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Original Research Article

A study on the utilization of rice husk as a biosorbent material for Cr (VI) removal from industrial effluent

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Abstract

Purpose: *To study Cr (VI) removal from waste water using chromium-resistant bacterial strains in combination with rice husk.*
Methods: *Two strains of Exiguobacterium sp. resistant to chromium (VI) were applied in the present work. Rice husk (RH) was used as an agricultural waste for Cr (VI) removal. The elimination of Cr from the husk was chemically facilitated using hydrochloric, sulphuric and citric acids, as well as formaldehyde and potassium dihydrogen phosphate Investigation of optimum physical factors such as pH, temperature, shaking speed and biomass concentration on Cr (VI) removal was carried out using citric acid-processed rice husk alone, and in combination of bacterial strains. Fourier transform infra-red (FTIR) spectroscopy was performed to determine the contributions of different functional groups involved in Cr (VI) binding. Scanning electron microscopy (SEM) of treated and untreated RH was also performed.*
Results: *Citric acid-processed RH was most effective in the removal of chromate (97.3 %). The two bacterial strains combined with rice husk proved highly efficient in Cr (VI) removal from sterile and non-sterile industrial effluents. FTIR spectra showed the involvement of esters, amines and aliphatic functional groups in Cr (VI) binding, while SEM displayed the damaging effects of Cr (VI) on the surface of RH; however, bacterial inoculation minimized the damage.*
Conclusion: *Exopolysaccharides from Exiguobacterium strains and citric acid-processed rice husk demonstrated high efficiency for Cr (VI) removal. Hence, RH with these bacterial strains are potential biosorbents for control of heavy metal contamination arising from industrial effluents.*

Keywords: *Rice husk, Cr (VI), Contamination, Effluents, Exiguobacterium, Citrate*

INTRODUCTION

Industries generate tons of harmful toxic effluents which contain large amounts of Cr (VI) [1]. The toxic effects of Cr (VI) range from skin reactions to lung carcinoma. Thus, there is need to eliminate the metal from waste water prior to channeling into the open water. Studies have identified a diverse range of organisms with high capacity for Cr (VI) reduction. These findings have been exploited as cheaper routes of treating waste water [2]. Several bacterial strains used in this bioremediation process include *Enterobacter, Bacillus*, *Pseudomonas and Agrobacterium.* Bacteria produce polymeric exopolysaccharides (EPS) which can be used for bioremediation of heavy metals [3]. Alternatively, the contaminants may be eliminated using adsorption process which is effective and cheap. Rice husk (RH) is one interesting biosorbent capable of purifying metal-contaminated water due to its unique characteristics. The effectiveness of RH as a biosorbent is due to its rich silica content and unique network of its cellulose structure [4]. Structural modification of RH improves its biosorbent characteristics by exposing more surface bioactive groups [5]. These groups arise from the lignin, cellulosic, hemi-cellulosic and pectin components of plant cell wall [6]. Chemical modification may introduce extra bioactive groups, or in some cases, inhibit existing reactive groups, thereby affecting biosorption properties.

Fourier transform infrared (FTIR) spectroscopy is a useful method for investigation of reactive groups involved in metal-polymer interactions [7]. Scanning electron microscopy (SEM) is a useful technique for studying alterations on polymer surfaces caused by binding of a metal e.g. Cr (VI).

The purpose of the present study was to investigate the Cr (VI) remediation potential of RH when applied alone, and when used in combination with exopolysaccharide-secreting strains of *Exiguobacterium.* In addition, the influence of chemical modification on the biosorbent effectiveness of RH was studied.

EXPERIMENTAL

Exiguobacterium strains and culture

Two strains of Cr (VI) resistant *Exiguobacterium* sp. were used in this study. They were cultured on L-agar containing potassium chromate (1 mg/ml at 37 ˚C).

Extraction of exopolysaccharides

Bacterial strains were grown in acetate minimal medium with and without supplementing K_2CrO_4 (1000 μg/ml) for 72 h at 37 °C and pH 7. The EPS was extracted with solvent extraction method [8].

Evaluation of RH as a biosorbent

Raw RH was collected from a local rice mill in Punjab, Pakistan. It was washed with water, oven dried and preserved at room temperature (30 °C). Then, in order to remove the yellow colour, it was boiled and oven-dried overnight at 65 °C.

Chemical processing

The dried RH was subjected to treatment with potassium dihydrogen phosphate (1.0 M) for 24 hr, sieved, rinsed in H_2O and oven-dried for 12 h. Modification with HCl was done by rinsing the RH with 1 % HCl first, and then with H_2O. The RH was chemically modified using citric acid [9], with 1 % formaldehyde and H_2SO_4 [10].

Determination of Cr (VI) bioremediation potential

Each 1.5 g portion of processed and unprocessed RH was steeped in 100 ml Cr (VI) solution (1 mg/ml), and incubated at 37 ˚C for 24 h. Thereafter, Cr (VI) levels were determined with diphenylcarbazide method [11].

Generation of various biosorbents

Various forms of biosorbent were produced through citric acid modification of RH, alone and after incubation with either E1 or E4 strain of *Exiguobacterium* at logarithmic growth phase.

Influence of duration of contact on Cr (VI) elimination

Citrate-processed RH (1.5 g %) was incubated with 1 mg/ml solution of Cr (VI) at neutral pH and E2 or E4 strain in a flask rotated at 150 rpm. After specific point interval, aliquots of the mixture were subjected to Cr (VI) analysis as before [11].

Determination of optimum physical factors

The Cr (VI) bioremediation potential of citrate-processed RH and RH + E1 or E4 were optimized with respect to physical factors such as temperature, pH, amount of biomass and rpm.

Column-based studies

A 60 x 4.5 cm glass column was washed with nitric acid, rinsed with H_2O and dried in a hot-air oven. The column was packed separately with the various RH-derived adsorbents in the presence of 1 % Cr (VI) under optimized physical conditions, and the bioremediation potential was measured colorimetrically as before [11].

Elimination of Chromium VI from effluent with RH

Effluent from a paint industry at Lahore, (sterile/non-sterile) was used. The bioremediation effectiveness of each of the various RH biosorbents was tested on Cr (VI). Each biosorbent was incubated with either E2 or E4 or alone at 37 ºC for 12 h, after which 1 ml of the medium was subjected to Cr (VI) analysis colorimetrically [11]. An incubation mixture containing only E2 or E4 was used as control.

Digestion of rice husk

To perform the digestion experiment, 0.5 g of each citric acid-processed rice husk was incubated separately in Cr (VI) solution (1 mg/ml) for 24 h. Rice husk incubated in ordinary H_2O served as control. After incubation, each adsorbent was taken in labeled flasks and digested by following Humphires method [12]. Samples were then subjected to chromium estimation [11].

Analysis of RH surface

Fourier Transform infrared spectroscopy

The reactive groups associated with chromium binding in each adsorbent were identified using FTIR by incubating 4 replicates of each adsorbent with Cr (VI) (1 mg/ml). The KBr disc procedure was used to determine spectra within the 400–4000 cm^{-1} range.

SEM

This was applied to examine the surface characteristics of biosorbents in the bound and unbound states. For this purpose, all five adsorbent materials (processed and unprocessed) were oven-baked and subjected to SEM (JSM-6480) at 15 V at 150 magnification.

Statistical analysis

Data are presented as mean ± standard error of the mean (SEM) and were analyzed by Student t-test using SPSS ver16 software. *P* = 0.05 was considered statistically significant.

RESULTS

The E1 and E4 strains of *Exiguobacterium* showed 83.4 and 75.8 % chromium (VI) removal, respectively, when grown at 1000 µg/ml. In the presence of chromium (VI) stress, EPS secretion levels by E2 and E4 were 180 and 142 mg/100 ml, respectively (Table 1).

Untreated rice husk adsorbed 29.89 % Cr (VI), while the value for boiled RH was 50.3 %. In contrast, HCHO- processed RH removed only 5.8 % of Cr (VI), while citrate-processed RH removed almost 97 % Cr (VI). The corresponding value for RH processed with K_2HPO_4, hydrochloric acid and sulphuric acid were 50.67, 30.33 and 40.5 %, respectively (Table 2). Thus, citric acid was the most effective agent. Therefore, it was used in subsequent experiments.

Table 1: Features of the *Exiguobacterium* sp. applied in the study

Characteristic	Bacterial strain	
	E1	*E4*
Microbe	*Exiguobacterium* sp.	*Exiguobacterium* sp.
Genbank accession no.	KC668296	KC668297
Gram staining	Gram positive rods	Gram positive rods
Maximum tolerable concentration of Cr (VI)	2250 µg/ml	2250 µg/ml
Cr (VI) removal	83.4±0.38 %	75.8 ±0.45 %
EPS Production with Cr (VI) (mg/100ml)	180 ± 0.5	142 ± 1.7
EPS Production without Cr (VI) (mg/100ml)	100 ± 1.8	57 ± 0.88

Mean ± standard error of the mean (SEM)

Table 2: Cr (VI) removal (%age) by processed and unprocessed rice husk (RH)

Rice husk	Cr (VI) removal (%)
Raw RH	29.89 ± 0.40
Boiled RH	50.30 ± 0.32
HCHO- processed RH	5.80 ± 0.43
citrate-processed RH	97.3 ± 0.623
K_2HPO_4 processed RH	50.67 ± 0.17
Sulphuric acid processed RH	40.5 ± 0.21
Hydrochloric acid processed RH	30.33 ± 0.25

Mean ± standard error of the mean (SEM)

The highest level of Cr (VI) elimination occurred within 2-4 h of incubation, while RH with E1 and E4 adsorbed 100 % K_2CrO_4 from the aqueous medium within 18 h. Stirring speed and pH had no effect on rate of Cr (VI) removal. A rise in temperature (26 to 37 ºC) raised Cr adsorption from 84.5 to 96.7 %. Combinations of E2 and E4 with RH produced 100 % elimination at 37 and 45 °C. There were reductions in Cr (VI) adsorption at reduced RH levels, and vice versa. Complete adsorption of Cr (VI) occurred after 18 h with 4.5 g RH mixed with E2 or E4 (Figure 1).

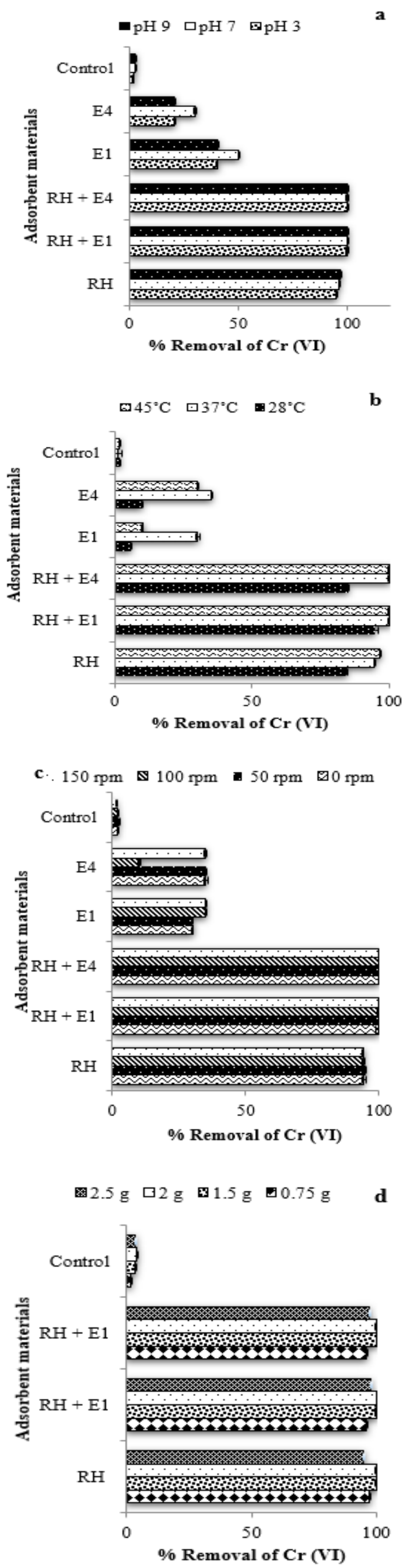

Figure 1: Investigations on optimum physical factors (a) variable pH (b) variable temperature (c) variable shaking speed (d) variable rice husk concentration

For RH, Cr (VI) uptake was observed to be 8.4 mg/g while the RH+E1 and RH+E4 exhibited 5.4 mg/g and 3.4 mg/g of Cr (VI) uptake, respectively. In sterile effluent, activity of RH and RH+E1 become suppressed. In case of non-sterile effluent, RH removed 99% of chromate (Figure 2).

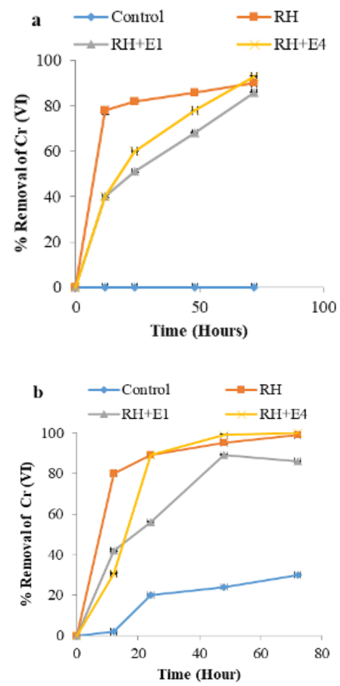

Figure 2: Elimination of Chromium VI from effluent with RH (a) sterile and (b) non-sterile

Amine and aliphatic functional groups were not detected in the various FTIR spectra. However, esters were present in all RH formulations (Figure 3).

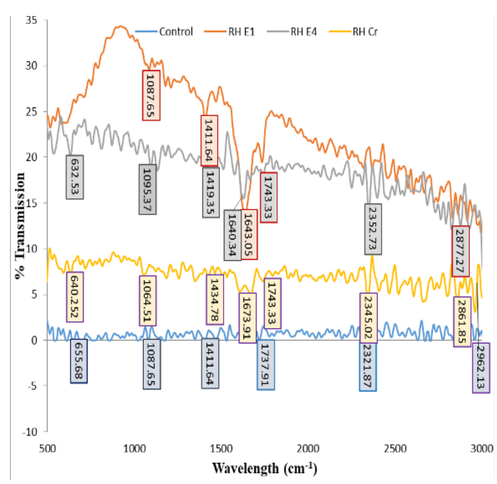

Figure 3: FTIR spectra of processed and unprocessed RH

Figure 4 reveals that SEM of RH depicted a rough surface containing minute pores. On incubation with citrate-processed RH with Cr (VI), the surface topology was lost. The SEM of RH in the presence of E2 and E4 showed lower level of damage due to bacterial secretion of EPS (Figure 4).

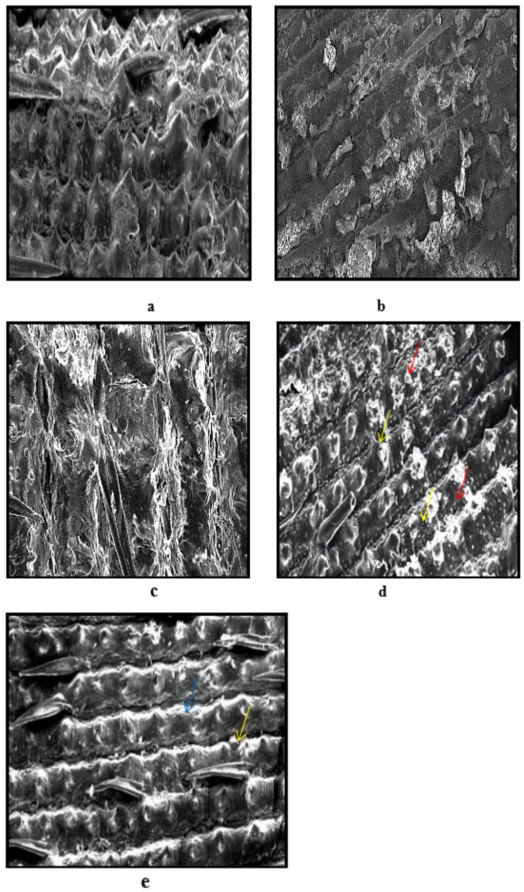

Figure 4: Scanning electron micrographs of (a) raw rice husk (b) citrate-processed RH (c) citrate-processed RH exposed to Cr (VI) stress (d) citrate-processed RH with E1 and Cr (VI); red coloured arrows indicated the distribution of bacteria; yellow coloured arrows represented the deposition of Cr (VI) particles adsorbed among the furrows of rice husk (e) citrate-processed RH with E4 and Cr (VI); blue coloured arrows indicated the dense bacterial EPS covering; yellow arrows represented the Cr (VI) adsorption

DISCUSSION

The two strains of *Exiguobacterium* E1 and E4 had very good EPS secretory and Cr (VI) adsorbent properties. Earlier studies on an *Exiguobacterium* sp. isolate revealed 66–69 % scavenging of Cr (VI) [13]. Bacterial EPS bind and sequester metal ion contaminants [14]. In a medium of Cr (VI), there were significant increases in the secretion levels of EPS by E2 and E4, probably as an adaptation strategy to cope with unfavorable growth medium. It is known that RH efficiently binds pollutants, especially metal ion contaminants in aqueous media due to presence of reactive functional groups, including OH^-.

The metal-binding capacity of RH is improved by various physical and chemical manipulations. In this study, boiling enhanced the Cr (VI) binding capacity of RH. An earlier study found that biosorption potential of RH was improved by boiling at 100 °C due to structural modification of lignin present on its surface [15]. Processing with HCHO markedly impaired the adsorbent effect of RH, most likely due to crosslinking of reactive OH' groups on RH. This is in agreement with a previous report which showed that HCHO markedly reduced the Cr (VI)-binding efficacy of biomass [16]. Acid processing had no appreciable effect on the chromium-binding capacity of RH, probably due to interference of the negatively-charged acid anions with the adsorption process. In contrast, citric acid enhanced the binding of Cr (VI) to RH. The heating of citric acid generated an anhydride which interacted with OH- of RH, forming esters which facilitated the binding of chromate [17].

The adsorption of Cr (VI) to RH in the presence of E2 and E4 was enhanced by increase in temperature to some extent, suggesting that it is an endothermic process. There was reduced uptake of Cr (VI) when E2 and E4 were present in the medium due to competition between the bacterial strains and RH for binding to the metal ion.

The difference in Cr (VI) adsorption by RH between the sterile and non-sterile effluents may be due to presence of other microflora which accentuated the adsorption process [18].

Raw rice husk showed uneven surface under scanning electron microscope, while citrate-processed RH exhibited several binding sites, and it became rougher. Chemical treatment with acid removed the impurities present on the surface of rice husk which made its fibers rough, and exposed many functional groups on the surface, including hydroxyl groups, thereby improving its binding capacity [19]. When the rice husk was used with bacterial strain E1 for the removal of hexavalent chromium, it was observed that both biological materials helped in the removal of Cr (VI) synergistically. Minimum damage was detected in the fibers of RH due to removal of Cr (VI) ions by bacteria.

CONCLUSION

This study has demonstrated a synergism between bacterial strains and RH in effective and fast adsorption of Cr (VI) from aqueous solution and industrial effluents. Thus, they are potential biosorbents for bioremediation of toxic industrial effluents.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

The authors declare that this work was done by the authors named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by them. Rida Batool conceived and designed the study; Aniqa Naeem performed the experiments, collected, analyzed the data and wrote the manuscript as well. Rida Batool critically reviewed the manuscript.

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REFERENCES

1. *Dhal B, Thatoi H, Das N, Pandey BD. Reduction of hexavalent chromium by Bacillus sp. isolated from chromite mine soils and characterization of reduced product. J Chem Technol Biotechnol. 2010; 85:1471-1479.*
2. *Soni SK, Singh R, Awasthi A, Singh M, Kalra A. In vitro Cr (VI) reduction by cell-free extracts of chromate-reducing bacteria isolated from tannery effluent irrigated soil. Environ Sci Pollut Res. 2013; 20: 1661-1674.*
3. *Borgio FJ, Bency BJ, Ramesh S, Amuthan M. Exopolysaccharide production by Bacillus subtilis NCIM 2063, Pseudomonas aeruginosa NCIM 2862 and Streptococcus mutans MTCC 1943 using batch culture in different media. Afr J Biotechnol. 2009; 8: 5454-5457.*
4. *Sadon FN, Ibrahem AS, Ismail KN. An overview of rice husk applications and modification techniques in wastewater treatment. J Purity Utility Reac Environ. 2012; 16: 338-364.*
5. *El-Shafey EI. Sorption of Cd (II) and Se (IV) from aqueous solution using modified rice husk. J Hazard Mater. 2007; 147: 546-555.*
6. *Demirbas A. Heavy metal adsorption onto agro-based waste materials: a review. J Hazard Mater. 2008; 157: 220-229.*
7. *Patel S. Preparation and characterization of porous adsorbent for chromium (VI) removal. National Institute of Technology, Rourkela. BTech. Thesis 2010.*
8. *Joo SH, Feitz AJ, Waite TD. Oxidative degradation of the carbothioate herbicide, molinate, using nanoscale zero-valent iron. Environ Sci Technol. 2004; 38: 2242–2247.*
9. *Marshall WE, Wartelle LH, Boler DE Johns MM, Toles CA. Enhanced metal absorption by soybean hulls modified with citric acid. Bioresour Technol. 1999; 69: 263-268.*
10. *Daffalla SB, Mukhtar H, Shaharun MS. Characterization of adsorbent developed from rice husk: Effect of surface functional group on phenol adsorption. J Appl Sci. 2010; 10: 1060-1067.*
11. *Rand MC, Arnod E, Michel J. Standard methods for the examination of water and waste water. American Public Health Association 1979.*
12. *Humphries EC. Mineral components and ash analysis, In: Modern Methods of Plant Analysis (Ed: Peach K and Tracy M.V) Springer Verlag, Berlin-Göttingen-Heidelberg., 1956; pp 468-502.*
13. *Sarangi A, Krishnan C. Comparison of in vitro Cr (VI) reduction by CFEs of chromate resistant bacteria isolated from chromate contaminated soil. Bioresour Technol. 2008; 99: 4130-4137.*
14. *Priester JH, Olson SG, Webb SM, Neu MP, Hersman LE, Holden PA. Enhanced exopolymer production and chromium stabilization in Pseudomonas putida unsaturated biofilms. App Environ Microbiol. 2006; 72: 1988-1996.*
15. *Bhatti HN, Khalid R, Hanif MA. Dynamic biosorption of Zn (II) and Cu (II) using pretreated Rosa gruss an teplitz (red rose) distillation sludge. Chem Eng J. 2009; 148: 434-443.*
16. *Bai RS, Abraham TE. Studies on enhancement of Cr (VI) biosorption by chemically modified biomass of Rhizopus nigricans. Water Res. 2002; 36: 1224-1236.*
17. *Dania A, Mukhtar HK, Naila K, Sara Nadia J, Fatima HB. Biosorption of chromium (VI) a low-cost milling agro waste black gram (Cicer arientenum) husk by flash column process. J Sci Res. 2009; 39: 1-8.*

18. Memon JR, Memon SQ, Saima Q, Bhanger MI, El-Turki A, Hallam KR, Allen GC. Banana peel: a green and economical sorbent for the selective removal of Cr (VI) from industrial wastewater. Colloids Surf B: Biointerfaces. 2009; 70: 232-237.

19. Ngah WSW, Hanafiah MAKM. Removal of heavy metal ions from wastewater by chemically modified plant wastes as adsorbents: A review. Bioresour Technol. 2008; 99: 3935-3948.