Chromium (VI) Biosorption from Aqueous Solutions by Free and Immobilized Biomass of *Oscillatoria* sp. H1 Isolated from Freshwater

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The *Oscillatoria* sp. H1 (Cyanobacteria) isolated from Mogan Lake was researched for the removal of chromium (VI) ions from aqueous solutions and were used free (dry biomass), immobilized (in Ca-alginate) live and immobilized heat-inactivated biomass as biosorbents. Particularly, the effects of physico-chemical parameters like pH, the temperature change, initial concentration, biosorption time and biosorbent dosages on the biosorption of Cr (VI) ions were investigated. The biosorption of Cr (VI) ions for all biosorbents was determined as a highest value at pH 6.0. The temperatures which changed between 20 and 40°C did not affect the biosorption capacity.

The biosorption of Cr (VI) ions on both free (dry) and immobilized (live and heat-inactivated) *Oscillatoria* sp. H1 biomass (mg/g) increased as the initial concentration of Cr (VI) ions was increased in the medium. Biosorption equilibrium was established in about 60 min. The retention of Cr (VI) increased with increasing the amount of the adsorbent up to 0.04 g and 30 bead. The results show that immobilized inactive cells (13.83 mg/g) and dry biomass (15.81 mg/g) had less biosorption capacity than that of the immobilized live form (20.82 mg/g). The Langmuir and Freundlich isotherm models were used to fit the equilibrium biosorption data. The biosorbent systems could be regenerated by washing with a solution of 10 mM HCl. The percent desorption achieved was as high as 98%. The biosorbents were reused in three biosorption-desorption cycles without significant loss of their initial biosorption capacity. The results indicated that the immobilized live *Oscillatoria* sp. H1 could be suitable for development of an efficient biosorbent for the removal of chromium (VI) from wastewater from some industrial processes and other ones.

KEY WORDS: Cr (VI); biosorption; microalgae; Ca-alginate; *Oscillatoria* sp.

1. Introduction

Widespread contamination of freshwater with various toxic and non-biodegradable heavy metals poses several health problems and consequently need to be decontaminated to standard permissible levels. The chromium (Cr) in the effluents of industries like leather tanning, electroplating, textile dyeing and metal finishing exists in hexavalent and trivalent forms and the former is more toxic and may cause cancer in the digestive tract and lungs of human beings.¹⁾ Paint manufacturing, ink formulating and dye house wastewaters contain chromium concentrations ranging from 0.4 to 7.5 mg/L, 150 mg/L and 300 mg/L, respectively. According to the US Environmental Protection Agency, the acceptable amount for heavy metal ions is usually less than 1.0 mg/L.²⁾ Moreover, in environment air, chromium particulates play a role in the oxidation of sulfur dioxide and the formation of acidic aerosols involved in global acid rain.³⁾

Conventional techniques for removing dissolved heavy metals include precipitation, adsorption and ion-exchange. The conventional methods for removing metals from waste-

waters are generally expensive and have many limitations. For iron and steel manufacturing, wastewater treatment systems typically include sedimentation to remove suspended solids, physical or chemical treatment such as pH adjustment to precipitate heavy metals, and filtration. In recent years, alternative methods for metal removal and recovery based on biological materials have been considered. Bioremoval involves a combination of active and passive transport mechanisms. The first stage, usually referred to as passive uptake, is an initial rapid and reversible accumulation step. This kind of adsorption is also termed biosorption.²⁾ Biosorption and/or bioaccumulation has emerged as a cost-effective and efficient alternative method. Biosorption utilizes the ability of biological materials to accumulate heavy metals from waste streams by either metabolically mediated or purely physico-chemical pathways of uptake. A wide range of non-living biomass like bark, lignin, peanut, hulls, as well as living biomass like fungi, bacteria,⁴⁾ yeast, moss, aquatic plants⁵⁾ and algae has been used as biosorbents.⁶⁻¹¹⁾

Algal biosorbents for metal removal from aqueous solu-

tion are in use for quite some time and for this purpose both live and dead mass of algae have been tried and reported as biosorbents.^{11,12)} Therefore, there are some reports on the use of immobilized algae as biosorbents.¹⁾

Cyanobacteria have a great potential for use as effective biosorbents, because they are easy to grow with simple nutrient requirements and unlike other microbial systems, they generally do not produce toxins. Further, cyanobacteria produce extracellular polysaccharides that have a tendency of binding metals. There are some reports on biosorption of heavy metals like copper, nickel, zinc and chromium by certain species of cyanobacteria and microalgae,¹⁾ but there are few studies on biosorption of metals by *Oscillatoria* sp. that can be conveniently used for biosorption studies. Also, as a biosorbent, this strain is both cost-effective and non-toxic.⁷⁾

The aim of this study was to determine the ability as a biosorbent of *Oscillatoria* sp. H1 for the removal of chromium (VI) from aqueous solutions and compare the adsorption behavior of different biosorbent forms: dry biomass, immobilized live and heat-inactivated *Oscillatoria* sp. H1.

2. Material and Methods

2.1. Microorganism and Media

The test organism *Oscillatoria* sp. H1 was isolated from freshwater samples (from Mogan Lake in Ankara, Turkey) and pure culture was grown on BG-11 medium [NaNO_3 , 15; K_2HPO_4 , 0.4; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.75; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.36; citric acid, 0.06; iron(III) ammonium citrate, 0.06; $\text{Na}_2\text{-EDTA}$, 0.01; Na_2CO_3 , 0.2 g/L, 1 mL; trace elements solution, (H_3BO_3 , 61; $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 169; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 287; $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 2.5; $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$, 12.5 mg/L) pH: 6.8] which is commonly used for growing blue-green algae in flasks. This medium contains only trace amounts of metal ions and allows rich growth. This strain was determined according to Bone *et al.*¹⁴⁾ and pure culture of the cyanobacterium was obtained by using standard isolation and culturing techniques.¹³⁾

The algal cultures in sterile shake flasks containing 100 mL BG 11 were maintained at a light intensity of 3 000 lux using cool fluorescent tubes at 25°C in an incubator (Minitron) suitable for photosynthesis. Fifteen-day-old algal cultures were harvested and the algal biomass was washed with distilled water and oven dried at 100°C for 2 h before use.

2.2. Immobilization of *Oscillatoria* sp. H1

The *Oscillatoria* sp. H1 was immobilized on Ca-alginate beads via entrapment by the method given by Chen¹⁵⁾ and Ergene *et al.*⁷⁾ Ca-alginate (2.0 g, from *Macrosytia pyrifera*, high viscosity, Sigma Chem., Co., U.S.A.) was dissolved in 100 mL of sterile distilled water and mixed with the algal biomass. The mixture was then introduced drop-wise into 0.1 M CaCl_2 solution with a burette, while stirring to prevent aggregation of Ca-alginate beads. The beads, approx. 4 mm in diameter, were cured in the solution for about 1 h, and then washed for three times with 150 mL of sterile distilled water. The cured beads were stored in 5 mM CaCl_2 solution at 4°C until use. The dry weight of the prepared biomass was determined after drying the alginate beads overnight in an oven at 50°C.

2.3. Reagents

All chemicals were of analytical reagent grade unless otherwise stated. Double distilled deionized water was used throughout the study. Chromium stock solution (1 000 mg/L) was prepared by dissolving a calculated amount of $\text{K}_2\text{Cr}_2\text{O}_7$ (Merck). The working solutions were prepared by diluting the stock solution to appropriate volumes. Britton-Robinson (B-R) buffer solution was prepared by dissolving 2.3 mL of glacial acetic acid, 2.7 mL of phosphoric acid and 2.472 g of boric acid in double distilled water and diluted to 1.0 L. 100 mL portions of this solution were taken and the desired pH was adjusted between 2.0 and 8.0 by addition of appropriate amount of 2.0 M NaOH.¹⁶⁾

2.4. Apparatus

A Philips PU 9285 model flame atomic absorption spectrometer (AAS) equipped with deuterium lamp background correction, hollow cathode lamp (HCL) and air acetylene burner was used for the determination of chromium. Absorption measurements were performed under the following conditions: wavelengths, 357.9 nm; fuel flow rate, 1.6 L min⁻¹; HCL lamp current, 9.0 mA; band pass, 0.5 nm and integration time 4 s. All pH measurements were performed with a METTLER-TOLEDO model digital pH meter.

2.5. Batch Procedure

Biosorption of Cr (VI) on dry biomass, immobilized live and heat-inactivated preparations from aqueous solutions was investigated in batch biosorption experiments at 25°C. The heat-inactivated preparation was obtained by boiling the live algae in water for 10 min. The removal of chromium was tested with synthetic chromium solutions (containing 10 mg of chromium in 100 mL). Dry biomass (0.04 g) was added to the synthetic solution. The pH of the solution was adjusted to 6.0 by addition of the Britton-Robinson (B-R) buffer. Then the mixture was agitated using a shaker for 60 min at 25°C. Determination of Cr (VI) ion was performed in liquid phase (supernatant) by flame atomic absorption spectrometry. In order to demonstrate the reusability of the biosorbents, the mixture was filtered through the filter and the retained chromium on the biomass was eluted by 50 mL of 10 mM HCl solution.

Each set of experiments was carried out in triplicate, and the mean values were used in the analyses of data. Five measurements for each sample were done to calculate the mean value. Calibrations were performed within a linear calibration range of Cr (VI), and the calibration curves with correlation coefficients less than 0.990 were repeated. The difference between the initial and remaining metal ion concentrations was assumed to be taken up by the biosorbent.

2.6. Desorption

In order to determine the reusability of the biosorbents, consecutive biosorption-desorption cycles were repeated four times using the same cyanobacteria preparations. Desorption of Cr (VI) was performed by HCl (10 mM, 50 mL). The dry biomass and alginate beads loaded with Cr (VI) were placed in the desorption medium and were stirred at 100 rpm for 1 h at 25°C. After each cycle of adsorption-desorption, biomass was washed with saline solution.⁸⁾ The

final chromium ion concentration in the aqueous phase was determined by using an AAS as described above. Desorption ratio was calculated from the amount of metal ions adsorbed on the dry biomass and immobilized preparations and the final metal ions concentration in the adsorption medium. Desorption ratio was calculated from the following equation:⁹⁾

Desorption ratio =

$$\frac{\text{Amount of Cr(VI) ions desorbed to the elution medium}}{\text{Amount of Cr(VI) ions adsorbed onto biosorbent}} \times 100$$

2.7. Selection of Microorganism

A filamentous cyanobacterium *Oscillatoria* sp. H1 was used in this study. It is known that the filamentous cyanobacterium has a wide polysaccharide capsule surrounding its entire filament.¹⁷⁾ Some cyanobacterial species such as *Oscillatoria*, *Spirulina* and *Gleocapsa* synthesize extracellular organic material such as siderophores with metal complexing properties. In cyanobacteria, Kelly¹⁸⁾ demonstrated that heavy metals are accumulated in intracellular storage compartments, called polyphosphate bodies, where metals accumulate, protect algal cells from toxicity. Also, *Oscillatoria* species (cyanobacteria) are easy to grow with simple nutrient requirements and unlike other microbial systems, they generally do not produce toxins. Therefore, this microorganism was selected for the removal of Cr (VI).

2.8. Properties of the Alginate as Support Material

Alginates were preferred over other materials because of their various advantages such as biodegradability, hydrophilic properties, presence of carboxylic groups and natural origin. Other additional advantages are their low density and mechanical stability that make them highly suitable for many biotechnological applications.⁶⁾ For these properties, in this study, the Ca-alginate beads were used as a support material for the entrapment of *Oscillatoria* sp.

3. Results and Discussion

3.1. Cr (VI) biosorption

3.1.1. Effect of pH and Temperature

Metal ion biosorption onto biosorbents is a pH dependent process. The metal biosorption depends on the protonation or unprotonation of these functional groups on the surface of the cell wall. The ionic forms of the metal ions in solution and the electrical charge of the algal cell wall components (*i.e.* functional groups carrying polysaccharides and proteins) depend on the solution pH.⁸⁾ Crist *et al.*¹⁹⁾ suggested that isoelectronic point would be found at pH 3.0 for the algal biomass. Above this pH, algal cells would have a net negative charge which would lead to increase in electrostatic attractions between positively charged metal ions and negatively charged binding sites, hence a rapid rise in binding efficiency between pH 4.5 and 5.0.

The effect of pH on the uptake of chromium ion (100 mg/L) by all biosorbents was studied in the pH range from 2.0 to 8.0 adjusting by a Britton-Robinson (B-R) buffer. And quantitative retention of chromium was obtained about at pH 6 (Fig. 1). Within the pH range of 2.0 to 6.0, Cr (VI) uptake capacities of all biosorbents increased rapidly. Kiran

Fig. 1. Effect of initial solution pH on biosorption of Cr (VI).

Fig. 2. Effect of temperature (°C) on biosorption of Cr (VI).

*et al.*¹⁾ reported that the maximum adsorption of Cr (VI) ions on immobilized *Lyngbya putealis* biomass was also observed at pH 2–3. Han *et al.*²⁰⁾ reported that the maximum adsorption of Cr (VI) ions on the dry biomass of *Chlorella miniata* was observed at pH -3. However, Congeevaram *et al.*²¹⁾ reported that the maximum removal of Cr (VI) for bacterial and fungal adsorbents was observed around pH 5–6. Baran *et al.*²²⁾ reported that the pH between 3 and 7 is widely accepted as optimum for metal uptake for almost all types of sorbents.

The effect of temperature on the metal biosorption experiments was investigated at five different temperatures (from 20 to 40°C) and the maximum biosorption capacity was found at 25°C (Fig. 2). Several researchers reported the similar finding for the optimum biosorption temperatures.^{23,8,20)} The temperature of the adsorption medium could be important for energy dependent mechanisms in metal biosorption by microbial cells. Generally, adsorption is an exothermic process, although some examples of endothermic adsorption have been reported.⁸⁾

3.1.2. Effect of Contact Time on the Biosorption of Cr (VI)

The effect of contact time on the uptake of Cr (VI) (100 mg/L) by dried biomass and live-heat-inactivated *Oscillatoria* sp. immobilized alginate beads was investigated in different durations (0, 5, 10, 15, 30, 60, 90, 120 min). The Cr (VI) adsorption rate was high at the beginning of adsorption and the saturation levels were completely reached at about 60 min for Cr (VI) ions (Fig. 3). After this equilibrium period, the amount of adsorbed Cr (VI) ions on

Fig. 3. Effect of contact time on the uptake of Cr (VI).

the biosorbents did not significantly change with time. This trend in binding of Cr (VI) ions suggests that the binding may be through interactions with functional groups located on the surface of the biosorbents.

Cr (VI) biosorption of dry biomass, immobilized live and heat-inactivated *Oscillatoria* sp. could be divided into two stages; a fast initial rate followed by a much slower biosorption rate. Similar results have been reported by other researchers. Arica *et al.*⁶⁾ divided the Cr (VI) biosorption of free and immobilized fungus into two stages; a first stage with a high rate and a much slower second one lasting approximately 150 min. To reach biosorption equilibrium. Chojnacka *et al.*¹¹⁾ attributed the fast initial metal biosorption rate to the surface binding by natural particles, and the following slower sorption to the interior penetration.

3.1.3. Effect of Initial Concentration of Cr (VI) on Biosorption

The effect of the initial concentration of chromium (VI) ion on the uptake of Cr (VI) by dry biomass, alginate beads and both immobilized live and heat inactivated *Oscillatoria* sp. was also studied in the range of 25–200 mg/L (Fig. 4). The results showed that the retained Cr (VI) increased by increasing the concentration of Cr (VI) in solution. When the initial Cr (VI) concentration was increased from 25 to 200 mg/L, the retained chromium increased approximately from 2.7 to 20.82 mg/g for the dry biomass, live and heat inactivated *Oscillatoria* sp., respectively. The results showed that immobilized inactivated cells and dry biomass had less available adsorptive sites for biosorption of metal ions than that of the immobilized live form (13.83, 15.81 and 20.82 mg/g). Arica and Bayramoğlu²⁴⁾ reported that the amount of biosorbed Cr (VI) ions on the CMC (carboxymethylcellulose), free and immobilized fungus preparation were 5.1, 18.9 and 32.3 mg/g dry adsorbents.

3.1.4. Effect of Amount of Adsorbent (Bead and Dry Weight)

The retention of Cr (VI) ions was examined in relation to the amount of adsorbent. For this purpose, the amounts of adsorbent were tested in a range of 0.02–0.1 g and 20–40 bead. It was found that the retention of Cr (VI) ions increased with increasing the amount of the adsorbent up to 0.04 g and 30 bead. Above these amounts, it practically did not change.

Fig. 4. Effect of initial Cr (VI) concentration (temperature: 25°C, pH: 6.0, agitation rate: 100 rpm, contact time: 60 min).

Fig. 5. The linearized Freundlich adsorption isotherms of Cr (VI).

3.2. Langmuir and Freundlich Adsorption Isotherms

An adsorption isotherm is characterized by certain constants the values of which express the surface properties and affinity of the sorbent. Out of several isotherm equations the two most commonly used, the Langmuir and Freundlich adsorption isotherms have been investigated in this study. In this study, both models were applied to describe the relationship between the adsorbed amount of chromium and its equilibrium concentration in solution.

During biosorption, a rapid equilibrium is established between adsorbed metal ions on biosorbent (q) and unadsorbed metal ions in solution (C). The Langmuir equation suggests the monolayer sorption on to a surface containing finite number of identical sites. Its linearized form is represented by the following equation.²⁵⁾

$$\frac{C}{q} = \frac{1}{Q_{\max} b} + \frac{C}{Q_{\max}}$$

where, C is the concentration of chromium in solution (mg/L) at equilibrium, q is the amount of adsorbed chromium per gram of adsorbent at equilibrium (mg/g), b is the Langmuir constant related to the energy of adsorption (l/mg) and $Q_{\max}$ is the adsorption capacity of the adsorbent (mg/g). Based on the linearized form of the adsorption isotherm derived from plots of C/q versus C , the constant $Q_{\max}$ values were calculated from the slope of graph.²⁵⁾ The empirical Freundlich equation based on sorption on a heterogeneous surface is given by the following equation:

$$q = K_F \cdot C^{1/n}$$

Table 1. Langmuir and Freundlich parameters for the adsorption isotherms of adsorbent for Cr (VI) ion.

	Langmuir			Freundlich		
	Q_m	b	R^2	K_F	n	R^2
Dried biomass	36.50	3.90×10^{-3}	0.984	0.27	1.27	0.980
Ca-Alginate	31.25	3.17×10^{-3}	0.987	0.18	1.24	0.990
<i>Oscillatoria</i> sp. (live)	39.06	5.80×10^{-3}	0.988	0.57	1.45	0.990
<i>Oscillatoria</i> sp. (inactivated)	34.25	3.40×10^{-3}	0.978	0.21	1.26	0.990

Q_m (mg/g)
 b is Langmuir adsorption constant (l/mg)
 R^2 is correlation coefficient

$$\log q = \log K_F + (1/n \log C)$$

where, K_F and n are the Freundlich constants. K_F and n are indicators of adsorption capacity and adsorption intensity, respectively. The Freundlich isotherm has generally been considered an empirical relationship and has been widely used to fit the experimental data (Fig. 3). The Freundlich constants K_F and n were obtained from the linearized logarithmic plot of Eq. 2.²⁶⁾ The plots of linearized Langmuir and Freundlich adsorption isotherms were obtained from the conducted equilibrium biosorption experiments at 25°C and pH 6. The Langmuir and Freundlich adsorption constants evaluated from these isotherms are also given in **Table 1**. The correlation coefficients of the isotherms are found to be high enough for all of the sorbate-sorbent systems.

Freundlich parameters indicated a serious increase in Cr (VI) uptake by the immobilization of *Oscillatoria* sp. in Ca-alginate beads. The maximum biosorption capacities of dried biomass and immobilized live *Oscillatoria* sp. were observed to be higher than the maximum adsorption capacity of the plain alginate beads and immobilized heat inactivated, respectively. Also, the maximum biosorption capacity of the immobilized live *Oscillatoria* sp. was found to be higher than the adsorption capacity of the dried biomass and immobilized heat inactivated counterpart. Although, the obtained low b values implied a weak bonding of Cr (VI) to the biosorbents, the magnitude of Freundlich constants, K_F and n , showed easy uptake of Cr (VI) ions from the aqueous medium.

3.3. Reusability of Biosorbent

Desorption of the adsorbed Cr (VI) ions from the biosorbent was studied in a batch system. In order to show the reusability of the biosorbent the adsorption-desorption cycle of Cr (VI) ions was repeated three times using the same preparations. The adsorption capacities for all the biosorbents did not noticeably change during the repeated (three times) adsorption-desorption operations. Also, the desorption efficiency of the biosorbed metal ions from immobilized live *Oscillatoria* sp. was determined as 98%. But, it is known that the adsorption capacity of the algal biomass for Cr (VI) ions decreased down to 2% during the repeated adsorption-desorption operations. A possible cause of reduction in the biosorption capacity of the algal cells could be attributed to the adverse effect of the desorbing agent on the binding sites of the algal cell wall components.

Table 2. A comparison of adsorption capacities of various biosorbents for Cr (VI).

Type of biosorbents	Cr (VI) uptake Q_m (mg Cr/g biomass)	References
<i>L. sajor-caju</i> (free- inactive)	18.9	[24]
<i>L. sajor-caju</i> (immobilized with CMC-active)	32.2	[24]
<i>Dunaliella</i> sp. (active)	58.3	[27]
<i>Dunaliella</i> sp. (active)	45.5	[27]
<i>Chlorella vulgaris</i> (inactive)	23.6	[28]
<i>Clodophora crispate</i> (inactive)	30.4	[29]
<i>Scenedesmus obliquus</i> (inactive)	15.6	[30]
<i>Synechocystis</i> sp. (inactive)	19.2	[30]
<i>Rhizopus nigricans</i> (free-inactive)	49.8	[31]
<i>Rhizopus nigricans</i> (immobilized-Ca- alginate, inactive)	34.7	[31]
<i>Oscillatoria</i> sp. (free-inactive)	36.4	This study
<i>Oscillatoria</i> sp. (immobilized with Ca-alginate, inactive)	34.2	This study
<i>Oscillatoria</i> sp. (immobilized with Ca-alginate, active)	39.1	This study

3.4. Evaluation of Immobilized and Free Biosorbents

In order to demonstrate the validity of proposed method, adsorption potential of immobilized live *Oscillatoria* sp. could be compared with other adsorbents used for this purpose. **Table 2** compares the maximum adsorption capacities obtained in the present work with those reported in other studies. Table 2 shows that immobilized live *Oscillatoria* sp. has good enough adsorption capacity when compared with other biosorbents. The differences of adsorption capacities are ascribed to the properties of different adsorbents such as structure, functional groups and surface area.

4. Conclusion

In this study, the Cr (VI) adsorption properties of dry biomass, immobilized live and heat-inactivated *Oscillatoria* sp. were studied. The results showed that the immobilized live *Oscillatoria* sp. had a higher biosorption capacity than the dry biomass and heat-inactivated *Oscillatoria* sp. Tien³²⁾ reported that *Oscillatoria limnetica* was a thin layer of diffuse mucilage, high surface area/dry weight ratio and the structure of mucilage may be able to explain the binding mechanisms. Therefore, there are no reports on biosorption of metals by *Oscillatoria* sp., which grows readily and forms a good mass that can be conveniently used for biosorption studies. Rapid growth and early harvestable biomass of this cyanobacterium ensures ready availability of this system as a biosorbent, which is both cost-effective and non toxic. Use of algal biomass in powder form is more in practice for biosorption purposes. However, separation of the algal powder from the wastewater after use becomes a major practical problem. Immobilization of the algae, on the other hand, not only avoids biomass-liquid separation, but also allows higher local cell density and retention of biomass within a definite working system that can be reused.

Consequently, the immobilized live *Oscillatoria* sp. may

be suitable for development of efficient biosorbent for the removal of chromium from wastewater. The Freundlich and Langmuir adsorption models were used for the mathematical description of the biosorption of Cr (VI). The biosorption was well described by Freundlich adsorption isotherms. The good fit of experimental data to the Freundlich sorption model was indicated the presence of the heterogeneous binding sites on algal surfaces.

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