after 4 days of incubation period (T: 25 ± 2 °C; stirring rate: 100 rpm).

boron removal yield was obtained at pH 9. According to these results pH 6 was selected as the optimum pH level for *B. cereus* in this process (Figure 1(a)).

On the other hand *A. versicolor* showed a good boron bioaccumulation yield at pH 5. The maximum boron removal yield was 24.39% at this pH value. The minimum boron bioaccumulation yield was recorded at pH 6 as 8.19% (Figure 1(b)).

According to the previous studies optimum pH values show difference between elements (Cr(VI), Cu(II), Ni(II), Cd(II)) (Dursun *et al.* 2003; Preetha & Viruthagiri 2007) and biomaterials (*Chlamydomonas reinhardtii)* (Flouty & Estephane 2012; Abboud & Wilkinson 2013).

Bioaccumulation experiments

The effect of increasing boron concentrations on bioaccumulation process by *A. versicolor* was investigated at 16.20, 19.20 and 24.17 mg/L initial boron concentrations. Cell free medium at these boron concentrations was used as the blank. There was no important change for the blank medium. Figure 2 shows the effect of increasing boron concentrations on fungal boron bioaccumulation yield. As seen in Figure 2 *A. versicolor* could accumulate only 25.02% boron at the second day of the incubation period at 16.20 mg/L boron concentration. *A. versicolor* reached 49.25% boron removal yield at the end of the incubation period at this

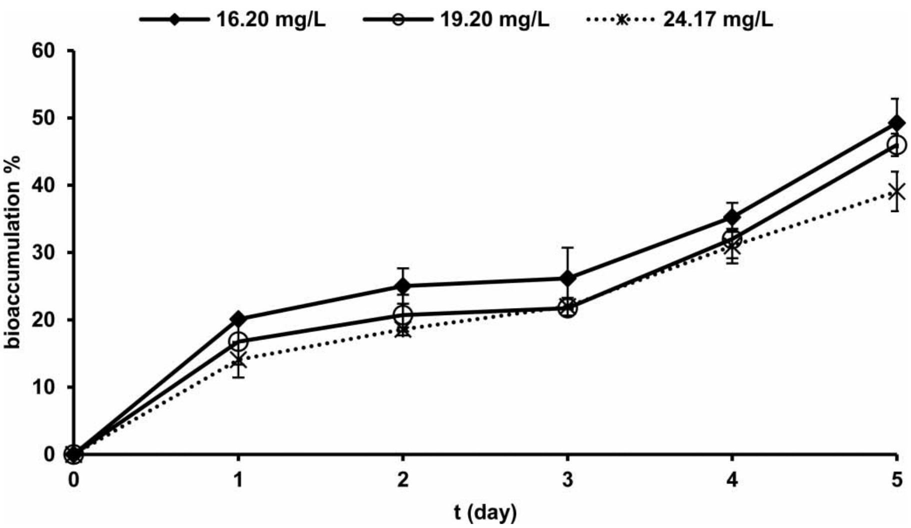

Figure 2 | The effect of increasing boron concentrations on the bioaccumulation yields (Y%) of *A. versicolor* in synthetic wastewater after 5 days of incubation period (T: 25 ± 2 °C; pH: 5; stirring rate: 100 rpm).

boron concentration. The minimum boron removal yield was obtained at the highest boron concentration. The boron accumulation yield was 39.08% at 24.17 mg/L boron concentration after 5 days of incubation.

The maximum specific boron uptake (q_m) was also calculated and is shown in Table 2. The highest q_m value was obtained at the highest boron concentration. The q_m was 21.45 mg/g at this concentration. On the other hand the minimum q_m value of 17.32 mg/g was obtained at the minimum boron concentration. The reason why the q_m value was higher at the highest boron concentration was due to the existing high boron concentrations in the culture media. Therefore the maximum fungal boron uptake was high at this concentration.

There are only a few boron bioremoval studies in the literature. In one of them *Lemna gibba* was tested for its boron removal efficiency at 0.3–10 mg/L boron concentration. Less than 2 mg/L boron was efficiently removed by *L. gibba* from water (Del-Campo Marín & Oron 2007). The other study revealed that novel isolate *Chlorella* sp. showed an excellent boron removal yield of 38.03% at 9.19 mg/L boron concentration (Taştan *et al.* 2012a).

Table 2 | The bioaccumulation yields (Y) and q_m values of *A. versicolor* at different boron concentrations in synthetic wastewater

C_0 (mg/L)	Bioaccumulation %	q_m (mg/g)
16.20	49.25 ± 3.58	17.32 ± 1.56
19.20	46.00 ± 1.66	20.53 ± 2.53
24.17	39.08 ± 2.93	21.45 ± 1.37

T: 25 ± 2 °C.
Stirring rate: 100 rpm.
Incubation time: 5 days.

Biosorption experiments

The fungal biosorption experiments were followed for 24 h at 16.20, 22.02 and 24.01 mg/L boron concentrations. Contrary to bioaccumulation experiments biosorption yield increased when boron concentration increased. The highest boron biosorption yield was obtained as 41.36% at 24.01 mg/L boron concentration at 15 minutes. The minimum boron biosorption was recorded at the minimum boron concentration (16.20 mg/L) as 5.32%. It took only 15 minutes to uptake 24.01 mg/L boron concentration in biosorption experiments instead of 5 days in bioaccumulation experiments. It can be suggested that biosorption is a more suitable method for the boron removal process at the highest concentrations. On the other hand bioaccumulation could be a more feasible method at the lowest boron concentrations.

As seen in Figure 3 biosorption yields increased at all of the boron concentrations up to 30 minutes and the maximum yields were obtained at this time. All of the boron removal yields decreased sharply after this time.

The maximum fungal boron uptake was also examined in biosorption experiments. As seen in Table 3 q_m values increased when initial boron concentrations increased. The maximum q_m was obtained at the highest boron concentration as 33.71%. When comparing the q_m values between bioaccumulation and biosorption experiments it is obviously

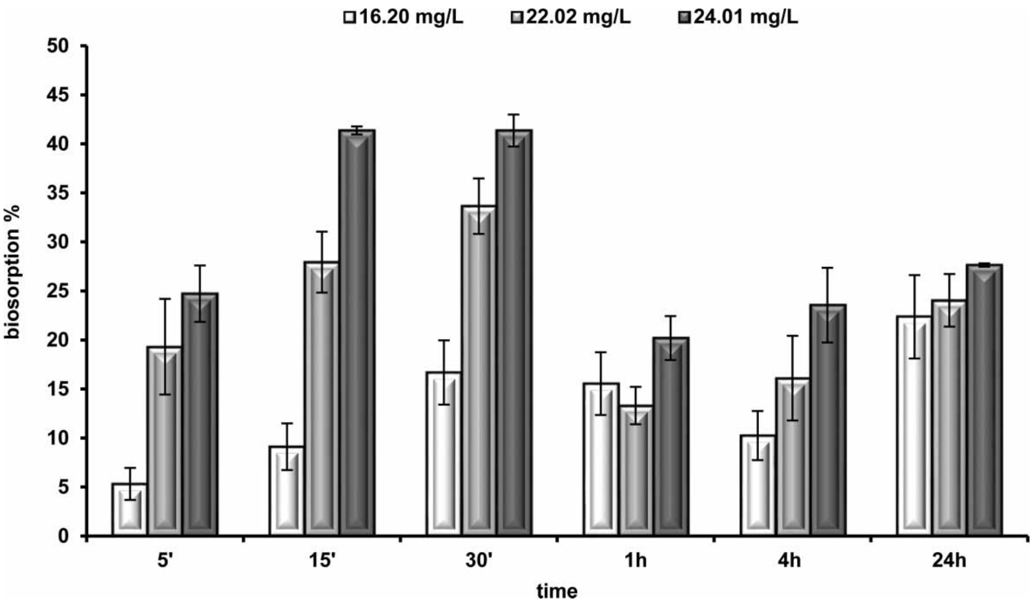

Figure 3 | The effect of increasing boron concentrations on the biosorption yields (Y%) of *A. versicolor* in dH_2O after 24 h of biosorption period (T: 25 ± 2 °C; pH: 5; stirring rate: 100 rpm).

Table 3 | Biosorption yields and q_m values (mg/g) at different boron concentrations of *A. versicolor* in dH_2O

C_0 (mg/L)	Biosorption %	q_m (mg/g)
16.20	16.68 ± 0.92	4.21 ± 0.13
22.02	33.64 ± 2.36	17.25 ± 0.06
24.01	41.36 ± 0.53	33.71 ± 0.46

T: 25 ± 2 °C.
Stirring rate: 100 rpm.
pH: 5.
Biosorption time: 30 min.

seen that biosorption is a more suitable method than bioaccumulation. Biosorption could serve as a fast method for boron removal; conversely bioaccumulation could serve as a high-capacity boron removal method.

As summarized in Table 4 fungal biomass not only has the ability to remove boron but also has the ability to remove other metals such as Cr(VI), Cu(II), Ni(II) and Zn(II). In our previous study *A. versicolor* accumulated Cr(VI), Cu(II) and Ni(II) at the highest removal yields (Taştan *et al.* 2010). For example the maximum Cr(VI)

Table 4 | Comparision of the bioaccumulation and biosorption yields of different elements by fungal biomass

Microorganism	Element	Concentration (mg/L)	Bioaccumulation %	Biosorption %	Reference
A. versicolor	B	16.20 mg/L	49.25		Present work
		24.01		41.36	Present work
A. versicolor	Cr(VI)	50 mg/L	99.89		Taştan *et al.* (2010)
	Cu(II)	50	29.06		
	Ni(II)	50	30.05		
A. versicolor	Cr(VI)			15.82	Taştan & Dönmez (2010)
	Cu(II)			28.47	
	Ni(II)			14.59	
Rhizopus arrhizus	Cr(VI)	25 mg/L	93.84		Preetha & Viruthagiri (2007)
	Cu(II)	25	95.52		Preetha & Viruthagiri (2007)
	Ni(II)	25	61.44		Preetha & Viruthagiri (2007)
A. niger	Ni(II)	30 mg/L		70.30	Amini *et al.* (2009)
Fusarium spp.	Zn(II)	100 mg/L		42.21	Velmurugan *et al.* (2010)

removal was 99.89% at 52.5 mg/L Cr(VI) concentration. The maximum specific Cr(VI) uptake was 7.78 mg/g at this concentration. In the present work the maximum boron bioaccumulation yield of *A. versicolor* was 49.25% and biosorption yield was 16.68% at 16.20 mg/L boron concentration. In another study the maximum boron bioaccumulation yield of *Chlorella* sp. was 38.03% at 9.19 mg/L boron concentration (Taştan *et al.* 2012a).

Many physical and chemical methods for boron removal are available in the literature. However, according to our best knowledge, there is no report available detecting bioaccumulation or biosorption yields of boron by *A. versicolor*.

CONCLUSIONS

Boron pollution is an important problem in the world, especially in the countries where boron deposits are high. Boron removal from the environment using efficient and economical methods is still a challenging problem. Our effort is to contribute to solving this problem by environmentally friendly methods and also to highlight the potential bioaccumulation effect of fungi. In this study different microorganisms were examined for their boron removal capacities in aquatic medium. The fungal boron removal process was also tested by bioaccumulation and biosorption methods. *B. cereus* and *A. versicolor* were found as the most boron-tolerant microorganisms. *A. versicolor* also showed a good tolerance against increasing boron concentrations. The maximum boron bioaccumulation yield was 49.25% and the maximum biosorption yield was 41.36% at 16.20 mg/L and 24.01 mg/L boron concentrations, respectively. However, the small size of the boron treatment process cannot fulfill the large-scale requirements in industrial applications, but our process can be a basis for and feasible in industrial applications due to the obtained effective results using synthetic wastewater, which was used in order to decrease the cost of the treatment process. Results obtained in biosorption experiments can also help to shorten the treatment method for industrial applications.

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