Cr⁶⁺ REMOVAL BY INDIGENOUS BACTERIA IN CONJUNCTION WITH DIFFERENT BIOWASTE MATERIALS: AN ECOFRIENDLY APPROACH

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ABSTRACT

Two Cr⁶⁺ reducing bacterial strains previously isolated from tannery effluents were used in the present study and identified as *Pseudomonas aeruginosa* Rb-1 and *Ochrobacterum intermedium* (Rb-2) by 16S rRNA sequencing. Different biowaste materials (waste tea leaves, carrot juice pulp, dry leaves of eucalyptus and rice husk) were assessed for sorption / removal of Cr⁶⁺ from aqueous solution of K₂Cr₂O₄. Feasibility of mono and mixed cultures of indigenous bacterial strains was evaluated for Cr⁶⁺ removal in conjunction with different biowaste materials. Among all tested biowastes materials, waste tea leaves showed optimum removal of Cr⁶⁺ from metal solution alone (77.1%) as well as in combination with bacterial strains (99.4%) after 720 minutes of contact time. Mixed culture of bacterial strains was found to be more efficient in Cr⁶⁺ removal than monoculture. The contact time of 720 minutes, pH 7, biomass concentration of 2.5 gram 100 mL⁻¹, 37°C and shaking speed of 100 rpm were found to be most optimum for optimum Cr⁶⁺ sorption alone as well as in combination with bacterial strains. Fourier transform infrared spectroscopy revealed that carboxyl, amino and OH groups present on the waste tea leaves played a significant role in the binding of Cr⁶⁺ ions with the biomass. The present study is unique in this respect that this approach involve both living and non-living materials and we could not find any report documenting such findings up to our knowledge.

Keywords: Biosorption, Cr⁶⁺, Tannery, Waste tea leaves, Fourier transform infra-red spectroscopy.

INTRODUCTION

The process of heavy metal removal by means of biological materials is known as biosorption. These biological materials are known as biosorbents¹. In the last few years, certain raw waste products from agricultural and industrial operations such as pine bark, grape stalks and crop milling waste has been tested as biosorbents for decontamination of heavy metals from environment². Agricultural and industrial waste by products, naturally available seaweeds and especially microbial biomass are regarded as effective materials for biosorption of heavy metals³. Microbial biomass has been extensively studied for the evacuation of heavy metals from the environment⁴. Conventional methods used for the removal of toxic heavy metals such as chemical precipitation, lime coagulation, ion exchange, reverse osmosis and solvent extraction are very expensive requiring large input of energy and chemicals and led to the production of toxic by-products. Biosorption is much advantageous over conventional technologies due to low operating cost, eco-friendly, high efficiency, no energy input and maximum possibility of metal recovery⁵. Thus keeping all this in view, feasibility of mono and mixed cultures of indigenous bacterial strains was evaluated for removal of Cr⁶⁺ in conjunction with different biowaste materials. Recent industrial activities are adversely affecting the environment through the production of novel substances⁶. Removal of toxic heavy metals is the key concern of the researchers because of their bio-accumulating properties and toxic effects on living organisms⁷. So, need of the hour is to quest for low cost and environment friendly techniques for the efficient removal of toxic heavy metals from environment. In this perspective, significant consideration has been given to the field of biosorption and efficiency of different biosorbent materials has been evaluated by various workers^{1,8,9}. The process of biosorption consists of a solid phase (biosorbent) i.e. biological material and a liquid phase (solvent) usually water containing a dissolved species to be sorbed (sorbate) i.e. metal ions¹⁰. Present section of research work deals with the screening and selection of non-conventional absorbents which efficiently remove the toxic Cr⁶⁺ from aqueous metal solutions alone as well as in combination with bacterial strains.

MATERIALS AND METHODS

Bacterial Strains and Growth Conditions

Pseudomonas aeruginosa Rb-1 (FJ870126) and *Ochrobacterum intermedium* Rb-2 (FJ870125), gram negative Cr⁶⁺ reducing bacterial strains previously isolated from tannery effluent were obtained from bacterial stock cultures of Department of Microbiology and Molecular Genetics, University of the Punjab, Lahore, Pakistan. They were normally grown in Luria Bertani (LB) agar (pH 7.0) at 37°C.

Identification of bacterial strain

Genomic DNA was extracted by using DNA extraction kit (Fermentas)

from overnight culture of Rb-1 (Luria Bertani broth) incubated at 37°C and 150 rpm shaking. Genomic DNA was sent to Macrogen Inc. Seoul, Korea for 16S rRNA gene sequencing. Reverse primer was converted to reverse complementary sequence with Chromas Lite 2.01 (Technelysium Pvt. Ltd, Australia). Forward, reverse and internal sequences were edited, aligned and assembled using CLC DNA Workbench Soft-ware. The consensus sequences were checked against GenBank using BlastN. Maximum homology of the query sequences to the database sequences was determined^{11,12}.

Preparation of biosorbents

The waste tea leaves and carrot juice pulp obtained from the tea bags and juice corner of the university cafeteria and dry leaves of eucalyptus grown in University of the Punjab, New Campus, Lahore, were used in the present study. Rice husk obtained from local rice mill and used as sorbent. Waste biomass collected from different source was crushed, sieved (1 mm) to obtain same sized particles autoclaved and preserved in sterilized glass bottles for further biosorption experiments. Distilled water contained Cr⁶⁺ concentration of 1000 µg mL⁻¹ without addition of bacterial strain as inoculum and biowaste material was used as control.

Time course studies of Cr⁶⁺ biosorption

Autoclaved biowaste material was added at concentration of 2 gram 100 mL⁻¹ of distilled water in conical flasks and metal concentrations were kept at 1000 µg mL⁻¹ at pH 7.0, 100 rpm and incubated at 37°C. For monoculture inoculation, bacterial strains were grown overnight in L broth. Cell pellet was obtained by centrifugation at 10,000 x g for 10 minutes and re-suspended in autoclaved distilled water. For monoculture inoculation, 1m L of bacterial suspension (log phase at 600 nm) was added to the properly labeled respective conical flask containing biowaste material whereas for mixed culture inoculation, bacterial suspension (log phase at 600 nm) of both the strains was added in equal amount. Five mL samples were drawn at specific time interval and filtered using Whatman filter paper no. 1. Cr⁶⁺ content in the filtrate were analyzed spectrophotometrically by diphenyl carbazide method¹³. Experiment was done in triplicate. Percentage biosorption was calculated.

Effect of pH, temperature, biomass concentration and shaking speed was observed on the biosorption studies. For this purpose, two gram biomass was dispersed in 100 mL⁻¹ of the aqueous solution of K₂CrO₄ having initial concentration of 1000 µg mL⁻¹.

Effect of pH on Cr⁶⁺ biosorption

Cr⁶⁺ sorption was monitored for pH range 2 to 8. NaOH and HCl were used to adjust the pH. All flasks were maintained at different pH values ranging from 2 to 8 for about 12 hours at 37°C. After specific interval of time (12 hours), samples were aseptically withdrawn and centrifuged (10,000 x g) as above. Supernatant was analyzed for the residual concentration of the Cr⁶⁺.

Effect of temperature on Cr⁶⁺ biosorption

Cr⁶⁺ sorption was monitored at three different temperatures 28, 37 and

48°C. All flasks were incubated at respective temperatures for about 12 hours. After twelve hours of incubation, samples were aseptically withdrawn and centrifuged (10,000 x g) as above. Supernatant was analyzed for the residual concentration of the Cr⁶⁺ spectrophotometrically.

Effect of biomass concentration on Cr⁶⁺ biosorption

Different weights of the waste tea leaves biomass ranging from 0.5 to 3 gram 100 mL⁻¹ were dispersed in aqueous solutions containing the 1000 µg mL⁻¹ K₂CrO₄. The aqueous solutions of K₂CrO₄ were adjusted to the optimum pH (7.0) at which optimum biosorption of the Cr⁶⁺ occurred. Bacterial inoculum was given in respective flasks and they were incubated for 12 hours on shaker at 37°C. The samples were taken after 12 hours of incubation and later on centrifuged at 10,000 x g. Residual Cr⁶⁺ concentrations in supernatant was determined by using diphenyl carbazide method.

Effect of shaking speed on Cr⁶⁺ biosorption

Optimum biosorbent concentration (2.5 gram 100 mL⁻¹) at pH 7.0 and 37°C was used to monitor the effect of shaking speed on Cr⁶⁺ biosorption. Experiments were carried out at three different shaking speeds (50, 100 and 150 rpm) for each culture. Bacterial inoculum was given in respective flasks and they were incubated for 12 hours on shaker at respective shaking speeds. The sample were collected after 720 minutes (12 hours) as above, centrifuged at 10,000 x g and analyzed for residual Cr⁶⁺ concentration.

Fourier Transform Infrared Spectral Analysis

The spectra of the native, chromium treated and Cr⁶⁺ and mixed culture

inoculated waste tea leaves (WTL) were obtained by using Perkin Elmer spectrum BX FTIR system (Beacon field Buckinghamshire HP9 1QA) equipped with diffuse reflectance accessory with the range of 500–4000 cm⁻¹. All spectra were acquired in transmission mode, by the KBr disc method to get the information specific to the functional groups. For the FTIR study, treated and non-treated waste tea leaves were centrifuged and lyophilized, followed by weighing. Then 20 mg of finely ground biomass was encapsulated in 200 mg of KBr (Sigma) in order to prepare translucent sample disks.

Statistical Analysis

Data was statistically analyzed using SPSS personal computer statistical package (version 16, SPSS Inc, Chicago). Analysis of variance (ANOVA) was performed and then means were separated using Duncan's multiple range test (P=0.05).

RESULTS AND DISCUSSION

Identification of bacterial strain

The 16S rRNA gene sequences of Rb-1 were compared with those in the NCBI sequence database (GenBank) through BLAST (www.ncbi.nlm.nih.gov/BLAST) which was most closely related to that of *Pseudomonas aeruginosa* (Fig. 1). The 16S rRNA gene sequence was submitted to NCBI GenBank with the accession number FJ870126.

Figure 1: Phylogenetic tree based on 16s rRNA gene partial sequences of *P. aeruginosa* (FJ870126) and NCBI reference strains. The evolutionary history was inferred using the UPGMA method¹⁴. The evolutionary distances were computed using the Maximum Composite Likelihood method¹⁵. Evolutionary analysis was conducted in MEGA5¹⁶.

Four different biowastes (waste tea leaves, eucalyptus leaves, rice husk, and carrot juice pulp waste) were evaluated for the Cr⁶⁺ removal ability from aqueous solution individually as well as in combination with bacterial strains. Various biological materials such as yeast, bacteria, sea weeds, agricultural byproducts like straws, coconut husks, pine needles, almond shells, cactus leaves, and charcoal¹⁷, husk of Bengal gram¹, powder of green coconut shell¹⁸, Eucalyptus bark¹⁹, tobacco leaf residues²⁰ and banana peel²¹ has been utilized as biosorbents because of significant metal binding properties. There are some disadvantages of biosorption such as metal desorption is required before further use of biosorbent due to binding of metal ions with its active sites, cells are not viable so having very little possibility of biological improvement (by genetic engineering) and there is no option of changing the valence state of metals biologically²². To overcome these shortcomings, application of biosorbent in combination with viable bacterial cells can be an effective technique.

Time course studies of Cr⁶⁺ biosorption

The effect of time course on Cr⁶⁺ removal was studied. The optimum Cr⁶⁺ removal occurred after 720 minutes contact time by all the tested biowaste materials and the waste tea leaves exhibited highest Cr⁶⁺ sorption ability. Significant increases in Cr⁶⁺ removal was observed with each bacterial strain in mono as well as in mixed culture inoculation as compared to non-inoculated treatment, with the most prominent increase by mixed culture of Rb-1 and Rb-2 (Table 1). Bacterization with Rb-2 resulted in significant augmented sorption capacity of the studied biowaste materials. The Cr⁶⁺ removal percentage was

found to be 95.35%, 84.60%, 77.56%, 87.78% and 27.96% for waste tea leaves, eucalyptus leaves, rice husk, carrot juice pulp waste and distilled water, respectively when co-incubated with Rb-1 after contact time of 720 minutes (Table 1). In all the tested biowaste materials, Rb-2 exhibited optimum removal in combination with waste tea leaves, as it exhibited 77.74% and 98.02% Cr⁶⁺ removal after contact time of 15 minutes and 720 minutes, respectively at pH 7 and 37°C with continuous shaking at 100 rpm (Table 1). Mixed culture of Rb-1 and Rb-2 significantly enhanced sorption rate of Cr⁶⁺ (99.44%) as compared to control after 720 minutes (Table 1). Therefore, the contact time of 720 minutes (12 hours) could be considered suitable for entire studies. Previously, waste tea had also been described as excellent biosorbent material for Cr⁶⁺ removal from aqueous solution²³.

Table 1: Effect of contact time on percentage Cr⁶⁺ removal by selected biowaste materials alone as well as in combination with bacterial strains (mono & mixed culture) from aqueous solution of K₂CrO₄

Bacterial strain / Biosorbent 15		Time (minutes)						
		30	45	60	120	360	720	
Rb-1	Waste tea	73.74±1.14(h)	83.79±0.37(k)	87.11±0.49(l)	88.81±0.74(l)	90.29±0.61(jkl)	93.56±0.40(lmn)	95.35±0.76(lmn)
	Eucalyptus	46.64±1.07(f)	56.44±0.19(fg)	66.88±1.05(fg)	70.43±1.00(f)	75.68±1.09(g)	80.92±1.14(hi)	84.60±0.82(i)
	Rice husk	41.76±0.40(e)	56.25±0.54(fg)	69.35±0.67(gh)	70.92±0.78(f)	72.36±0.64(g)	73.37±1.50(g)	77.56±0.59(h)
	Carrot pulp	31.87±1.47(d)	58.39±0.83(gh)	72.44±0.70(hi)	82.48±0.80(jk)	85.58±0.83(hi)	87.78±1.12(jk)	89.59±0.51(ijkl)
	Water	2.64±0.14(a)	5.80±0.17(b)	7.56±0.19(b)	9.48±0.17(b)	10.83±0.18(b)	19.53±1.01(b)	27.96±1.01(b)
Rb-2	Waste tea	77.74±0.52(hi)	85.19±0.10(kl)	88.90±0.62(l)	90.51±0.49(l)	91.85±1.02(kl)	97.86±0.95(n)	98.02±0.55(mn)
	Eucalyptus	47.64±0.90(f)	58.59±0.57(gh)	72.12±0.70(hi)	78.49±0.76(hi)	83.17±0.81(h)	89.77±0.87(jkl)	90.83±0.88(jkl)
	Rice husk	41.57±1.00(e)	54.64±0.77(f)	64.84±0.92(f)	73.67±0.56(fg)	83.87±0.68(hi)	85.31±0.25(ij)	86.27±0.64(ij)
	Carrot pulp	33.89±1.08(d)	65.73±0.64(i)	77.93±0.75(k)	83.4±0.81(jk)	87.49±0.85(hij)	90.05±0.87(jkl)	91.13±0.88(jkl)
	Water	3.63±0.15(a)	6.54±0.23(bc)	9.36±0.42(bc)	13.45±0.71(c)	18.08±0.53(c)	26.50±0.82(c)	33.53±0.64(c)
Rb-1 + Rb-2	Waste tea	79.3±0.48(i)	87.39±0.23(l)	89.32±0.29(l)	91.98±0.43(l)	93.72±0.68(l)	95.45±0.46(mn)	99.44±0.06(n)
	Eucalyptus	50.53±0.58(f)	60.77±0.59(h)	73.71±1.19(ij)	79.84±0.77(ij)	84.80±0.82(hi)	91.56±0.89(klm)	93.26±0.90(klm)
	Rice husk	47.19±1.11(f)	57.17±0.55(fg)	70.52±0.68(ghi)	82.69±0.80(jk)	86.24±0.83(hij)	88.10±0.46(jk)	89.04±0.90(ijk)
	Carrot pulp	40.96±1.12(e)	67.7±0.66(i)	77.05±0.45(jk)	84.21±0.82(k)	88.31±0.85(ijk)	92.18±0.89(klm)	95.20±0.92(lmn)
	Water	4.62±0.24(a)	8.78±0.26(c)	12.05±0.40(c)	16.70±0.36(c)	21.55±0.39(c)	29.50±1.55(c)	45.94±2.37(d)
Control	Waste tea	67.49±0.23(g)	72.06±0.41(j)	73.63±0.43(ij)	75.67±0.29(gh)	76.25±0.60(g)	77.00±0.42(gh)	77.06±0.23(h)
	Eucalyptus	21.6±0.21(c)	34.42±0.33(e)	46.65±0.45(e)	58.80±0.57(e)	65.27±0.63(f)	66.00±0.35(f)	66.01±0.53(g)
	Rice husk	14.76±0.26(b)	23.19±0.22(d)	37.39±0.36(d)	55.57±0.74(e)	59.64±0.25(e)	60.01±0.50(e)	60.08±1.22(f)
	Carrot pulp	15.51±0.15(b)	21.36±0.14(d)	35.61±0.23(d)	47.4±0.24(d)	52.11±0.59(d)	52.25±0.27(d)	52.71±1.22(e)
	Water	0.00±0.00(a)	0.00±0.00(a)	0.01±0.00(a)	0.01±0.00(a)	0.02±0.00(a)	0.02±0.00(a)	0.02±0.00(a)

Mean of 04 values ± standard error of the mean. In each column, figures followed by different letter (s) in parenthesis indicate significant difference by Duncan's multiple range test (P<0.05).

As waste tea leaves appeared as efficient biosorbent for Cr⁶⁺ removal, different factors (pH, temperature, biomass concentration, shaking speed) influencing this efficiency were studied by using waste tea leaves as biosorbent in combination with the Rb-1 and Rb-2 (mono and mixed culture inoculation) at 37°C, initial Cr⁶⁺ concentration of 1000 µg mL⁻¹ and contact time of 720 minutes (12 hours) at 100 rpm.

Effect of pH on Cr⁶⁺ biosorption

One of the key factors for the effective biosorption of heavy metal ions from aqueous solution is pH^{20,24}. The percentage removal of Cr⁶⁺ increased from 10.1 to 71.16% with increasing pH from 2 to 7. Therefore, pH 7 could be regarded as optimal pH for Cr⁶⁺ biosorption by waste tea leaves along with bacterial strains. For the biosorption of Cr⁶⁺, pH value 7.0 has been reported as suitable earlier²⁵. In monoculture inoculation, Rb-2 showed more Cr⁶⁺ removal (12.20%, 18.29%, 25.50%, 65.14%, 90.75% and 98.02%) whereas Rb-1 in combination with waste tea leaves removed 11%, 15.24%, 21%, 53.49%, 87.50% and 95.35% Cr⁶⁺ after contact time of 720 minutes over pH range of 2 to 7 as compared to non-inoculated treatment. Mixed culture of Rb-1 and Rb-2 in combination with waste tea leaves showed significantly augmented Cr⁶⁺ removal. Decrease in Cr⁶⁺ removal was observed at pH 8.0 (Fig. 2A). Mixed culture bacterial inoculation along with biowaste exhibited optimum synergistic Cr⁶⁺ removal / sorption relative to sole biosorbents or bacterial culture. pH alters the functional group activity of biomass, metal solution chemistry as well as metallic ions competition²⁶. At low pH the surface charge on the biosorbent is positive which strongly attract the negatively charged metal ion i.e. Cr₂O₇²⁻, thus increasing the rate of Cr⁶⁺ biosorption. Reduced Cr⁶⁺

biosorption at lower pH values may possibly be due to change in the chemical nature and surface characteristics of biosorbent (waste tea) as most of the carboxylic groups do not dissociate at low pH and cannot bind to metal ions though they are involved in complexation reactions and bacterial growth is also reduced at low pH^{27,28}.

Effect of temperature on Cr⁶⁺ biosorption

The effect of varying temperature (28, 37 and 48°C) on the Cr⁶⁺ biosorption was observed at pH 7. For optimum Cr⁶⁺ removal, 37°C was found to be optimum. Waste tea leaves could remove 67.49% and 77.06% Cr⁶⁺ from aqueous solution after contact time of 15 and 720 minutes, respectively. In monoculture inoculation, Rb-2 exhibited more (98.02%) Cr⁶⁺ removal than Rb-1 (95.35%) in combination with waste tea leaves after contact time of 720 minutes. Mixed culture inoculation of Rb-1 and Rb-2 in combination with waste tea leaves was more efficient (79.3% & 99.44% after contact time of 15 and 720 minutes, respectively) in removing Cr⁶⁺ from aqueous solution than monoculture inoculation. Increase in temperature caused reduction in potential of Cr⁶⁺ removal (Fig. 2B). Temperature < 50°C best for optimum Cr⁶⁺ biosorption from aqueous solution by used black tea has also described previously²⁹. Lower temperature caused decrease in the rate of Cr⁶⁺ removal in the present study so the interaction between metal ion and biosorbent can be concluded as exothermic interaction because in case of exothermic interactions higher temperature increase binding of metal ions to the biosorbent³⁰. Increasing temperature may produce a swelling effect in internal structure of waste tea thus increasing space for the penetration and binding of large metal ions³¹.

Figure 2: Percentage removal / sorption of Cr^{+6} by bacterial strains (mono and mixed culture) alone as well as in combination with waste tea leaves (WTL) from aqueous solution of potassium dichromate. (A) at variable temperatures (B) at variable pHs. Mean of 04 replicates and bars represent standard error of the mean. Different letter (s) represent significant difference by Duncan's multiple range test ($P < 0.05$).

Effect of biomass concentration on Cr^{+6} biosorption

Biomass concentration is one of the significant factors for effective biosorption. Cr^{+6} reduction / sorption found to be increased from 36.17% to 67.49% with increasing biomass concentration from 0.5 to 2.5 gram 100 mL^{-1} while it was decreased at dosage of $3.0 \text{ g } 100 \text{ mL}^{-1}$. Thus, the concentration of $2.5 \text{ gram } 100 \text{ mL}^{-1}$ was found to be most suitable for optimum Cr^{+6} removal (67.49%) by using waste tea leaves as a sorbent. Significant increases in Cr^{+6} removal from aqueous solution was observed with mixed culture of *P. aeruginosa* Rb-1 and *O. intermedium* Rb-2 along with increasing concentration of the waste tea leaves when compared to control (Fig. 3A). This increase in Cr^{+6} removal with increasing biomass concentration is due to increment in the binding sites available to metal ions for complexation. Similarly, this kind of trend in heavy metal biosorption with varying biomass concentration has reported earlier³². Decrease in Cr^{+6} removal at higher biomass concentration is due to cell agglomeration and subsequent decline in inter-cellular distance which is reported to create a 'screen effect' between the dense layer of cells. Ultimately, resulted in blocking of binding sites for metal ions³³.

Effect of shaking speed on Cr^{+6} biosorption

Cr^{+6} biosorption is highly affected by the shaking speed of the sorbent system. Positive impact of all the shaking speeds on Cr^{+6} removal by biosorbent whether alone or in combination with mono and mixed bacterial culture was observed as compared to stationary system. Under non inoculated conditions, optimum removal of hexavalent chromium (67.49%) was observed at 100 rpm whereas at shaking speed of 50 and 150 rpm comparatively lower Cr^{+6} removal (39.59 and 42.04% respectively) was observed. Bacterial inoculation considerably increased the percentage removal of Cr^{+6} at the shaking speed of 100 rpm over the stationary system. In monoculture inoculation, Rb-2 was more efficient in removing Cr^{+6} along with waste tea leaves at all the shaking speeds. At 150 rpm, decrease in Cr^{+6} removal percentage was observed in inoculated as well as non-inoculated treatments (Fig. 3B). These findings revealed that at 100 rpm solid (biomass) and liquid (metal ions) phases are in good contact with each other and thus facilitating optimum Cr^{+6} removal from aqueous solution. Biosorptive removal of Cr^{+6} by *Rhizopus arrhizus* at 100 rpm and by *Rhizopus*

nigricans and Bengal gram husk at 120 rpm has reported previously^{1, 34, 35}.

Figure 3: Percentage removal / sorption of Cr^{+6} by bacterial strains (mono and mixed culture) alone as well as in combination with waste tea leaves (WTL) from aqueous solution of potassium dichromate (A) at variable biomass concentration (waste tea leaves) (B) at variable shaking speeds (rpm). Mean of 04 replicates and bars represent standard error of the mean. Different letter (s) represent significant difference by Duncan's multiple range test ($P < 0.05$).

No significant increase in Cr^{+6} reduction / sorption at other studied shaking speeds was observed. May be appropriate aeration or mixing is required for getting better binding of the metal ions with the biomass.

Fourier transform infrared spectroscopy

Fourier transform infrared spectral (FTIR) analysis was carried out in the range of $500\text{--}4000 \text{ cm}^{-1}$ (wave number). The spectrum pattern of native waste tea leaves showed absorption peaks in the region of $3747.82\text{--}3350 \text{ cm}^{-1}$ indicative of alcoholic group and NH stretching peak, 3331.72 cm^{-1} indicative of carboxylic acid, 2921.15 cm^{-1} indicative of CH stretch and peaks at 2357.98 and 2341.40 cm^{-1} indicated the existence of amines. Sharp absorption peaks at 2035.61 and 1968.83 cm^{-1} were due to aromatic rings whereas peak at 1620.70 cm^{-1} was due to aromatic ring vibration. Absorption peaks at 1540.88 and 1507.99 cm^{-1} corresponded to the secondary amide group. The absorption peaks in the region of $1000\text{--}1500 \text{ cm}^{-1}$ indicative of $-\text{CH}_3$, CH_2 and CF functional groups while $750\text{--}1000 \text{ cm}^{-1}$ absorption peaks showed existence of S = O, CC and C-Cl functional groups (Fig. 4).

The FTIR spectrum of waste tea leaves after treatment with Cr^{+6} exhibited prominent absorption peaks in the region of $3500\text{--}4000 \text{ cm}^{-1}$ were due to stretching of OH group. The absorption peaks at 895.15 cm^{-1} and 815.83 cm^{-1} were diminished (Fig. 5). The FTIR spectrum of waste tea leaves after treatment with Cr^{+6} and mixed culture of Rb-1 and Rb-2 showed characteristic pattern of absorption peaks. The bending pattern of absorption peaks in the region of $500\text{--}1000 \text{ cm}^{-1}$ were due to stretching of alkyl halides in comparison with the spectrum of native waste tea leaves (Fig. 6). Cr^{+6} stress and inoculation of mixed culture of bacterial strains resulted in shift in the absorption peaks at different regions. These alterations suggested binding of metal ions with certain functional groups³⁶. FTIR spectroscopic analysis of native biomass of waste tea leaves (control) revealed its complex nature due to presence of various absorption peaks representing different functional groups with affinity towards various metallic ions³⁷. Increased Cr^{+6} removal by waste tea leaves in combination with mixed culture might be due to production of certain metabolites such as polysaccharides which increased the availability of binding

sites for metal ions. Increased production of polysaccharides is also supported by FTIR analysis of waste tea leaves in combination with mixed culture of bacterial strains in the region of 1000-500 cm^{-1} (Fig. 6). On the other hand, bacterial cell walls also reported as ideal binding sites for metal ions³⁸. FTIR analysis of waste tea leaves (WTL) before and after metal treatment showed that carboxyl, amino and OH groups present on the waste tea leaves played a significant role in the binding of Cr^{+6} ions with the biomass^{22,39}.

Figure 4: FTIR spectra of waste tea leaves (WTL) treated as control.

Figure 5: FTIR spectra of waste tea leaves (WTL) after treatment with 1000 $\mu\text{g mL}^{-1}$ of Cr^{+6} .

Figure 6: FTIR spectra of waste tea leaves (WTL) after treatment with 1000 $\mu\text{g mL}^{-1}$ of Cr^{+6} in combination with *P. aeruginosa* Rb-1 and *O. intermedium* Rb-2.

Waste tea leaves is a cheap waste material so its utilization alone as well as in combination with *Pseudomonas aeruginosa* Rb-1 and *Ochrobacterium*

intermedium Rb-2 in industrial waste water treatment plants for the removal of toxic hexavalent chromium could be more convenient. The Cr^{+6} removal / sorption are strongly dependent upon pH, initial Cr^{+6} concentration, temperature and biomass concentration. FTIR studies clearly demonstrated the difference in spectra of native and Cr^{+6} treated biowaste indicated the binding of Cr^{+6} ions with the biowaste. However, the exact mechanism for the removal of Cr^{+6} need to be explored.

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