Chapter 3

Materials and Methods

3.1 REDUCTION OF Cr(VI) BY INDIGENOUS BACTERIA

3.1.1 SAMPLE COLLECTION AND CHARACTERIZATION

Mine water from a chromite mine in Sukinda Valley of Jajpur district, Orissa, India was collected in sterile polypropylene bottles and stored at 4°C until used for microbiological analysis. Water samples were analyzed for physical parameters such as pH, total dissolved solids, total suspended solids, conductivity and dissolved oxygen. Chemical analysis was conducted to quantify phosphate, chloride, nitrate and sulphate by titrimetry method. Soluble metal content in mine water was analyzed by inductively coupled plasma-optical emission spectroscope (AAnalyst 400, PerkinElmer, Shelton, CT, USA) following the standard methods of American Public Health Association (2012).

3.1.2 ESTIMATION OF CHROMIUM

A colorimetric method was employed to analyze the concentration of Cr(VI) in the supernatant. Samples were acidified with 0.2 N H$_2$SO$_4$ and reacted with 1,5-diphenylcarbazide to produce a purple colour. The measurement was carried out at 540 nm wavelength (Cheung and Gu 2007). Total Cr was measured using a Flame Atomic Adsorption Spectrophotometer (AAnalyst400/HGA 900, Perkin Elmer, USA) equipped with a 35 mA chromium hollow cathode lamp at a wavelength of 359.9 nm. The minimum detection limit was 0.002 mg /l. Before analysis using the AAS, samples were acidified by 1 N HNO$_3$ to dissolve chromium hydroxide precipitates and to extract adsorbed Cr(VI). Cr(III) concentration was determined as the difference between total Cr and Cr(VI) concentration. Each set of experiments were carried out in triplicate to ensure reproducibility and standard error was calculated.

3.1.3 ISOLATION AND CULTURE CONDITIONS
To isolate hexavalent chromium resistant bacteria, 1 ml of mine water sample was serially diluted in sterile distilled water and plated on M9 minimal medium agar plates containing 10 mg/l of Cr(VI) supplemented as K$_2$Cr$_2$O$_7$. M9 minimal medium composes of M9 salts (Na$_2$HPO$_4$.7H$_2$O, KH$_2$PO$_4$, NaCl, NH$_4$Cl) MgSO$_4$, glucose and CaCl$_2$. The bacterial colonies were observed after 24h of incubation at 37ºC. Isolated colonies were plated onto nutrient agar medium and pure culture of each isolate was obtained.

3.1.4 MAXIMUM TOLERANCE TEST

The maximum tolerable concentration (MTC) of the indigenous isolates were determined by well diffusion method in PYG (peptone yeast glucose) medium with Cr(VI) concentrations ranging from 10 to 1000 mg/l. The maximum concentration of Cr(VI) in the medium which supported growth of organism was taken as the MTC (Malik 2004; Sundar et al 2011).

3.1.5 SELECTION, SCREENING AND CHARACTERIZATION OF THE INDIGENOUS ISOLATES FROM MINE WATER SAMPLE

3.1.5.1 MORPHOLOGICAL CHARACTERISTICS

3.1.5.1.1 COLONY MORPHOLOGY

The colony morphology of pure culture of the isolates was studied on Nutrient Agar plates. Colony morphology was described based on shape, size, texture, elevation and effect on growth medium.

3.1.5.1.2 CELL MORPHOLOGY

The cell morphology was studied using Gram’s staining. The Gram’s stain was used to classify bacteria based on the basis of their forms, sizes gram reaction and cellular morphologies. Gram positive bacteria appear violet and Gram negative appears pink.

3.1.5.2 BIOCHEMICAL CHARACTERIZATION

3.1.5.2.1 CATALASE TEST
Aerobic microorganisms produce hydrogen peroxide (H₂O₂) as their final end product of aerobic respiration. This H₂O₂ when accumulates in environment becomes toxic to the organism. To prevent this, some organisms produce catalase enzyme that oxidizes hydrogen peroxide to oxygen and water. The production of this enzyme can be analyzed by adding 3 % hydrogen peroxide to culture sample of an organism smeared on a glass slide. If catalase enzyme is present, vigorous bubbling will occur.

3.1.5.2.2 OXIDASE TEST

This test is performed to analyze the production of the enzyme cytochrome oxidase. This enzyme in presence of atmospheric oxygen oxidizes the colorless substrate tetramethyl-p-phenylenediamine dihydrochloride to form a dark-purple compound.

3.1.5.2.3 INDOLE TEST

This test is done to check the production of indole from tryptophan using the enzyme tryptophanase. The bacterium to be tested was inoculated in basal medium supplemented with aminoacid tryptophan and incubated overnight. After incubation Kovac’s reagent was added and the formation of a pink or red colored ring at the top was taken as positive.

3.1.5.2.4 METHYL RED (MR) TEST

MR test is performed to check the ability of the organism to produce and maintain acidic end product from glucose fermentation. Some bacteria can produce large amount acids from glucose fermentation, overcoming the buffering actin of the system. Methyl red is a pH indicator, which remain red in colour at pH 4.4 or less. Development of red colour is taken as positive. MR negative organisms produce yellow colour.

3.1.5.2.5 VOGES-PROSKAUER (VP) TEST

VP test is a general test in detecting mixed acid producers. After incubation, 40% KOH and alpha-naphthol were added to the test broth and exposed to the atmospheric O₂. If acetoin is present, it will be oxidized in the presence of air and KOH to form diacetyl. Guanidine components of peptone reacts with diacetyl in the presence of alphanaphthol
to produce red color, which is taken as a positive test. The negative ones must be held maximum for one hour to check for color development after addition of reagents.

3.1.5.2.6 CITRATE UTILISATION TEST

Citrate utilization test is used to identify the ability of an organism to exploit citrate as the sole source of carbon and energy. Citrate is broken down by the enzyme citritase to oxaloacetate and acetate. Oxaloacetate further breaks down to pyruvate and CO₂. Utilization of sodium citrate and ammonium salt produces Na₂CO₃ as well as NH₃, which results in alkaline pH. This changes the medium’s color from green to blue. Bacterial colonies were inoculated into the Simmon’s citrate agar and incubated 37°C for overnight. The green color of medium changes to blue depending on the organism’s ability to utilize citrate.

3.1.5.2.7 HYDROGEN-SULPHIDE PRODUCTION TEST

Tryptone yeast extract agar slants were prepared and inoculated with the bacterial colonies. This was then incubated for 7 days. A typical brown bluish black or black color change of the substrate indicates the H₂S production. The incubated strains were compared with un-inoculated controls.

3.1.5.2.8 UREASE TEST

Urease test was performed to find out the ability of bacterial isolate to produce urease, which hydrolyzes urea into ammonia and carbon dioxide. This test is performed in a broth with two pH buffers, a very small amount of nutrients, urea, and phenol red. If the bacterium degrades urea into ammonia, an alkaline environment would be created, which will turn the media to pink.

3.1.5.2.9 PHENYLALANINE DEAMINATION TEST

Phenylalanine deamination test is performed to find out the ability of bacteria to produce deaminase. If the enzyme is produced, the amine group from the amino acid phenylalanine is removed and is released as free ammonia and phenylpyruvic acid.
The bacterium is inoculated in the phenylalanine deaminase medium which contains nutrients and DL-phenylalanine. After incubation, 10% ferric chloride was added to the media. If phenylpyruvic acid was produced, it will turn dark green after reacting with the ferric chloride. If the straw color of medium is retained, then the organism is considered as negative for the production of phenylalanine deaminase.

3.1.5.2.10 NITRATE REDUCTION TEST

Nitrate reduction test is performed to check the ability of bacteria to produce nitrate reductase. Nitrate broth is used as the test medium which contains nutrients and potassium nitrate as a source of nitrate. The bacterium after incubation is added with sulfanilic acid and α-naphthylamine. If the nitrate is reduced to nitrite by the bacteria, the nitrites will form nitrous acid in the medium. This nitrous acid reacts with sulfanilic acid and forms diazotized sulfanilic acid. This further reacts with α-naphthylamine to form a red-colored compound, which is considered a positive result for nitrate reduction.

If the medium does not turn red, it means either the organism was unable to reduce the nitrate, or the organism had denitrified the nitrate or nitrite to produce ammonia. In order to confirm further, a small amount of powdered zinc is added after the addition of the nitrate reagents. The tube would turn red after addition of reagents, if the unreduced nitrate was present. Hence, a red color in the second step is a negative result.

3.1.5.3 MOLECULAR CHARACTERIZATION

16S rRNA sequencing was performed for the chosen isolate to confirm its identity. The 16S rRNA nucleotide sequencing was performed by fluorescent dye terminator method (ABI Prism Big dye terminator cycle sequencing ready reaction kit v.3.1). The sequence obtained was then analysed using BLAST. The nearest neighbouring sequences were downloaded and aligned using Clustal W version 1.6. Phylogenetic tree was constructed with the sequences aligned by the neighbour joining algorithm using CLC free workbench 3.2 software. Morphological, biochemical and 16S rRNA gene analysis were carried out and the taxonomic identity of the strain was confirmed.

3.1.6 BACTERIAL GROWTH
Growth of bacterial isolates were studied in nutrient broth with and without Cr(VI) in medium. A volume of 100 ml nutrient broth in 250 ml Erlenmeyer flask was sterilized and adjusted to an initial pH 7.5. Growth study was carried out at 30 ºC; Growth was measured in terms of optical density at 600 nm. Specific growth rates were calculated from the microbial growth data obtained from the various batch experiments.

3.1.7 VIABLE CELL COUNT AND DRY WEIGHT OF BIOMASS

Pour plate and colony counting methods were used to enumerate the viable cells with the colonies grown on nutrient broth and plate count agar. Samples were collected at regular time from the experimental batches and were added to the agar plate. The bacterial count measured after 24 h incubation period was reported as colony forming units (CFU) per ml of the sample.

Dry weight of biomass per litre of media was obtained to calculate the reduction capacity of bacterial cells. Cells grown in nutrient broth were withdrawn by sterile pipette and centrifuged at 12000 x g for 10 min at 2 ºC. The pellet was separated and collected in a dry watch glass. The pellet was dried at 105ºC in an oven. To correlate the CFU count to biomass concentration, a direct conversion factor was determined by measuring the dry weight of biomass with a known viable cell concentration (CFU/ml) during the exponential growth phase.

3.1.8 ADAPTATION

To enhance the Cr(VI) reduction rate and growth rate of bacteria, each isolate was adapted serially at 10, 20, 30, 50, 80 and 100 mg/l of Cr(VI). The period for sequential adaptation varied with increasing concentration of Cr(VI). The adaptation was carried out in each concentration till the indigenous isolates showed a maximum growth upto higher concentration till 100 mg/l similar to growth observed in control medium [nutrient broth without Cr(VI)]. The adapted isolates were maintained at 100 mg/l Cr(VI).

3.1.8.1 PHASE CONTRAST MICROSCOPY

Changes in the bacterial cells on adaptation to Cr(VI) was studied in comparison to the un-interacted indigenous strain through the phase contrast microscopy (Ackereley et al
Thin smear of adapted isolates at increasing Cr(VI) concentration were prepared and studied under Phase Contrast Microscope (Leica DM 2500, GMBH) and the micrographs were captured by Lexica DFC 295 camera.

3.1.9 ANTAGONISTIC/SYNERGISTIC ASSAY AND DEVELOPMENT OF CONSORTIUM

To achieve higher reduction rate of Cr(VI), consortium of the adapted isolates was developed based on antagonistic/synergistic assay. Two isolates were taken and grown in nutrient broth supplemented with 100 mg/l of Cr(VI) separately. After 4 hours of incubation, 100 µl of one culture broth was poured onto the surface of nutrient agar supplemented with 100 mg/l of Cr(VI) and a loop full culture of the other isolate was streaked in the middle of the plate. It was incubated at 37°C for 24 hours and then plate was observed. Zone of inhibition between two isolates was absent for all the adapted isolates, which shows that they lack competitive inhibition. This shows that they have synergistic effect which is very important for the development of consortium. Three binary consortiums and one ternary consortium were developed. Growth and Cr(VI) reduction studies were carried out at 5, 10, 20, 50 and 100 mg/l initial concentration of Cr(VI).

3.1.10 Cr(VI) REDUCTION STUDIES

The Cr(VI) stock solution was prepared in sterilized distilled water and then filter sterilized with 0.45 µm Whatman syringe filter. The medium was supplemented with different initial Cr(VI) concentrations 5, 10, 20 mg/l for un-adapted isolates and 5, 10, 20, 50, 100 mg/l. Experiments were carried out in separate flasks for each interval and all the tests were done in duplicates. All the experiments were done at pH 7.5. Initial inoculum containing 1.5 x 10^8 per ml bacterial cells was added to each flask. Nutrient broth without bacterial culture was maintained as control.

To measure the quantity of Cr(VI) reduced by bacterial cells, the samples from each flask was centrifuged (12000 x g for 10 min at 2 ºC) and harvested. The supernatant was separated and analyzed for Cr(VI) concentration by 1, 5-diphenylcarbazide (DPC). Reduction kinetics for chromium reduction has been conducted.
3.1.11 REDUCTION CAPACITY

The Cr(VI) reduction capacity of the bacterial cells was determined as the concentration of Cr(VI) reduced per amount of viable bacterial cells during incubation which is given by the following equation (Molokwane et al 2008)

\[
R_c = \frac{C_o - C}{X_o - X}
\]

where \(R_c\) = Cr(VI) reduction capacity (mg Cr(VI) removed/ mg cells), \(C_o\) = initial Cr(VI) concentration (mg/l), \(C\) = Cr(VI) concentration at time of incubation \(t\), \(X_o\) = initial viable cell concentration (mg/l) and \(X\) = cell concentration at time of incubation \(t\). A viable cell conversion factor was followed for every isolate to convert cell count (CFU) to the mass concentration.

3.1.12 FT-IR STUDY

The surface chemical characteristics of the isolated bacteria were characterized by Fourier Transform-Infra Red Spectrometer (Nicolet 6700 ,Thermo Scientific instruments groups, U.S.A). One mg of each lyophilized bacterial sample (interacted, un-interacted with Cr(VI), adapted and consortium) was mixed with 100 mg KBr and the fine powdered mixture was then pressed in a mechanical die press to form a pellet by applying a pressure of 1200 psi for about 5 min. The transparent tablets were inserted in the instrument and the spectra were recorded from 4000 to 400 cm\(^{-1}\).

3.1.13 SCANNING ELECTRON MICROSCOPY

SEM was performed to characterize the surface morphology of \(B.\ subtilis, A.\ junii, E.coli\) and their consortium. The smear of bacterial biomass was made on the glass slide and air dried. The dried smear was washed by ethanol and then air dried. The glass slide was fixed on specimen mount with carbon tape. The gold was sputtered on the sample in argon atmosphere. The surface morphology of bacteria was observed under Scanning Electron Microscopy (S-400, HITACHI, and Tokyo, Japan) (Paul et al 2012). The surface elemental analysis of unadapted and Cr(VI) interacted adapted bacteria was carried out by Energy Dispersive X-ray spectroscopy. The gold sputtered samples were analyzed and the spectra were recorded using JEOL JSM-5510 equipment.
3.2 ADSORPTIVE REMOVAL OF Cr(VI) BY INDIGENOUS BACTERIAL ISOLATES

3.2.1 PREPARATION OF ALGINATE BEADS

A 4% (w/v) slurry of sodium alginate was prepared by continuous stirring in hot (60°C) distilled water. After cooling the alginate slurry was extruded using a 0.45 x 13 mm gauge syringe into 0.2 M CaCl₂·2H₂O for bead formation. The beads were of 1 mm diameter and were kept in the polymerizing medium for 4 h. Then the beads were washed in saline and distilled deionized water to remove the unbound or loosely bound CaCl₂.

3.2.2 PREPARATION OF BACTERIAL BIOMASS

The three chromite mine isolates obtained from Sukinda mine water, *Bacillus subtilis* VITSUKMW1, *Acinetobacter junii* VITSUKMW2 and *Escherichia coli* VITSUKMW3 (GenBank number: JF309279, JF346549 and JN393206 respectively) were adapted serially till 100 mg/l of Cr(VI), and finally a ternary bacterial consortium was developed from these adapted isolates. The cells were harvested from the bacterial growth media at their logarithmic phase by centrifuging at 4,000 rpm for 20 min. The collected biomass were washed thoroughly using double distilled water (MilliQ), before conducting sorption experiments.

3.2.3 IMMOBILIZATION OF BIOMASS

A 5% (w/v) slurry of sodium alginate was prepared by continuous stirring in hot (60°C) distilled water. After cooling varying quantities (5%, w/v) of adapted bacterial consortium were added and stirred. The alginate – adapted bacterial consortium mixture was then extruded using a 0.45 x 13 mm gauge syringe into 0.2 M CaCl₂·2H₂O as cross linking agent for polymerization and bead formation. The resultant beads were of 1 mm diameter and were kept in the polymerizing medium for 4 h. Then the beads were washed in saline and distilled deionized water to remove the unbound or loosely bound CaCl₂.
3.2.4 BATCH BIOSORPTION STUDIES

Adsorption of chromium by adapted bacterial consortium, alginate beads and adapted bacterial consortium immobilized alginate beads were carried out in batch.

3.2.4.1 OPTIMIZATION OF PARAMETERS

Batch studies were conducted for biosorption at initial Cr(VI) concentration of 100 mg/l and 100 mg sorbent dose in 100 ml metal solution at 30 °C for 180 min with varying pH from 2.0–7.0. Effect of contact time was studied at initial Cr(VI) concentration of 100 mg/l and 100 mg sorbent dose in 100 ml solution at 30 °C and optimized pH. Samples were analyzed for Cr(VI) at time intervals of 30, 60, 90, 120, 180 and 240 minutes. The effect of temperature on sorption was determined through batch experiments carried out at different temperatures (25, 30, 35, 40 and 45 °C). Batches of sorption studies were carried out at optimized sorption parameters and 100 mg sorbent dose in 100 ml metal solution for different initial Cr(VI) concentrations in the range 5–200 mg/l to determine the variation in sorption with initial metal ion concentration. Sorbent dosage was optimized by varying the sorbent dosage, 25, 50, 100, 200, 300 and 400 mg in 100 ml metal solution of 100 mg/l Cr(VI). The optimization studies were carried out for adapted bacterial consortium, alginate beads and adapted bacterial consortium immobilized alginate beads.

3.2.4.2 MEASUREMENT OF BATCH ADSORPTION CAPACITY

Metal sorption efficiency of biosorbent was determined by the sorption capacity which is given as the amount of hexavalent Cr adsorbed per unit of biosorbent (mg metal ions g⁻¹ dry biosorbent). Sorption capacity and the percentage of biosorption by the biosorbent were obtained by the following equations.

\[ q = \frac{V(C_0 - C_e)}{ma} \]  
\[ \text{Biosorption} \% = \left(\frac{C_0 - C_e}{C_0}\right) \times 100 \]  

35
Where \( q \) is the sorption capacity in mg/g, \( V \) is the volume of solution (L), \( C_0 \) is the initial Cr(VI) concentration in mg/l, \( C_e \) is the Cr(VI) concentration at equilibrium and \( m \) is the weight of biosorbent (g).

### 3.2.5 BATCH ADSORPTION ISOTHERM

Adsorption isotherms explain the interaction of a sorbate molecule to the sorbent and are considered as a critical parameter for designing sorption systems. The adsorption equilibrium data at 30 °C were modeled using Langmuir, Freundlich and Dubinin-Radushkevich isotherms to study the mode of interaction of Cr(VI) ions with sorbent when the metal solution phase and sorbent solid phase are in equilibrium.

The linearized Langmuir isotherm applied for Cr(VI) sorption is expressed by the equation [Langmuir 1918]:

\[
\frac{C_e}{q_e} = \frac{1}{q_0 b} + \frac{C_e}{q_0}
\]

where \( C_e \) is the metal residual concentration at equilibrium , \( q_e \) is the chromium concentration adsorbed on the sorbent at equilibrium, \( q_0 \) is maximum specific uptake of metal and \( b \) is the Langmuir constant. The theoretical specific sorption capacity measured from the plot of \( C_e/q_e \) versus \( C_e \) was compared to the corresponding experimentally determined values.

The linear form of Freundlich isotherm is given by the following equation.

\[
\log_{10} (q_e) = \log_{10} (K_f) + \frac{\log_{10} (C_e)}{n}
\]

where \( q_e \) is the amount of chromium adsorbed at equilibrium, \( K_f \) is the Freundlich constant, \( C_e \) is the residual chromium concentration in solution, \( n \) is adsorption intensity. The values of \( K_f \) and \( n \) are obtained from the plots of \( \log_{10} (q_e) \) versus \( \log_{10} (C_e) \). Larger the \( K_f \) and \( n \) values, the higher the sorption capacity.

Sorption data was also subjected to Dubinin–Radushkevich model and is represented by the following equation.
\[ \log_{10} q_e = \log_{10} q_D - 2B_D R^2 T^2 \log_{10} \left( 1 + \frac{1}{c_p} \right) \]  \hspace{1cm} (6)

where \( q_D \) is the predicted saturation capacity (mg/g), \( B_D \) is a constant related to adsorption energy (mol\(^2\)K/J\(^2\)), \( R \) is the gas constant (KJ/mol/K) and \( T \) is the temperature (K). The constant \( B_D \) is inversely proportional to mean free energy of molecule for sorption which is determined by the following equation.

\[ ED = \frac{1}{\sqrt{2B_D}} \]  \hspace{1cm} (7)

3.2.6 BATCH ADSORPTION KINETICS

Dynamics of sorption process describes the rate at which a solute gets adsorbed onto the biosorbent. Different sorption models have been used to explain the metal uptake rate. Pseudo-first order and pseudo-second order models were applied to the sorption data. The model with the highest correlation coefficient value (\( r^2 \)), close to unity was considered the best fit.

Pseudo-first order or Lagergen kinetic model is based on the biosorbent capacity and is represented by the following equation.

\[ \frac{dq}{dt} = k_1(q_e - q_t) \]  \hspace{1cm} (8)

where \( q_e \) and \( q_t \) are the sorption capacities (mg/g) at equilibrium and at time ‘t’ respectively, \( k_1 \) is the pseudo-first order rate constant (min\(^{-1}\)). The logarithmic derivative of this equation is the most used form and is represented by the following equation.

\[ \log (q_e - q_t) = \log_{10} q_e - \left( \frac{k_1}{2.303} \right) t \]  \hspace{1cm} (9)

The plot of \( \log (q_e - q_t) \) vs. \( t \) gives a linear relationship from which the slope \( k_1 \) is determined.

The pseudo-second order kinetic model sorption kinetics equation is represented by the following equation.
\[ \frac{dq}{dt} = k_2 (q_e - q_t)^2 \]  

(10)

where \( k_2 \) is the pseudo-second order rate constant for sorption (g\(^{-1}\) min\(^{-1}\)). Equation (9) can be rearranged to obtain more useful form given by the following equation.

\[ \frac{1}{(q_e - q_t)} = \frac{1}{q_e} + k_2 t \]  

(11)

The plot of \( 1/(q_e - q_t) \) vs. \( t \) gives a linear relationship if the pseudo second order is applicable.

3.2.7 THERMODYNAMICS OF SORPTION

Thermodynamic parameters such as changes in standard free energy (\( \Delta G^o \)), standard enthalpy (\( \Delta H^o \)) and standard entropy (\( \Delta S^o \)), were calculated for the evaluation of feasibility of the adsorption reaction and fully understand the nature of adsorption. It was possible to determine the thermodynamic parameters for the sorption reaction by using the equilibrium constants under the variable experimental conditions.

Based on the thermodynamics, the relation between \( \Delta G^o \) and the equilibrium constant (\( K \)) is known by the equation

\[ \Delta G^o = -RT \ln (K) \]  

(12)

Again, \( \Delta H^o \) and \( \Delta S^o \) of the reaction at constant temperature is related to the \( \Delta G^o \) according to the following equation

\[ \ln (K) = \frac{\Delta S^o}{R} - \frac{\Delta H^o}{RT} \]  

(13)

Where, \( K \) can be considered as the Langmuir constant, \( b \) if the system follows Langmuir isotherm and \( R \) is the universal gas constant (8.314 J K\(^{-1}\) Kgmol\(^{-1}\)). The thermodynamic parameters determine the spontaneity of the reaction and randomness of the system during the sorption process.

The \( \Delta H^o \) and \( \Delta S^o \) values of sorption were measured from the slope and the intercept of the plot between \( 1/T \) verses \( \ln (K) \). Here \( K \) value was taken as the Langmuir constant \( b \), obtained for sorption by alginate beads, adapted bacterial consortium and adapted bacterial consortium immobilized alginate beads.
3.2.8 Cr(VI) & Cr(III) SYNTHETIC MIXTURE STUDIES

Effect of Cr(III) on the adsorption of Cr(VI) by adapted bacterial consortium immobilized alginate beads were studied by mixing Cr(VI) and Cr(III) at different concentrations (95+5, 70+30, 50+50, 30+70, 5+95) in distill deionized water, keeping the total initial concentration to be 100 mg/l. The adsorption experiments were carried out at optimized condition (pH 3.0, 30°C temperature, 1g/l biosorbent dosage and 180 min equilibrium time).

3.2.9 PACKED BED REACTOR STUDIES

Continuous sorption experiments (Figure 1) were conducted in a glass column (1.5 cm Internal diameter and 30 cm height), packed with a known quantity of adapted bacterial consortium immobilized alginate beads. 100 mg/l of Cr(VI) solution was pumped through the column, at desired flow rates using a peristaltic pump. The optimized parameters were used for the adapted bacterial consortium immobilized alginate beads. The Cr(VI) concentration was quantified at each interval from the sampling point. The packed bed reactor study was carried out at pH 3.0 and 30°C temperature. The effect of flow rate on Cr(VI) adsorption was studied by varying the flow rates, 3, 9 and 15 ml/min, keeping bed height and initial Cr(VI) concentration constant at 10 cm and 100 mg/l, respectively. Effect of bed height on sorption of Cr(VI) was evaluated by varying the bed height, 10, 15 and 20 cm, while flow rate and inlet Cr(VI) concentration were constant at 3 ml/min and 100 mg/l respectively. Effect of influent Cr(VI) concentration on breakthrough point and sorption capacity was studied by varying influent Cr(VI) concentration, 100, 200 and 300 mg/g respectively keeping the bed height and flow rate (20 cm and 3 ml/min) constant.

The total quantity of Cr(VI) adsorbed in the column ($W_{ad}$) is calculated from the area above the breakthrough curve [outlet Cr(VI) concentration Vs. Time] multiplied by flow rate. Adsorption capacity ($q$) of the adapted bacterial consortium immobilized alginate beads, is calculated on dividing $W_{ad}$ by sorbent mass (M). The total amount of Cr(VI) ions entering the column can be calculated from the equation:
\[ W = \frac{C_0 F t_e}{1000} \]  

(14)

Where \( C_0 \) is the inlet Cr(VI) concentration (mg/l), \( F \) is the volumetric flow rate (ml/h) and \( t_e \) is the exhaustion time (h). Total Cr(VI) removal percentage can be calculated from the equation:

Total Cr(VI) removal percentage = \[ \frac{W_{ad}}{W} \]  

(15)

Fig. 1 Schematic diagram of continuous flow reactor

3.2.9.1 ADAMS–BOHART MODEL

Bohart and Adams based on the surface reaction theory established an equation, which describes the relationship between \( C_t/C_0 \) and \( t \) in a continuous system. This model assumes that that equilibrium is not instantaneous. It is used for describing the initial part of the breakthrough curve. The expression is as follows:
where $C_0$ and $C_t$ are the influent and effluent concentration (mg/l), respectively; $K_{AB}$ is the kinetic constant (L/mg min), $N_0$ is the saturation concentration (mg/l), $Z$ is the bed depth of the packed bed reactor (cm) and $U_0$ is the superficial velocity (cm/min) defined as the ratio of the volumetric flow rate $Q$ (cm$^3$/min) to the cross-sectional area of the bed $A$ (cm$^2$). The range of $t$ was taken into consideration from the beginning to the end of breakthrough. The parameters $K_{AB}$ and $N_0$ can be calculated from the linear plot of $\ln(C_t/C_0)$ against $t$ (graph not provided).

### 3.2.9.2 THOMAS MODEL

Thomas model is used to describe the performance theory of the sorption process in packed bed reactor. The linearized form of this model can be described by the following expression.

$$\ln \left( \frac{C_t}{C_0} \right) = K_{TH} \frac{q_0}{q} - K_{TH} q_0 t$$

(17)

Where, $K_{TH}$ is the Thomas model constant (ml/min mg), $q_0$ is the adsorption capacity (mg/g), and $t$ stands for total flow time (min). The values of $K_{TH}$ and $q_0$ can be determined from the linear plot of $\ln [(C_0/C_t) - 1]$ against $t$ (graph not provided).

### 3.2.9.3 YOON–NELSON MODEL

Yoon and Nelson developed a model to investigate the breakthrough behavior of adsorbate gases on activated charcoal. The Yoon–Nelson model is based on the postulation that the rate of decrease in the probability of sorption for each sorbate molecule is proportional to the probability of sorbate sorption and the probability of sorbate breakthrough on the adsorbent. The linearized Yoon–Nelson model is expressed as:

$$\ln \left( \frac{C_t}{C_0 - C_t} \right) = K_{YN} t - K_{YN}$$

(18)
where $K_{YN}$ is the rate constant (min) and $s$ is the time required for 50% adsorbate breakthrough (min). A linear plot of $\ln[C_t/(C_0 - C_t)]$ against $t$ determined the values of $K_{YN}$ and $s$ from the intercept and slope of the plot.

3.2.10 TREATMENT OF Cr(VI) SPIKED NATURAL WATER

Adsorption of spiked Cr(VI) from different environmental water matrices was investigated. Water samples from different locations [Ground water (GW1) – Soloor, Ground water (GW2) – Suthipattu, Lake water (LW) – VIT, Waste water (WW) – VIT] were collected, and adsorption experiments were carried out at optimized pH 3, 3 ml/min flow rate, 20 cm bed height of sorbent (adapted bacterial consortium immobilized alginate beads) and 100 mg/l of influent Cr(VI) concentration. The Cr(VI) adsorption capacity of adapted bacterial consortium immobilized alginate beads in different environmental water matrices was obtained.

3.2.11 CHARACTERIZATION OF SOLID SORBENT

3.2.11.1 BET SURFACE AREA AND PORE SIZE DISTRIBUTION

Surface area, pore volume, pore diameter and porosity of the alginate bead and adapted bacterial consortium immobilized alginate bead are determined with BET instrument (Micromeritics Autopore IV, USA). The isotherm plots are used to compute the average pore diameter and specific surface area ($N_2$/BET method) of beads, while pore volume is estimated from the volume of $N_2$ adsorbed at $p/p_0$ 0.99.

3.2.11.2 SCANNING ELECTRON MICROSCOPY AND EDX ANALYSIS

Interacted and un-interacted adapted bacterial consortium immobilized alginate beads were attached to 10 mm metal mounts using carbon tape and sputter coated with gold under vacuum in argon atmosphere. The surface morphology of the coated sample was analyzed using scanning electron microscope (Hitachi S400, Japan). The surface elemental analysis of Cr(VI) interacted and un-interacted adapted bacterial consortium immobilized alginate beads were carried out by Energy Dispersive X-ray spectroscopy.
The gold sputtered samples were analyzed and the spectra were recorded using JEOL JSM-5510 equipment.

3.2.12 STATISTICAL ANALYSIS

Each set of the experiment was performed in triplicates (in batch sorption experiments). One-way Anova with Dunnette’s post test and Tukey's Multiple Comparison Test was carried out using GraphPad Prism 5.0 software to check statistical significance of the results obtained experimentally.
3.3 ADSORPTIVE REMOVAL OF Cr(VI) BY FRESHWATER ALGAL ISOLATES

3.3.1 PREPARATION AND IMMOBILIZATION OF ALGAL CONSORTIUM

The algae, *Oocystis sp*, *Nostoc sp*, *Syncoccus sp* and *Desimococcus sp* were isolated from Retteri Lake, Chennai, India and grown in BG 11 broth under shaking condition (120 rpm) at 30 °C. The Cr concentration in lake water measured by Atomic absorption spectrophotometer (AAS) was below detection level. The genus level identification was made with the assistance of Centre for Advanced Studies (CAS) Botany in University of Madras, India. The algal cells were adapted sequentially from 1 mg/l to 100 mg/l of Cr(VI). The adapted algal isolates were tested for antagonistic/synergistic effect. The adapted algal cells having synergistic effect were grown together as consortium. An algal consortium was developed to enhance the Cr(VI) sorption capacity. For sorption study, the algal cells were grown both as individual and consortium. The cells were harvested by centrifuging the algal culture media at 4,000× g for 20 min. The collected algal cells were washed using double distilled water before conducting the sorption experiments.

A 4% (w/v) slurry of sodium alginate was prepared by continuous stirring in hot (60 °C) distilled water. A 4% (w/v) of adapted algal consortium was added to sodium alginate slurry after cooling and stirred. The alginate–adapted algal consortium was then extruded using a 0.45 × 13 mm gauge syringe into 0.2 M CaCl₂·2H₂O as a cross linking agent for polymerization and immobilized bead formation. The beads were of 1 mm diameter and were kept in the polymerizing medium for 4 h at 4 °C. Then the beads were washed in saline and distilled water to remove the unbound or loosely bound CaCl₂. In another novel development, the algal consortium was immobilized to improve the Cr(VI) sorption capacity obtained by free cells of algal consortium.
3.3.2 SUSPENDED/IMMOBILIZED BATCH REACTOR STUDIES

3.3.2.1 OPTIMIZATION OF PARAMETERS

The adsorption of chromium by individual algal sp (Oocystis sp, Nostoc sp, Syncoccus sp and Desimococcus sp), adapted algal consortium and adapted algal consortium immobilized alginate beads were carried out in batch. The batch studies were conducted for biosorption at 100 mg/l of Cr(VI) concentration and 100 mg sorbent dose in 100 ml metal solution at 30 °C for 180 min at pH varying from 2.0–7.0 by adding 0.01 N HCl. The effect of contact time was studied at 100 mg/l of Cr(VI) concentration and 100 mg sorbent dose in 100 ml solution at 30 °C and optimized pH. The samples were analyzed for Cr(VI) at 30, 60, 90, 120, 180 and 240 minutes. The effect of temperature on sorption was determined through batch experiments carried out at different temperatures (25, 30, 35, 40 and 45 °C). The effect of temperature on Cr(VI) solubility was tested by control experiments conducted in the absence of sorbent. The batches of sorption studies were carried out at optimized sorption parameters and 100 mg sorbent dose in 100 ml metal solution for different initial Cr(VI) concentrations in the range of 5–300 mg/l to determine the variation in sorption with initial metal ion concentration. The sorbent dosage was optimized by varying the sorbent dosage at 25, 50, 100, 200, 300 and 400 mg in 100 ml metal solution of 100 mg/l Cr(VI).

3.3.2.2 MEASUREMENT OF BATCH ADSORPTION CAPACITY

The metal sorption efficiency of biosorbent was determined by the sorption capacity which is given as the amount of Cr(VI) adsorbed per unit of the biosorbent (mg metal ions/g dry biosorbent). The sorption capacity and the percentage of biosorption by the biosorbent were obtained by the following equations:

\[ q = \frac{V(C_o - C_e)}{m} \]  \hspace{1cm} (19)

\[ \text{Biosorption} \% = \frac{C_o - C_e}{C_o} \times 100 \]  \hspace{1cm} (20)
Where, $q$ is the sorption capacity in mg/g, $V$ is the volume of solution (L), $C_o$ is the initial Cr(VI) concentration in mg/l, $C_e$ is the Cr(VI) concentration at equilibrium and $m$ is the weight of biosorbent (g).

3.3.3 BATCH ADSORPTION ISOTHERM

The adsorption isotherms explain the interaction of a sorbate molecule to the sorbent and are considered as a critical parameter for designing sorption systems. The adsorption equilibrium data at 30 °C were modeled using Langmuir, Freundlich and Dubinin-Radushkevich isotherms to study the mode of interaction of Cr(VI) ions with sorbent when the metal solution phase and sorbent solid phase are in equilibrium.

The linearized Langmuir isotherm applied for Cr(VI) sorption is stated by the equation (Langmuir 1918):

$$\frac{C_e}{q_e} = \frac{1}{q_0b} + \frac{C_e}{q_o}$$  \hspace{1cm} (21)

where $C_e$ is the Cr(VI) residual concentration at equilibrium, $q_e$ is the chromium concentration adsorbed on the sorbent at equilibrium, $q_o$ is maximum specific uptake of metal and $b$ is the Langmuir constant. The theoretical specific sorption capacity measured from the plot of $C_e/q_e$ versus $C_e$ was compared to the corresponding experimentally determined values.

The linear form of Freundlich isotherm is shown by the following equation.

$$\log_{10}(q_e) = \log_{10}(K_f) + \frac{\log_{10}(C_e)}{n}$$  \hspace{1cm} (22)

where $q_e$ is the amount of chromium adsorbed at equilibrium, $K_f$ is Freundlich constant, $C_e$ is the residual chromium concentration in solution, $n$ stands for adsorption intensity. $K_f$ and $n$ values are calculated from the plots of $\log_{10}(q_e)$ versus $\log_{10}(C_e)$. Larger the $K_f$ and $n$ values, the higher the sorption capacity.

Sorption data was also subjected to Dubinin–Radushkevich model and is represented by the following equation.
where $q_D$ is the theoretical saturation capacity (mg g$^{-1}$), $R$ is the gas constant (KJ mol$^{-1}$ K$^{-1}$), $B_D$ is a constant related to adsorption energy (mol$^2$ KJ$^{-2}$), and $T$ is the temperature (K). The constant $B_D$ is inversely proportional to mean free energy of molecule for sorption which is determined by the following equation.

$$ED = \frac{1}{\sqrt{2B_D}}$$  \hspace{1cm} (24)

3.3.4 BATCH ADSORPTION KINETICS

The dynamics of sorption process describes the rate at which a solute gets adsorbed onto the biosorbent. The pseudo-first order and pseudo-second order models were applied to the sorption data. The model with the highest correlation coefficient value ($r^2$) close to unity was considered the best fit.

Pseudo-first order or Lagergen kinetic model is based on the biosorbent capacity and is represented by the following equation.

$$\frac{dq}{dt} = k_1 (q_e - q_t)$$  \hspace{1cm} (25)

where $q_e$ and $q_t$ are the sorption capacities (mg/g) at equilibrium and at time $t$ respectively, $k_1$ is the pseudo-first order rate constant (min$^{-1}$). The logarithmic derivative of this equation is the most used form and is represented by the following equation.

$$\log (q_e - q_t) = \log_{10} q_e - \left(\frac{k_1}{2.303}\right)t$$  \hspace{1cm} (26)

The plot of $\log (q_e - q_t)$ vs. $t$ gives a linear relationship from which the slope $k_1$ is determined.

The pseudo-second order kinetic model sorption kinetics equation is represented by the following equation.

$$\frac{dq}{dt} = k_2 (q_e - q_t)^2$$  \hspace{1cm} (27)
where $k_2$ is the pseudo-second order rate constant for sorption ($g^{-1} \text{ min}^{-1}$). Equation (9) can be rearranged to obtain more useful form given by the following equation.

$$\frac{1}{(q_e - q_t)} = \frac{1}{q_e} + k_2t$$

(28)

The plot of $1/(q_e - q_t)$ vs. $t$ gives a linear relationship if the pseudo second order is applicable.

3.3.5 THERMODYNAMICS OF SORPTION

The thermodynamic parameters such as changes in standard free energy ($\Delta G^o$), standard enthalpy ($\Delta H^o$) and standard entropy ($\Delta S^o$) were calculated for the evaluation of feasibility of the adsorption reaction and to fully understand the nature of adsorption. The thermodynamic parameters were calculated for the sorption reaction by deeming the equilibrium constants under several experimental conditions. Based on the thermodynamics, the relation between $\Delta G^o$ and the equilibrium constant ($K$) is provided by the equation

$$\Delta G^o = -RT \ln (K)$$

(29)

Again, $\Delta H^o$ and $\Delta S^o$ of the reaction at constant temperature is related to the $\Delta G^o$ according to the following equation

$$\ln (K) = \frac{\Delta S^o}{R} - \frac{\Delta H^o}{RT}$$

(30)

Where, $K$ can be considered as the Langmuir constant, $b$ if the system follows Langmuir isotherm and $R$ is the universal gas constant (8.314 J K$^{-1}$ Kg mol$^{-1}$). The thermodynamic parameters determine the spontaneity of the reaction and randomness of the system during the sorption process.

The $\Delta H^o$ and $\Delta S^o$ values of sorption were determined from the slope and the intercept of the plot between $1/T$ verses $\ln (K)$. Here $K$ value was taken as the Langmuir constant $b$, obtained for sorption by adapted algal consortium immobilized alginate beads.
3.3.6 CHARACTERIZATION OF ALGAL CONSORTIUM IMMOBILIZED ALGINATE BEADS

3.3.6.1 BET SURFACE AREA AND PORE SIZE DISTRIBUTION

Surface area, pore volume, pore diameter and porosity of the alginate beads and adapted algal consortium immobilized alginate beads were determined with a BET analyzer (Micromeritics Autopore IV, USA). The isotherm plots were used to calculate the average pore diameter and the specific surface area (N\textsubscript{2}/BET method) of the beads, while pore volume was calculated from the volume of N\textsubscript{2} adsorbed at p/p\textsubscript{0} 0.999.

3.3.6.2 SCANNING ELECTRON MICROSCOPY AND EDX ANALYSIS

The surface morphology of algal consortium immobilized alginate beads were studied under scanning electron microscope. The interacted and un-interacted algal consortium immobilized alginate beads were dried and mounted on 10 mm metal stubs using a carbon tape. The samples were then sputtered coated with gold under vacuum in argon atmosphere. The analysis was made using potential difference of 20 kV for the tungsten filament. The surface morphology of the coated sample was observed by a scanning electron microscope (Hitachi S400, Japan).

The surface elemental analysis of Cr(VI) interacted and un-interacted algal consortium immobilized alginate beads was carried out by Energy Dispersive X-ray spectroscopy. The gold sputtered samples were analyzed and the spectra were recorded using JEOL JSM-5510 equipments.

3.3.7 APPLICATION STUDIES IN IMMOBILIZED BED CONTINUOUS FLOW REACTOR

The continuous sorption experiments were conducted in a glass column (1.5 cm ID and 30 cm height), packed with a known quantity of adapted algal consortium immobilized alginate beads. A 100 mg/l of Cr(VI) solution was pumped through the column, at desired flow rates using a peristaltic pump. The optimized parameters were used for the algal consortium immobilized alginate beads. The Cr(VI) concentration was
quantified at each interval from the sampling point. The packed bed column study was carried out at pH 3.0 and 30 °C temperature.

The total quantity of Cr(VI) adsorbed in the column ($W_{ad}$) is calculated from the area above the breakthrough curve [outlet Cr(VI) concentration vs. time] multiplied by the flow rate. The adsorption capacity ($q$) of algal consortium immobilized alginate beads is calculated on dividing $W_{ad}$ by sorbent mass ($M$). The total amount of Cr(VI) ions entering the column can be calculated from the equation [31]:

$$W = \frac{C_0 \cdot F \cdot t_e}{1000}$$  \hspace{1cm} (31)

where, $C_0$ is the inlet Cr(VI) concentration (mg/l), $F$ is the volumetric flow rate (ml/h) and $t_e$ is the exhaustion time (h). The total Cr(VI) removal percentage can be calculated from the equation:

Total Cr(VI) removal percentage = $\frac{W_{ad}}{W}$  \hspace{1cm} (32)

The effect of flow rate on Cr(VI) adsorption was studied by varying the flow rates, 3, 9 and 15 ml/min, keeping bed height and initial Cr(VI) concentration constant at 10 cm and 100 mg/l respectively. The effect of bed height on sorption of Cr(VI) was evaluated by varying the bed height, 10, 15 and 20 cm, while, flow rate and inlet Cr(VI) concentration were constant at 3 ml/min and 100 mg/l respectively. The effect of influent Cr(VI) concentration on breakthrough point and sorption capacity was studied by varying influent Cr(VI) concentration, 100, 200 and 300 mg/g respectively keeping the bed height and flow rate (20 cm and 3 ml/min) constant.

The reusability of biosorbent was studied by four cycles of alternating sorption/desorption experiments with the supplement of 300 mg/l of Cr(VI) at the beginning of each cycle. About 0.01 N NaOH was used to desorb Cr(VI) from column.
3.3.7.1 ADAMS–BOHART MODEL

Bohart and Adams based on the surface reaction theory established an equation, which describes the relationship between \( C_t/C_0 \) and \( t \) in a continuous system. This model assumes that the equilibrium is not instantaneous. It is used for describing the initial part of the breakthrough curve. The expression is as follows:

\[
\ln \left( \frac{C_t}{C_0} \right) = K_{AB} C_0 t - K_{AB} N_0 \left( \frac{Z}{U_0} \right)
\]  

(33)

where \( C_0 \) and \( C_t \) are the influent and effluent concentration (mg/l), respectively; \( K_{AB} \) is the kinetic constant (L/mg min), \( N_0 \) is the saturation concentration (mg/l), \( Z \) is the bed depth of the packed bed reactor (cm) and \( U_0 \) is the superficial velocity (cm/min) defined as the ratio of the volumetric flow rate \( Q \) (cm\(^3\)/min) to the cross-sectional area of the bed \( A \) (cm\(^2\)). The range of \( t \) was taken into consideration from the beginning to the end of breakthrough. The parameters \( K_{AB} \) and \( N_0 \) can be calculated from the linear plot of \( \ln(C_t/C_0) \) against \( t \) (graph not provided).

3.3.7.2 THOMAS MODEL

Thomas model is used to describe the performance theory of the sorption process in packed bed reactor. The linearized form of this model can be described by the following expression.

\[
\ln \left( \frac{C_0}{C_t} - 1 \right) = K_{TH} \frac{q_0}{q} t
\]  

(34)

Where, \( K_{TH} \) is the Thomas model constant (ml/min mg), \( q_0 \) is the adsorption capacity (mg/g), and \( t \) stands for total flow time (min). The values of \( K_{TH} \) and \( q_0 \) can be calculated from the linear plot of \( \ln \left( [(C_0/C_t) - 1] \right) \) against \( t \) (graph not provided).

3.3.7.3 YOON–NELSON MODEL

Yoon and Nelson developed a model to investigate the breakthrough behavior of adsorbate gases on activated charcoal. The Yoon–Nelson model is based on the postulation that the rate of decrease in the probability of sorption for each sorbate
molecule is proportional to the probability of sorbate sorption and the probability of sorbate breakthrough on the adsorbent. The linearized Yoon–Nelson model is expressed as:

\[
\ln \left( \frac{C_t}{C_0 - C_t} \right) = K_{YN} t - s K_{YN}
\]  

(35)

where \( K_{YN} \) is the rate constant (min) and \( s \) is the time required for 50% adsorbate breakthrough (min). A linear plot of \( \ln[C_t/(C_0 - C_t)] \) against \( t \) determined the values of \( K_{YN} \) and \( s \) from the intercept and slope of the plot.

3.3.8 TREATMENT OF Cr(VI) SPIKED NATURAL WATER

The application of optimized Cr(VI) sorption was investigated in different natural water and tannery effluent matrices. The samples from different locations [ground water (GW1, GW2) from Soloor and Suthipattu in Vellore, India; lake water (freshwater) and domestic waste water from VIT University, Vellore, India) were collected, and adsorption experiments were carried out at optimized pH 3, 30 °C temperature, 1 g/l sorbent (algal consortium immobilized alginate beads) dosage and equilibrium time 180 min. Initially, 300 mg/l of Cr(VI) was spiked in all the samples. Both initial and final concentrations of Cr(VI) were studied by 1, 5 Diphenyl carbazide assay. The total Cr was quantified by Atomic Absorption Spectrophotometer. The Cr(III) concentration was calculated by the difference in total Cr and Cr(VI).

3.3.9 STATISTICAL ANALYSIS

Each set of experiment was carried out in triplicates. One-way Anova with Dunnette’s post test and Tukey's Multiple Comparison Test was carried out using GraphPad Prism 5.0 software to check statistical significance of the results obtained experimentally.
3.4 FATE OF BIO-SORBED Cr(VI)

3.4.1 Cr(VI) BIOSORPTION AND BIOREDUCTION STUDY

The biosorption and bioreduction of Cr(VI) by adapted bacteria consortium immobilized alginate beads was carried out under optimized condition for Cr(VI) adsorption with increase in time (pH: 3.0, temperature: 30 °C, initial concentration of Cr(VI): 150 mg/l, and sorbent dosage: 1 g/l) with increased interaction time of 240 h.

The adsorption and bioreduction of Cr(VI) by adapted algal consortium immobilized alginate beads was carried out under optimized condition for Cr(VI) adsorption with increase in time ((pH: 3.0, contact time: 180 min, temperature: 30 °C, initial Cr(VI) concentration: 150 mg/l, sorbent dosage: 1g/l)) with increased interaction time of 240 h.

3.4.2 SPECIATION OF Cr RELEASED FROM Cr(VI) LOADED SORBENT

After equilibrium sorption, the Cr(VI) loaded algal consortium immobilized alginate beads were collected and suspended in distilled under optimized sorption parameters without Cr(VI). The setup was kept in shaker for 240 h (10 days) and the samples were collected at 24 h interval and analyzed for Cr(VI) and Cr(III). The surface adsorbed Cr was found to be released into water. The released Cr was quantified by DPC and AAS.

3.4.3 MECHANISTIC STUDIES

3.4.3.1 ELECTRON PARAMAGNETIC RESONANCE (EPR)

The solid state spectra of bacterial and algal consortium immobilized alginate beads interacted with Cr(VI), was recorded at room temperature by Varian E-112 Electron Paramagnetic Resonance spectrometer (9.5 GHz) operating at X-band frequency with a 100 kHz modulation frequency. The spectrum was recorded under the following conditions:- microwave power: 3.1 mW, modulation amplitude: 3 G.
3.4.3.2 FOURIER TRANSFORM INFRA RED SPECTROSCOPY (FT-IR)

The surface chemical characteristics of the biosorbent were characterized by IR Affinity–1 Fourier Transform-Infra Red Spectrometer (Shimadzu, Japan). The Cr(VI) interacted and un-interacted samples of both algal consortium immobilized alginate beads and bacterial consortium immobilized alginate beads were each mixed separately with 100 mg KBr and the fine powdered sample was then pressed in a mechanical die press to form a pellet by applying a pressure of 1200 psi for about 5 min. The transparent tablets were inserted in the instrument and the spectra were recorded from 4000 to 400 cm\(^{-1}\).