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Bioremoval of trivalent chromium using *Bacillus* biofilms through continuous flow reactor

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ABSTRACT

Present study deals with the applicability of bacterial biofilms for the bioremoval of trivalent chromium from tannery effluents. A continuous flow reactor was designed for the development of biofilms on different substrates like glass beads, pebbles and coarse sand. The parameters for the continuous flow reactor were 20 ml/min flow rate at 30 °C, pH4. Biofilm biomass on the substrates was in the following sequence: coarse sand > pebbles > glass beads (4.8×10^7, 4.5×10^7 and 3.5×10^5 CFU/cm^2), which was confirmed by CLSM. Biofilms developed using consortium of *Bacillus subtilis* and *Bacillus cereus* on coarse sand had more surface area and was able to remove 98% of Cr(III), SEM-EDX proved 92.60% Cr(III) adsorption on biofilms supported by coarse sand. Utilization of *Bacillus* biofilms for effective bioremoval of Cr(III) from chrome tanning effluent could be a better option for tannery industry, especially during post chrome tanning operation.

1. Introduction

Vellore district is mushroomed with many tanneries. There is lot of unorganized tanning industries; which forms the livelihood of Vellore population. Cr(III) is used as a tanning agent in the leather industries; the wastewater resulting from chrome tanning processes contains high amount of chromium metal, which is harmful for the environment and human health [1]. Toxicological studies of Cr(III) compounds reported skeletal and neurological disorders [2]. Cr(III) compounds are cytotoxic and forms DNA adduct [3].

Though there are many methods for effluent treatment like precipitation, chemical oxidation or reduction, lime neutralization, ion exchange, filtration, electrochemical treatment, reverse osmosis, membrane technologies and evaporation recovery. All these methods are expensive and will produce solid sludge containing toxic compounds [4]. And also chromium at low concentrations in the effluent waters cannot be removed by conventional methods [5]. Thus an alternate treatment strategy is required, which would be environment friendly. Indigenous chromium tolerant bacterial strains might be a better choice for the development of biofilms towards chromium removal from tannery effluent.

The purpose of the present study was to isolate Cr(III) tolerant bacterial species from tannery effluent polluted sites in Palar river basin and to develop a bacterial biofilm on different substrates like glass beads, pebbles, coarse sand for bioremoval of Cr(III). The study focuses on the bioremoval of Cr(III) using indigenous chromium tolerant bacterial biofilms through continuous flow reactor. The biofilm bioreactor would be a better choice for tanneries to alleviate Cr(III) pollution in the effluent waters. This technology could be adopted in an industrial scale for environmental problems of tanneries.

2. Materials and methods

2.1. Isolation, screening and characterization of effective strains

Soil and water samples were collected from chromium polluted sites in the Palar river basin of Vellore district, Tamilnadu, India. Samples were processed for the isolation of Cr(III) tolerant bacterial strains as per APHA [6]. Trivalent chromium tolerant bacteria were isolated using nutrient agar plates amended with $Cr(NO_3)3{\cdot}9H_2O$ at pH 4 and incubated at 30 °C for 3–5 days.

The effective strains were screened based on maximum tolerable concentration (MTC), Cr(III) bioremoval ability and exopolysaccharide (EPS) production [7]. The selected strains were characterized morphologically, biochemically and physiologically following Gerhardt et al. [8]. The taxonomical identifications of the selected bacterial strains were confirmed by 16S rRNA gene sequencing.

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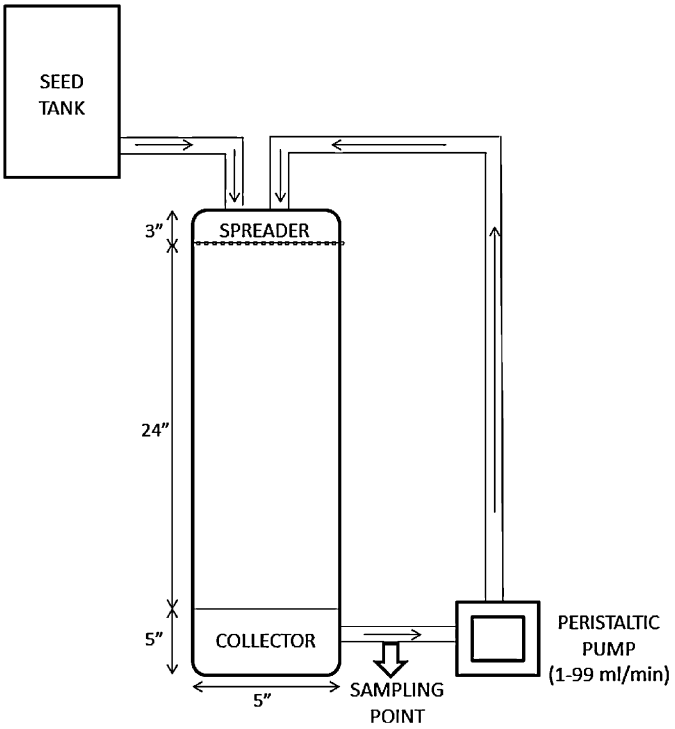

Fig. 1. Schematic diagram of continuous flow biofilm cultivation reactor.

2.2. Biofilm formation by tissue culture plate assay

The bacterial biofilm estimation was carried out using standard tissue culture plate (TCP) assay as described by Christensen et al. [9]. In the present study, two bacterial strains (*Bacillus subtilis* VITSCCr 01 and *Bacillus cereus* VITSCCr 02) were observed for their ability to form biofilm on tissue culture plate. Overnight cultures of *Bacillus* strains were diluted to 1×10^6 CFU/mL in fresh Nutrient Broth (NB) with 0.5% glucose after adjusting the OD at 600 nm. Aliquots (200 μL) of various concentrations of Cr(III) (10, 25, 50, 75, and 100 mg/l) was transferred onto 96-well flat-bottom tissue culture plates, uninoculated broth served as control. Plates were incubated in static and dynamic conditions for 48 h at 30 °C. After incubation, the content of each well was gently removed by tapping the plates. The wells were washed twice with 200 μL of deionized water to remove free-floating 'planktonic' bacteria. Biofilms in plates were dried at 60 °C for 1 h and adherent bacteria were stained with 200 μL of 0.1% crystal violet for 5 min. The plates were rinsed twice with deionized water to remove excess stain and dried at 37 °C for 2 h. Stained adherent cells were detached from the plates using 200 μL of 30% (w/v) glacial acetic acid for 10 min with shaking at 300 rpm. The OD of stained biofilm was determined at 492 nm (Power Wave XS2, Biotek Corp, Microplate reader). The mean OD value obtained from the media control well was deducted from all the test OD values.

2.3. Continuous flow reactor design for biofilm development

Simplified continuous flow reactor was designed for the cultivation of biofilm and for the treatment of chrome tanning effluent. The continuous flow reactor was fabricated using silicate glass material attached with a two channel peristaltic pump (Ravel Hiteks, RH-P 100 S–100–2H) to enable the uninterrupted flow of culture. The description of the reactor set up is given in Fig. 1.

The overnight culture of *B. subtilis* VITSCCr 01 and *B. cereus* VITSCCr 02 and their consortium were used as seed inoculum for biofilm development. The reactor was packed with sterile glass beads (5 mm)/pebbles10–15 mm)/coarse sand (3–5 mm) up to 2/3 of the column length. The bacterial inoculum was fed through the substrates at different flow rates of 10, 20, 30, 40 and 50 ml/min, up to 72 h at 30 °C. Intermittent samples were collected at 24, 48 and 72 h and were further characterized.

2.4. Characterization of developed biofilms

2.4.1. Biomass estimation

The developed biofilms were estimated for total bacterial biomass by viable plate count method. 1 cm^2 biofilm samples were scraped from the substrates using a sterile scalpel and dispersed in 10 ml of sterile water. The dispersed biofilm was plated on nutrient agar plates and incubated at 30 °C for 24 h. The NA plates were calculated for colony forming units and there by biomass per cm^2 were calculated.

2.4.2. Stability of biofilms

Stability of biofilm on the substrates was studied to check the irreversible biofilm formation. After the biofilm development, sterile water was passed through the column at 50 ml/min flow rate for 24 h. Stable biomass was calculated by detecting biomass adhered on the substrate/cm^2 after water flow from initial biomass calculated as in Section 2.4.1.

2.4.3. Microscopic examination

Morphological characterization of biofilms was done by Scanning Electron Microscope (SEM), Atomic force microscope (AFM) and Confocal laser scanning microscopy (CLSM). For SEM the biofilms samples were fixed with 2.5% glutaraldehyde, ethanol (dehydrated) and coated with gold under vacuum in an argon atmosphere. The surface morphology of the gold coated samples was visualized by a Scanning Electron Microscope (Hitachi S4000). SEM allowed the identification of any interesting structural features on the morphology of biofilms.

For AFM imaging the biofilms were fixed with 0.4% paraformaldehyde for 5 min and fixed cells were imaged using Atomic Force Microscopy (Non contact mode) (Nanosurf Easy Scan2, Nanosurf Inc.; USA). This mode of AFM enhances the topography of biofilm by scanning the tip above the surface without damaging the biofilm.

For CSLM biofilm samples from all substrates were thoroughly washed thrice with 0.05 M phosphate buffer, pH 7.4 and immersed in 1 ml solution of phosphate buffered-saline for 5 min. Samples were stained with acridine orange (0.01%, w/v) for 10 min at room temperature in the dark. Then, samples were washed with PBS and fixed with 4% glutaraldehyde for 1 h. Laser confocal scanning microscope (Leica SP2; Leica Microsystems, Heidelberg, Germany) was used for the imaging of samples. The Leica confocal software was used for analysis of biofilm images, which allowed for collection of *z*-stacks three-dimensional (3D) reconstruction. Excitation and emission wavelength were set to 460 and 650 nm respectively by adjustable spectrum slit.

2.5. Cr(III) bioremoval studies in continuous flow reactor using Bacillus biofilms

The chromium uptake studies were performed with standard chromium solutions (25, 50 and 100 mg/l) and chrome tanning effluents (1:10 and 1:100 diluted with distilled water). After biofilm formation, the biofilm bed was washed out and the Cr(III) solutions or the sterile chrome tanning effluents were passed continuously through the column with a flow rate of 20 ml/min. Cr concentration at the inlet (initial concentration) as well as at the outlet (final concentration) of the column was measured by Atomic

Absorption Spectroscopy (VARIAN SPECTRAA-240) at wavelengths of 357.9 nm, 425.4 nm and 520.8 nm. Optimizations of temperature, pH, column size and flow rate were performed to achieve maximum bioremoval.

Surface morphology of the biofilms used for Cr(III) bioremoval was visualized by Scanning Electron Microscope (Hitachi S4000) with combined Energy Dispersive X-ray Analyser at a voltage of 10 keV. SEM allowed the identification of any interesting structural features on the morphology of biofilms. Elemental composition of the insoluble precipitates in Cr-incubated biofilms was verified using energy dispersive X-ray analysis.

2.6. Adsorption isotherms

Two isotherm equations have been tested in this study and details of the equations and parameters are presented below [10].

(A) The general Langmuir sorption model is expressed by

$$Q_e = \frac{(Q_{max} b C_e)}{(1 + bC_e)} \quad (1)$$

Q_e (mg/g) is the amount of metal ion sorbed by the biofilm at equilibrium, Q_{max} (mg/g) is the maximum metal sorption, C_e (mg/l) is the concentration of metal in solution at the equilibrium and b (1 mg^{-1}) is the Langmuir adsorption equilibrium constant.Freundlich isotherm is expressed by

$$Q_e = K_f C_e^{1/n} \quad (2)$$

Q_e and C_e are the same as in the Langmuir equation, and K_f and n relate to the capacity and intensity of adsorption, respectively

2.7. Statistical analysis

Each set of experiments was carried out at least in duplicate, and in triplicate in some cases. Experiments were repeated separately to ensure reproducibility. In each set of repeated experiments, standard deviations and standard error showed 95% confidence interval.

3. Results and discussion

3.1. Isolation, screening and characterization of Cr(III) tolerant biofilm forming bacteria

Forty five indigenous Cr(III) tolerant bacteria were isolated from water and soil samples of tannery polluted sites in Palar river basin. Bacterial strains were screened based on Maximum tolerance concentration to Cr(III), Cr(III) bioremoval ability and EPS production [7]. Among the isolates two bacterial strains were selected and observed for biofilm formation by tissue culture plate assay (TPC), both the bacterial strains showed maximum biofilm formation in microtitre plates, which was confirmed with crystal violet staining. Further, they were characterized as *B. subtilis* [7] and *B. cereus* (showing 99% similarity in BLAST search to *B. cereus* and the sequence size was 1423 bp) by 16S rRNA sequencing (GenBank accession number GQ395343 and GQ395344). Isolation of bacteria from tannery environments represents an appropriate method for chromium removal and bioremediation [11]. It has been already reported, that chromium resistant bacterial strains isolated from chromium polluted environment are capable of reducing chromate [12]. Exopolysaccharide plays a major role in the biofilm formations and also sequestration of metallic ions [13], hence EPS production is one of the important criteria for selection of effective strain.

Table 1
Biomass quantification of biofilms of *Bacillus subtilis* and *Bacillus cereus* consortium on different substrates.

Substrate	After 24 h (CFU/cm^2)	After 48 h (CFU/cm^2)	After 72 h (CFU/cm^2)
Glass beads	1.6×10^2	4.2×10^4	3.5×10^5
Pebbles	2.4×10^2	1.3×10^5	4.3×10^7
Coarse sand	2.7×10^2	2.2×10^5	4.8×10^7

3.2. Biofilm formation on different substrates by continuous flow reactor

The *B. subtilis* VITSCCr01 and *B. cereus* VITSCCr02 strains were used for biofilm development on glass beads, pebbles and coarse sand by continuous flow reactor. Optimization of reactor for flow rate, substrate quantity and time taken for the biofilm formation was done for each substrate. The optimum flow rate for biofilm formation was 20 ml/min for glass beads and pebbles and it was 30 ml/min for coarse sand. The substrate quantity was fixed as 2/3 of the total column length for all the substrates. Time taken for biofilm formation was varied among the substrates in the following order; coarse sand 24 h, glass beads – 48 h and pebbles – 72 h.

Studies by Quintelas et al. [14,15] and Lin et al. [16] developed biofilms on granular activated carbon, kaolin, zeolite and chitosan bead for the removal of hexavalent chromium and other metals. But there are no reports on biofilm development on substrates like glass beads, coarse sand and pebbles for Cr(III) removal studies, using continuous flow reactor design. Moreover, continuous flow biofilm reactor design using indigenous bacterial strains to remove Cr(III) would be an ideal green technology for tanneries.

3.3. Characterization of biofilms

The biofilms were analyzed periodically at regular intervals (24, 48 and 72 h). The developed biofilms were observed for their increase in biomass, stability and morphology variations.

3.3.1. Biomass

The biomass quantification was estimated by bacterial enumeration method. The biomass of biofilms on different used are: coarse sands > pebbles > glass beads and it was 4.8×10^7 (after 24 h), 4.5×10^7 (after 48 h) and 3.5×10^5 (after 72 h) CFU/cm^2 respectively (Table 1). The biofilm formation was more on coarse sand and thereby bacterial biomass was more in coarse sand than pebbles and glass beads. The rough surface and hydrophobic nature may be a probable reason for it.

3.3.2. Stability

The biofilm stability studies were carried out on different substrates under force flow condition. The biomass withstanding the force flow was calculated as the stable biofilm. After 24 h of continuous flow glass beads the stable biomass was found to be 2.4×10^4 out of 3.5×10^5 in glass beads, 3.6×10^6 out of 4.8×10^7 in coarse sands and 3.1×10^6 out of 4.5×10^7 CFU/cm^2 in pebbles. The more stable biofilm was found on coarse sand and pebbles than glass beads.

3.3.3. Morphology

Bacterial biofilms of *B. subtilis* VITSCCr01 and *B. cereus* VITSCCr02 were obtained individually and as consortium in all the substrates and were analyzed with and without chromium using SEM, AFM and CLSM.

The bacterial biofilms interacted with Cr(III) and uninteracted biofilms were analyzed for SEM imaging. Fig. 2a shows the diversity

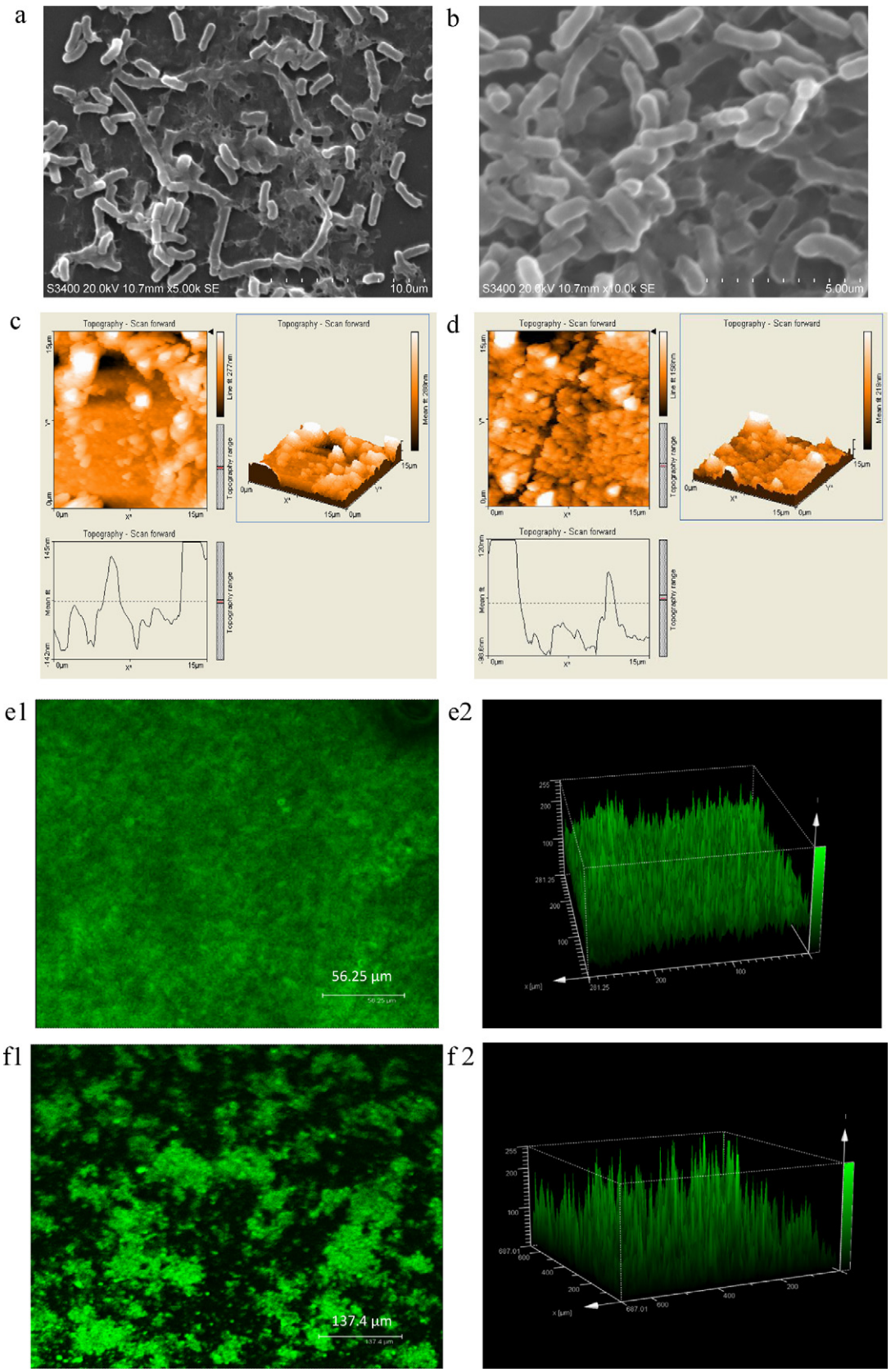

Fig. 2. (a) SEM imaging of *Bacillus subtilis* and *B. cereus* consortium biofilms without Cr(III). (b) SEM imaging of *B. subtilis* and *B. cereus* consortium biofilms with Cr(III) interaction. (c) AFM imaging of immature biofilms of *B. subtilis* and *B. cereus* consortium during biofilm formation. (d) AFM imaging of mature biofilms of *B. subtilis* and *B. cereus* consortium. (e1) CLSM imaging of *B. subtilis* and *Bacillus cereus* consortium biofilms without Cr(III). (e2) 3D structural imaging of *B. subtilis* and *B. cereus* consortium biofilms without Cr(III). (f1) CLSM imaging of *B. subtilis* and *B. cereus* consortium biofilms with Cr(III) interaction. (f2) 3D structural imaging of *B. subtilis* and *B. cereus* consortium biofilms with Cr(III) interaction.

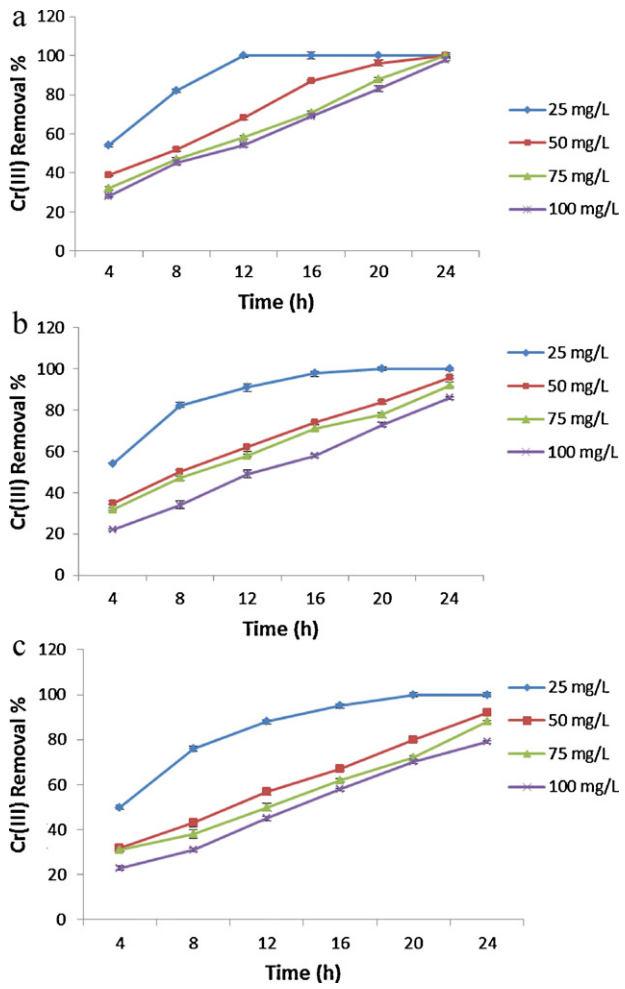

Fig. 3. (a) Bioremoval of Cr(III) from synthetic chromium solutions by *Bacillus subtilis* and *Bacillus cereus* consortium biofilms supported on coarse sand. (b) Bioremoval of Cr(III) from synthetic chromium solutions by *B. subtilis* and *B. cereus* consortium biofilms supported on pebbles. (c) Bioremoval of Cr(III) from synthetic chromium solutions by *B. subtilis* and *B. cereus* consortium biofilms supported on glass beads.

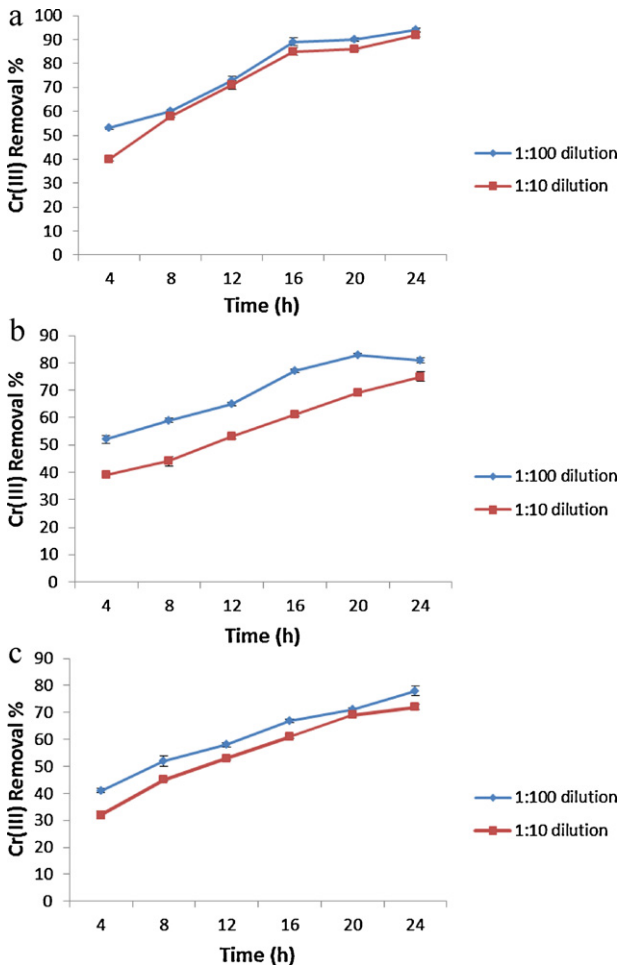

Fig. 4. (a) Bioremoval of Cr(III) from chrome tanning effluent by *Bacillus subtilis* and *B. cereus* consortium biofilms supported on coarse sand. (b) Bioremoval of Cr(III) from chrome tanning effluent by *B. subtilis* and *B. cereus* consortium biofilms supported on pebbles. (c) Bioremoval of Cr(III) from chrome tanning effluent by *B. subtilis* and *B. cereus* consortium biofilms supported on glass beads.

of the mixed culture of *B. subtilis* and *B. cereus*, with the presence of rod shaped bacteria in multilayers. The bacterial cells covered with EPS (Exopolymeric substances) layers were seen during biofilm formation on all the substrates. Extracellular polymeric material released by the bacteria was more in biofilms interacted with chromium. 'The precipitation of insoluble Cr(III) may be covered by extracellular polymeric materials in the bacterial biofilms. The cells after interaction with Cr(III) showed a clear difference in the cell morphology and size enlargement (pleomorphism) and surface modification (Fig. 2b). There was surface modification changes between biofilms developed after 24 h than that after 48 h (Fig. 2b and c).

The Cr(III) interacted biofilms on coarse sand showed more thickness of biomass than pebbles and glass beads. Fig. 2e1 shows average projections of CLSM z-series images from uninteracted biofilms on coarse sand and Fig. 2e2 shows the thickness of biofilm with *z*-stacks three-dimensional (3D) reconstruction. Thickness of the uninteracted biofilms was calculated as $78 \pm 2.55\,\mu m$. The thickness of biofilm decreased to $65 \pm 3.21\,\mu m$ for Cr(III) interacted biofilms (Fig. 2f1 and f2) after 24 h on coarse sand. The biomass were comparatively less when the biofilms developed in the presence of Cr(III) this in turn affect the biofilm thickness.

3.4. Chromium bioremoval studies using biofilms

The bioremoval of Cr(III) in different concentrations (25, 50, 75 and 100 mg/l) of synthetic chromium solution were studied using biofilms of *B. subtilis* and *B. cereus* consortium. Biofilms supported on coarse sand showed maximum chromium bioremoval than pebbles and glass beads. The Cr(III) bioremoval percentage for biofilms coated on coarse sand was 100% Cr(III) at 25, 50 and 75 mg/l synthetic chromium solution. In 100 mg/l synthetic chromium solution the bioremoval was 98% after 24 h (Fig. 3a). Biofilms coated on pebbles and glass beads showed 86 and 79% Cr(III) removal at 100 mg/l concentration of synthetic chromium solution (Fig. 3b and c). The chromium bioremoval by biofilms were more effective than the planktonic cells.

The Cr(III) bioremoval was also studied with real chrome tanning (1:10 and 1:100 dilution) using biofilms of *B. subtilis* and *B. cereus* consortium. The Cr(III) concentration in raw effluent was 3400 mg/l so it was diluted for bioremoval studies. Biofilms supported on coarse sand showed maximum Cr(III) bioremoval from tannery effluent compared to pebbles and glass beads. Biofilms coated on coarse sand were able to remove 92% Cr(III) for 1:10 dilution and 94% Cr(III) at 1:100 dilution after 24 h (Fig. 4a). Biofilms coated on pebbles showed 81% and 75% Cr(III) removal for 1:10 and 1:100 dilution after 24 h respectively. Biofilms on glass beads

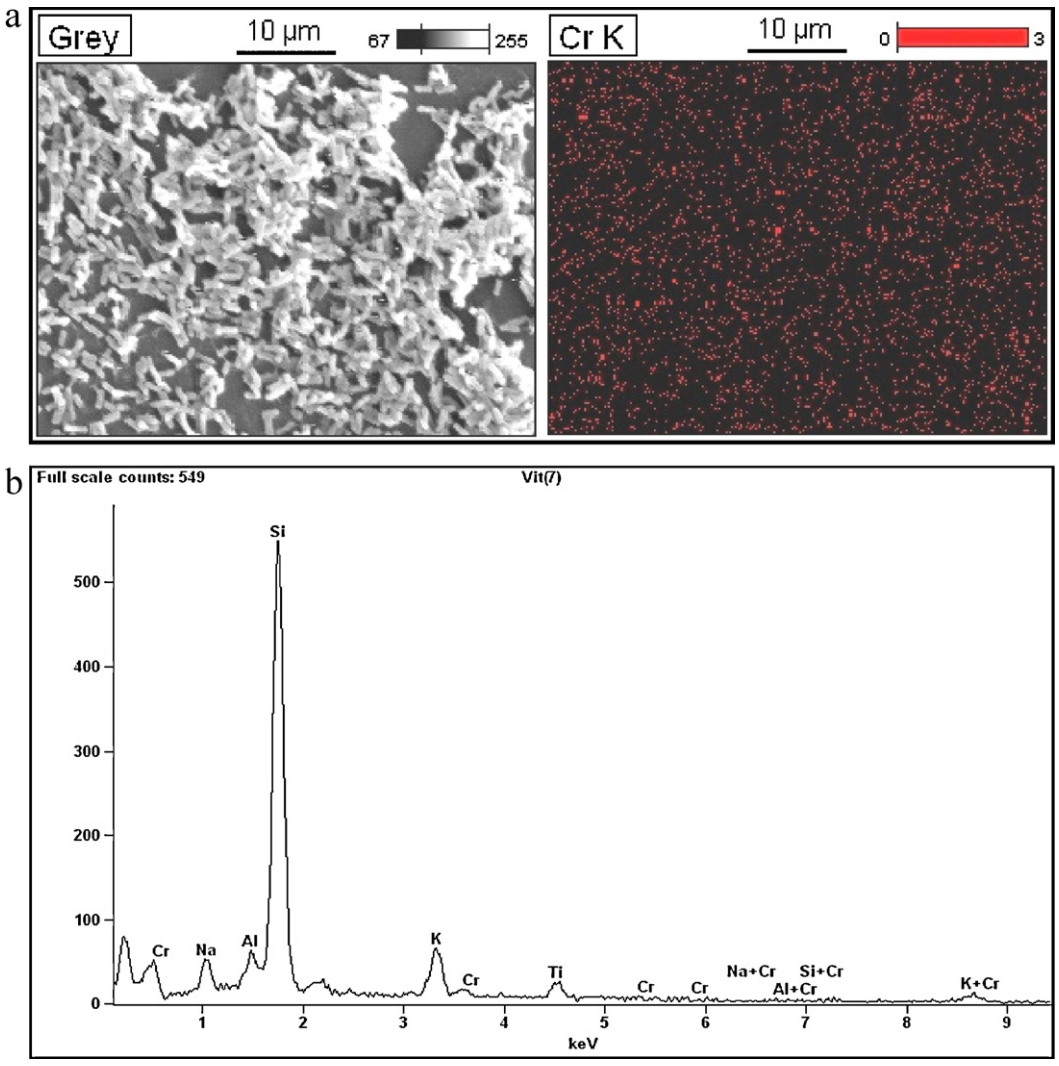

Fig. 5. (a) SEM imaging and chromium mapping of *Bacillus subtilis* VITSCCr01 and *Bacillus cereus* VITSCCr02 biofilms after chromium interaction by SEM-EDX. (b) EDX spectra of *B. subtilis* VITSCCr01 and *B. cereus* VITSCCr02 biofilms after chromium interaction, Si peak due to glass slide and Al peak originates from sample holder.

removed 78% and 72% Cr(III) at 1:100 and 1:10 dilutions respectively (Fig. 4b and c).

The physico-chemical properties of chrome tanning effluent before and after biofilm treatment are given in Table 2. There was a significant difference in the phsico-chemical properties of chrome tanning effluent after biofilm treatment.

Table 2
Physico-chemical properties of raw and biofilm treated tannery effluent.

Parameters	Concentration (mg/l) ± SD before treatment	Concentration (mg/l) ± SD after treatment
Color	Dark green	Colorless
Odor	Foul smell	No odor
Temperature (°C)	25.50 ± 0.33	25.50 ± 0.23
pH	2.5 ± 0.61	5.5 ± 0.85
Conductivity (μ Ω)	10.50 ± 0.71	4.40 ± 0.75
Total dissolved solids	815 ± 30.0	523 ± 18.0
Biochemical oxygen demand	650.00 ± 56.9	150.00 ± 16.0
Chemical oxygen demand	1264.00 ± 85.9	460.00 ± 35.0
Chloride	680.00 ± 10.0	430.00 ± 11.0
Sulphite	21.00 ± 1.5	15.00 ± 1.8
Sulphate	33.00 ± 5.0	12.00 ± 4.4
Ammonia nitrogen	142.00 ± 8.0	82.00 ± 6.0
Total nitrogen	729.00 ± 1.0	426.00 ± 1.5
Total hardness	460.00 ± 32.0	180.00 ± 22.0
Sodium	44.70 ± 5.2	24.70 ± 3.2

SEM-EDX showed higher percentage of chromium in the biofilms of *B. subtilis* VITSCCr01 and *B. cereus* VITSCCr02 than other elements. The percentage of chromium concentration present was 92.60 wt% and 90.43 at% (Table 3). Fig. 5a shows SEM imaging and chromium mapping of *B. subtilis* and *B. cereus* consortium biofilms after synthetic Cr(III) interaction by SEM-EDX. EDX spectra of *B. subtilis* and *B. cereus* consortium biofilms after chromium interaction showed, Si peak due to substrate (coarse sand), Ti and Al peak originates from sample holder (Fig. 5b).

Biosorption of Cr(VI) by a *Bacillus coagulans* biofilm supported on granular activated carbon (GAC) removed 46.86% Cr(VI) [17]. Lameiras et al. [18], studied biosorption of Cr(VI) using a bacterial biofilm supported on granular activated carbon and on zeolite and obtain 42% biosorption. Quintelas et al. [10], have performed bioremoval of Cr(VI) in a pilot-scale bioreactor through a biofilm of *Arthrobacter viscosus* supported on GAC. Smith and Gadd [19] studied Cr(VI) removal using SRB (sulphate reducing bacteria) biofilms. There are no reports on the bioremoval of Cr(III) in a pilot scale bioreactor through bacterial biofilms. This study deals with the bioremoval of trivalent chromium from real tanning effluents using chromium tolerant bacterial biofilms. As these bacterial strains were adapted to higher chromium and salt ion concentrations they

Table 3
EDX elemental analysis of developed biofilms of *Bacillus subtilis* VITSCCr01 and *Bacillus cereus* VITSCCr02.

Element line	Net counts	Net counts error	wt%	at%	Formula
Na K	57	±62	2.40	12.54	Na
Al K	856	±31	6.54	8.42	Al
Si K	7137	±88	–	–	
K K	934	±34	4.96	4.96	K
K L	298	±18	–	–	
Ti K	306	±22	2.20	2.15	Ti
Ti L	975	±36	–	–	
Cr K	10	10	–	–	
Cr L	24	±17	92.60	90.43	Cr
Total			100	100	

can be used in bioremoval of chromium from tannery effluent, which is used to have high chromium and salt ions.

3.5. Adsorption isotherms

The adsorption studies were conducted at a fixed biosorbent dosage, by varying the initial concentration of trivalent chromium in the effluent by 1:10 and 1:100 dilutions for 4, 8, 12, 16, 20 and 24 h. According to the Langmuir adsorption isotherm the adsorbed layer will be only one molecule thick and all the sides of the adsorbent will have equal affinity for the metal ions. Therefore the presence of adsorbed molecule at one side will not affect the adsorption of molecules at the adjacent side. The Langmuir correlation coefficient (R^2) values are 0.97783, 0.95704 and 0.9825 for coarse sand, pebbles and glass beads respectively at 1:100 dilution (Fig. 6a). For 1:10 dilution the Langmuir correlation coefficient (R^2) values are 0.96196 (coarse sand), 0.93674 (pebbles) and 0.92921 (glass beads) (Fig. 6b). The Freundlich adsorption isotherm assumes that the adsorbent consists of a heterogeneous surface, which is composed of different classes of adsorption sites. The correlation coefficient values (R^2) and the Freundlich constants K_f and n are calculated and as the values of intensity of adsorption isotherm ($1/n$) are greater than 1 ($1/n > 1$), the sorption process is known to be homogeneous. Further the applicability of Langmuir and Freundlich adsorption isotherms is derived on the basis of correlation coefficient (R^2), as in this case the R^2 values for Langmuir plot is higher than the Freundlich plot the sorption obeys Langmuir adsorption isotherm.

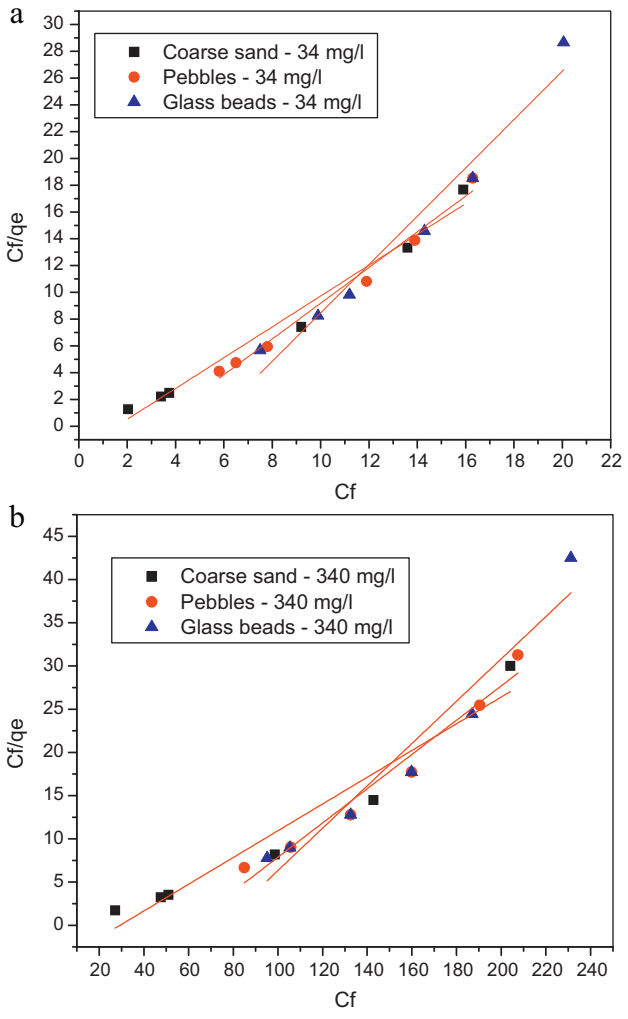

Fig. 6. (a) Langmuir adsorption isotherm of 1:100 dilution chrome effluent (34 mg/l Cr) for coarse sand, pebbles and glass beads. (b) Langmuir adsorption isotherm of 1:10 dilution chrome effluent (340 mg/l Cr) for coarse sand, pebbles and glass beads.

4. Conclusion

Present study attempted a novel approach towards bioremoval of Cr(III) in a plot scale bioreactor design using indigenous *Bacillus* biofilms. Since Cr(III) poses a threat to humans and environment, it is pertinent to study a biofilm based chromium remediation strategy. Simplified flow through system was designed for biofilm development on economically feasible substrates for Cr(III) removal. This study gives an insight into in situ remediation of chromium, as these bacterial strains were indigenous for tannery environment. These design and substrates can be recycled for desorption of Cr(III) and could be continuously reused for further Cr(III) bioremoval in tanning industries.

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