Bioremoval of chromium from wastewater of tannery factory in Iraq

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Abstract

Bioremoval of chromium from wastewater of tannery factory in Iraq was studied. The bacteria *Proteus vulgaris* 7E showed an enhanced capability in biosorping chromium when its concentration increased in the solution, reaching a maximum of 476,7 mg/ ml out of 492 mg/ ml under optimum conditions at pH 6 and 50°C at one hour contact time and biomass of 1 mg/ml.

The present results showed that dead cells of *P. vulgaris* 7E biosorbed 87.41 mg/ml of chromium in comparison with 91.18 mg/ml of chromium biosorbed by living cells, this indicates the insignificant effect of physiological state of cells.

It was found that the above biosorption is physico-chemical process depends upon electrostatic attraction forces.

The results has illustrated that the most efficient eluting solution was 0.1M HCL which recovered 85% of biosorbed chromium.

P. vulgaris 7E was able to remove completely all chromium from the waste water taken from tannery factory.

Introduction

The heavy metal industry and its waste impose a hazardous effect on life; hence so many physical and chemical methods have been designed to remove the heavy metals ions from the industrial waste water. However, those methods are impractical from commercial point of view due to high expensive cost in addition to the complicated treatment of its end products(1).

Chromium is one of the important pollutants found in the waste water of different industries (e.g.tannery and electroplating) which characterized by its high toxicity (2).

The use of biological processes for the treatment of metal enriched wastewaters can overcome some of the limitations of physical and chemical treatments and provide a means for cost- effective removal of metals. A great deal of interest has recently been generated using different kinds of inexpensive biomass for adsorbing and removing heavy metals from wastewater (3,4).

Metal binding is achieved by a direct mechanism involved an electrostatic interactions between bacterial cell wall negatively charged groups and the positively charged metal ion (5,6).

Beveridge and Murray (7) suggested a mechanism of two steps explaining the binding of metal ion with the bacterial cell wall; first step involved a stoichiometric interaction of the metal ion with reactive sites in the cell wall followed by the second step; these sites act as nucleation sites for more metal ions precipitation.

pH is of a great importance in charging these sites which in turn affects the bacterial efficiency in heavy metal biosorption (5). In addition to pH effect there is also another factors affect this efficiency such as biomass concentration, ion competition and contact duration (8).

The aim of the present study is to investigate the ability of bacteria isolated from wastewater of tannery factory in Iraq for chromium removal from this wastewater as well as aquous solutions and to optimize the

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biormeoval conditions for the most efficient isolate.

Materials and Methods

Sampling

Water samples were collecting from the industrial waste water treatment unit tannery factory in Baghdad during March, 2004 from the main tank; one sample per week over a period of month, as triplicate,in sterile glassware and plasticware containers.The sample was divided into two parts; the first one (in plasticware container) was obtained to estimate the temperature, pH and chromium concentration, while the other part (in glassware container) was obtained to isolate bacteria.

Also, several samples were collected from the primary tank and water discharged to the river after treatment, as triplicates, in order to estimate the chromium concentration in the filtrate after it was filtered through 0.45 µm Millipore filters, using flame atomic absorption spectrophotometer (Shimadzu, Japan).

Bacterial isolation

After good mixing, six decimal dilutions for each sample were done. 0.1 ml of each dilution was cultured on Nutrient agar, MacConky agar and Shigella-Salmonella agar via spreading using L shaped glass spreader on two plates per dilution. All plates were cultivated at 37 °C for 24 h. Identification was completed according to Bergey's manual (9) and api 20 E system.

Chromium – bacteria contact

Bacterial isolates were propagated in Brain Heart Infusion Broth pH 7.2 incubated at 37°C for 24h.Thereafter, growing cells were harvested by cooled centrifugation(4 °C) at 5000 rpm for 30 min. Then, it was washed with deionized distilled water (DDW) three times,collected in sterile test tubes and resuspended in small amount of DDW. One milliliter was dried in the oven at 100 °C in order to estimate the dry weight.

Biosorbents (bacterial cells) were added to 100 mg/l,pH5 and 20ml of chromium solution, as triplicates for one hour at 50°C. Control chromium solution (free of bacteria) was prepared as well. After that, all chromium solution were centrifuged at 4°C at 5000 rpm for 30min and the chromium concentration was estimated in the supernatant using flame atomic absorption spectrophotometer (10). The amount of biosorbed chromium was taken to be the difference between the chromium concentration in control and chromium concentration in supernatant

Factors affecting chromium biosorption

These experiments aimed to optimize the chromium biosorption by the most efficient isolate. Each factor developed higher biosorption will be considered in the next experiment. All experiments were performed in triplicate.

Effect of pH

P. vulgaris 7E cells were added chromium solutions of pH values (1, 2, 3, 4, 5 and 6), pH above 6 did not consider since chromium ions have precipitated. pH values were adjusted using 2M HCl and 2M NaOH. Same procedure mentioned in Chromium – bacteria contact was followed.

Effect of temperature

P.vulgaris 7E cells were added to chromium solutions (pH6) and incubated at different temperatures:(10,20,30,40and50)°C.Same procedure mentioned in Chromium–bacteria contact was followed.

Effect of Chromium initial concentration

P.vulgaris 7E cells were added to chromium solutions (pH 6) of different initial concentrations:5,10,25,50,50,75,100,200,500 and 1000)mg/l,same procedure mentioned in Chromium–bacteria contact was followed.

Effect of Biomass concentration

P.vulgaris 7E cells were added to chromium solutions at different biomass concentrations:(0.25,0.5,1.0,2.0 and 2.5) mg/ml at pH 6.Same procedure mentioned in Chromium – bacteria contact was followed.

Effect of time of contact

P.vulgaris 7E cells were added to chromium solutions (pH6) incubated for different periods: (0.05, 0.5,1.0,3.0, and 24) hours.Same procedure mentioned in Chromium–bacteria contact was followed in

exception of using 1.0mg/l as biomass concentration.

Effect of physiological state

P.vulgaris 7E live and dead cells (killed by boiling at 100°C for 10 min., then cultured on nutrient agar for viability check) were added to chromium solutions (pH 6) in two different flasks. Same procedure mentioned in Chromium – bacteria contact was followed.

Chromium desorption

Same procedure mentioned in Chromium – bacteria contact was followed in addition to resuspending bacterial cells, loaded with chromium, in 10 ml of 0.1 M, Na_2CO_3 pH 11.7, 0.1 M, HCl and 0.1 M, EDTA pH 7.5 for one hour at 50 °C then centrifuged and the chromium was measured in supernatant.

Efficiency of the isolate Proteus vulgaris 7E in chromium removal from the waste water of the tannery factory

P. vulgaris 7E cells were added to a sample taken from waste water of the tannery factory according to the procedure explained in Chromium – bacteria contact.

Statistical analysis

t- test and LSD at 95% confidence limit were employed

Results and Discussion

Parameters measured in situ

Table (1) showed, clearly, temperature was in the range (28.5 – 29.2) °C and the pH values were within 7.5 – 8. This rise in pH attributed to soda and calcium hydroxide addition, a matter agreed with Alnasrawi (11) results.

Table (1): Mean pH values and temperature of samples taken from main hole

Sample	pH	Temperature (°C)
First week (sample 1)	8.5	28.5
Second week (sample 2)	7.5	29.5
Third week (sample 3)	8.4	29
Forth week (sample 4)	7.6	30
Range	8-7.5	29.2-28.5

As it is shown in table (2), the chromium concentration in the main hole found to be 200 mg/l, however, it decreased to 22 mg/l when the wastewater reached the primary tank. Such decrease could be attributed to the dilution effect caused by: the addition of chromium free-water and other fluids which come from other departments of factory, and the addition of calcium hydroxide as a precipitant may contribute in dilution effect (11).

Alnasrawi (11) reported that the total concentration of chromium discharged to the river was to be 0.16 mg/l, a result disagreed with the present study results which found it about 5 mg/l (Table 2), obviously, it is considered above the safe levels in drinking water which should be about 0.05 mg/L (U.S. EPA), and the recommended discharge level (less than 5 mg/l) (12), a matter confirms the insufficiency of the treatment procedure followed by the factory.

Table (2): Mean chromium concentration in waste water of the tannery factory

Sample	Chromium concentration (mg/l)		
	Main hole	Primary tank	Discharged to the river
First week	200	22.5	4
Second week	170	21.5	5
Third week	230	24	6
Forth week	200	20	5
Mean	200	22	5

Efficiency of bacterial isolates in chromium biosorption

Seven isolates were isolated from the waste water of the tannery factory.*P. mirabilis* 1E showed lowest biosorption amount(3 mg/l),whereas Proteus mirabilis 7E developed higher biosorption amount (91.18 mg/l) (Table 3).Accordingly, this isolate was chosen for the subsequent experiments.

Table (3): Mean chromium biosorption efficiency of the bacterial isolates (after 30 minutes of contact)

Isolate	code	Biosorped chromium ± SD [1] (mg/l)
Proteus vulgaris	7E	91.18 ± 4.3 a
Pseudomonas aeruginosa	6E	83 ± 3.7 a
Klebsiella pneumoniae	5E	68 ± 3.1 b
Salmonella typhi	4E	28 ± 2.6 c
Escherichia coli	3E	25 ± 2.8 c
Proteus vulgaris	2E	22 ± 2.3 c
Proteus mirabilis	1E	3 ± 0.7 d

[1]significant differences (P≤ 0.05%) between each different letters.

Factor affecting the chromium biosorption pH effect

Figure(1) depicted the relationship between pH and chromium biosorption. Biosorbed chromium concentration increased with a rise in pH up to pH6 (91.18 mg/l). However, there were insignificant differences(P>0.05) between pH5 and 6. Many authors have mentioned the biosorption of heavy metals is very sensitive to pH,since the biosorption rate increases with the increase of pH,up to certain pH value (13,14,15).the present study results agreed with other authors as they found the optimal biosorption occurred at pH 5 to 6 (16,17,18).

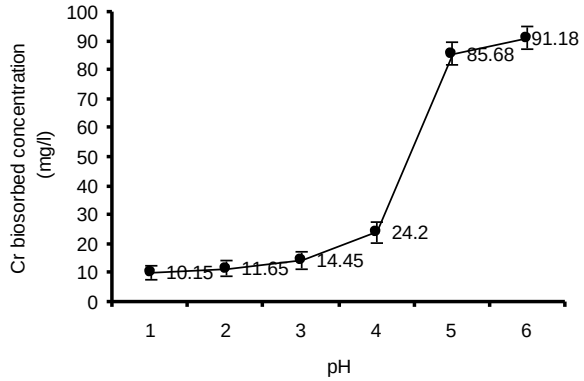

Figure (1): Effect of pH on chromium biosorption by *P. vulgaris* 7E

Biosorption of the chromium pH plays a vital role due to the nature of chemical interactions of each metal with the functional groups present on the microbial cell surface. At pH values above the iso-electric point of the cells, there is a negative charge on the cells. At low pH, overall surface charge on the cell is negative and facilitates biosorption of negatively charged $Cr_2O_7^{-2}$ (15).

Sheng *et al.* (19) suggested that high pH values are preferred in cations removal like Cr^{+3} and Cd^{+2}, while high removal of anions occurred at low pH values.

Such behaviour could be explained as follows: cation biosorption increased as pH raises due to deprotonation from the reactive sites, thereafter,the cation binds with the negatively charged site.While at low pH the protons will increased in number and compete with the cations on reactive sites and the overall biosorption capacity will be decline (20,21).

Effect of temperature

Temperature has a dramatic effect on biosorption of chromium by P.vulgaris 7E. As it observed from figure(2),the biosorption rate increased with temperature rise up to 50 °C (91.18 mg/l), in this manner, present results suggest that chromium biosorption by *P* vulgaris 7E is an endothermic process. However, insignificant differences were noticed at 30, 40 and 50 °C.

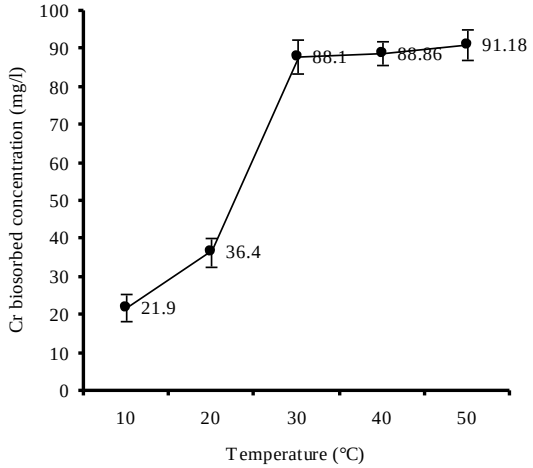

Figure (2): Effect of temperature on chromium biosorption by *P. vulgaris* 7E

Loukiodou et al.(6) found that Aeromonas caviae binds more chromium as the temperature increased from 20 to 60°C. Also(10) found similar results as they ment- ioned that optimum biosorption of Cr by Staphylococcus saprophyticus occurred at 30 °C.

Increased biosorption of heavy metals with increasing temperature has been ascribed to bond rupture that perhaps enhances the number of active sites involved in metal sorption or higher affinity of sites for metal (12).

Effect of Chromium initial concentration

The influence of chromium initial concentration on chromium biosorption can be evaluated from figure (3).The experimental results indicate that the isolate *P. vulgaris* 7E removed completely all chromium ions from the aqueous solutions with concentrations range 5to50mg/l.however the highest biosorption rate was noticed at500mg/l,nevertheless, a declination in biosorption was observed at 1000 mg/l. Such results could be attributed to the saturation of the reactive sites with metal ions which led to weakening the

electric attraction between the reactive site and metal ion at the far end of the metal ion aggregate (7).

Sen and Dastidar (22)pointed out to the specific removal of chromium increased with increase in initial Cr concentration,up to500 mg/l.

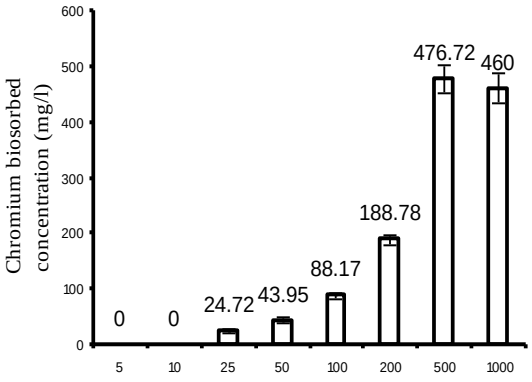

Figure (3): Effect of initial concentration on biosorption of Cr by *P. vulgaris* 7E

Effect of Biomass concentration

Results demonstrated in figure(4) a marked effect of biomass concentration on chromium biosorption since biosorption of chromium ions increased from 62.3 to 90.49 mg/l with increase in cell concentration from 0.1mg/ml to 0.25mg/ml,respectively.

Thereafter,insignificant increase (P> 0.05) has happened in biosorption rate up to 1.0 mg/ml, followed by a gradual decrease in biosorption 82.88 and 76.43 mg/l at 2 and 2.5 mg/ml biomass concentration respectively.

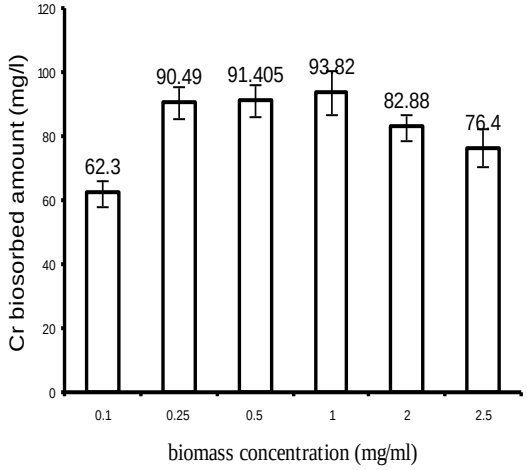

Figure (4): Effect of biomass concentration on Cr biosorption by *P. vulgaris* 7E

Availability of chromium adsorption sites increases with increasing cell mass concentration, but due to agglomeration of biomass, total adsorption sites are not all available and hence the biosorption rate decreases (23).

Effect of time of contact

Biosorption process reached the equilibrium state within one hour given that P.vulgaris 7E biosorbed 93.8 mg/l.However, this capacity declined after 1.5 hour of contact reaching to its lower rate after 24 hours (figure 5).

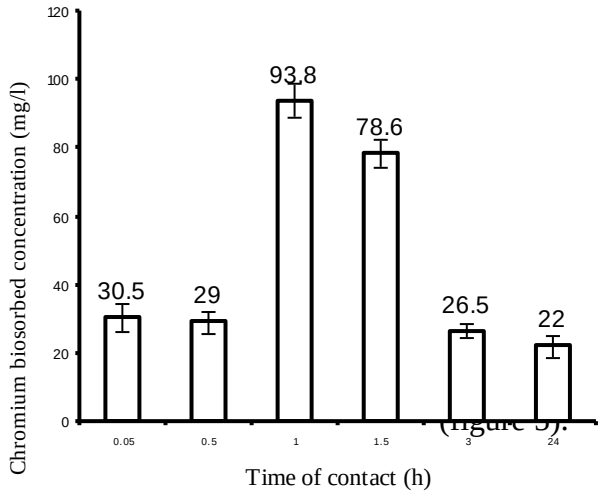

Figure (5): Effect of time of contact on chromium biosorption by *P. vulgaris* 7E

These results agreed with the results of Sheng *et al*.(19) as they mentioned that the Bioremoval of chromium reached the equilibrium within one hour by the marine alga *Sargassum* and *Padina*. While others pointed out that chromium biosorption accomplished by *Sargassum* spp. within 6 hours and they suggested that the reactive sites has saturated within this period (4).

Effect of physiological state

Biosorption capacity by heat killed cells and viable cells of *P.vulgaris* 7E nearly the same (insignificant differences P>0.05) as the dead cells biosorbed 87.41mg/l and the viable cells biosorbed 91.18mg/l.Such results agreed with other studies suggested that the physiological state has no effect on the biosorption (24,25).

Chromium desorption

Results illustrated in figure (6) showed the efficiency of diluted HCl in chromium desorption capability over other desorption

solutions since it desorbed 77.5mg/l (85%) from 91.18mg/l chromium biosorbed on *P. vulgaris* 7E cells.

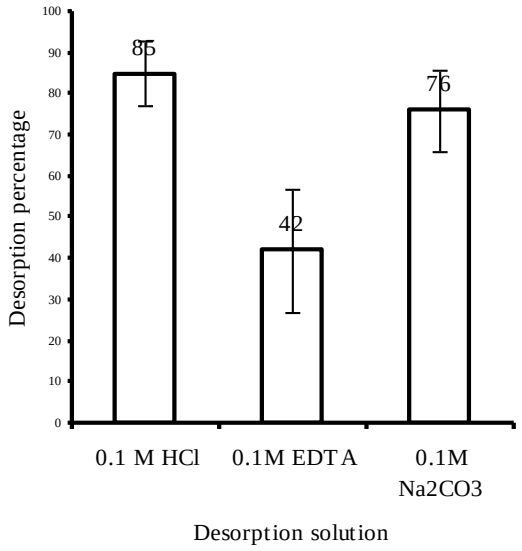

Figure (6): Chromium desorption by *P. vulgaris* 7E

Diluted HCl was the best desorption solution in recovery about 13.8mg/g from 16.3mg/g of Cd biosorbed on *Zoogloea ramigera* (25).

From all above results we can conclude that the isolate *P. vulgaris* 7E showed an enhanced capability in biosorping chromium when its concentration increased in the solution, reaching a maximum of 476,7 mg/ ml under optimum conditions at pH 6 and 50°C within one hour contact time and biomass of 1 mg/ml., accordingly, it may consider one of the most efficient microorganism employed in chromium biosorption from aqueous solution.

Efficiency of the isolate Proteus vulgaris 7E in chromium removal from the waste water of the tannery factory

The isolate *P. vulgaris* 7E was able to remove nearly all Cr ions from the sample taken from waste water of the tannery factory, a matter confirm its outstanding ability in chromium Bioremoval.

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الازالة الحيوية للكروم من الفضلة الصناعية لمعمل الدباغة في العراق

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الخلاصة

لغرض الإزالة الحيوية للكروم من مياه المخلفات الصناعية لمعمل الدباغة التابع للشركة العامة للصناعات الجلدية تم أختبار قابلية عزلات بكتيرية مختلفة معزولة من وحدة المعالجة الصناعية. وكانت العزلة 7E *Proteus vulgaris* الأكفأ فقد أظهرت أعلى كفاية إمتزاز بلغت 476,72 مكغم / مل من تركيز أساس 492 ملغم / لتر في ظروف مثلى، وهي أس هيدروجيني 6 عند 50 °م، ولمدة ساعة واحدة، وتركيز كتلة حياتية 1ملغم/مل.أظهرت النتائج أن الخلايا المقتولة بالحرارة امتزت كمية من الكروم مقاربة (فرق غير معنوي $P>0.05$) لما إمتزتها الخلايا الحية، إذ بلغت87.41 و 91.18 مكغم / مل على التوالي، مما يدل على عدم وجود تأثير للحالة الايضية للخلايا.أثبتت الدراسة الحالية من النتائج أعلاه أن عملية الإمتزاز الحيوي للكروم بوساطة البكتريا 7E *P. vulgaris* هي عملية فيزيوكيميائية تعتمد على قوى تجاذب الكهربائية الساكنة، وبينت نتائج التجارب التي أجريت لتبيان كفاية محاليل الغسل المستخدمة لاسترجاع الكروم من الكتلة الحياتية كفاية محلول حامض الهيدروكلوريك المخفف (0.1 مولاري) إذ أمكنه استرجاع 85% من كمية الكروم الممتزة. كما بينت النتائج قدرة البكتريا 7E *P. vulgaris* على إزالة الكروم من مياه المخلفات الصناعية وبنسبة مقاربة الى 100% .