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Concomitant bioremediation of chromium (VI) and pentachlorophenol from the tannery effluent by immobilized *Brevibacterium casei*

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Abstract: - A *Brevibacterium casei* previously isolated from treated tannery effluent in our laboratory was selected in this study. The bacterium was tolerant to a maximum of 850 mg/L Cr(VI) and 1000 mg/L PCP concentration and concomitantly reduced 69% Cr(VI) and degraded 72% PCP within 168 h at pH 7.5 and 35°C temperature under shaking condition (120 rpm). The bacterial cells immobilized on different matrices (agar, agarose and calcium alginate) showed that calcium alginate was found to be the best support material as the immobilized bacterial biomass was capable to reduce 74% Cr(VI) and simultaneously degraded 80% PCP within 168 h at pH 7.5 and 35°C with 120 rpm aeration at an initial concentration of 850 mg/L Cr(VI) and 1000 mg/L PCP. Further, the efficiency of simultaneous Cr(VI) and PCP bioremediation in the treated tannery effluent was higher in bioreactor trials by using calcium alginate immobilized *Brevibacterium casei* cells. A significant decrease in the physicochemical properties of raw treated tannery effluent such as color, odor, pH, electrical conductivity, total solids, suspended solids, dissolved solids, BOD, COD, As(III), Fe(II) and Ni(II) was also obtained after treatment with calcium alginate immobilized *Brevibacterium casei.* Moreover, the immobilized cells has potency to almost 60% remove the PCP and Cr(VI) of the effluent within 24 h. This bacterial strain could be an efficient tool for the bioremediation of Cr(VI) and PCP co-contaminated sites and has a potential application in environmental restoration.

Keywords: - *Bioremediation; Bioreactor; Chromate; Immobilization; Pentachlorophenol*

I. INTRODUCTION

Chromium and pentachlorophenol (PCP) are often together discharged into the environment beyond the permissible limit through various industrial wastes such as leather industry, textile manufacturing, dying, petroleum refining, wood preservation, etc. and has lead to severe environmental pollution [1,2]. It is of great concern as they cause environmental pollution, ecological disturbances and has hazardous effects on human beings and other living organisms. This is a major factor that is making Indian leather industry globally less competitive. Chromium is a transition metal that usually occurs in nature in hexavalent [(VI), chromate] and trivalent (III). The Cr(VI) compounds are soluble hence highly toxic, mutagenic, carcinogenic and teratogenic, whereas, Cr(III) is relatively insoluble and less toxic [3,4]. Tanneries use chromium sulphate [Cr(III)] as a tanning agent, that results in severe ground water contamination around tanneries, which is finally transformed into the hexavalent chromium.

Further, the Cr(VI) species is non-degradable, thus once discharged into the environment, it tends to persist, circulate and eventually accumulate at different trophic levels in members of the food chain [5-7]. Thus, reduction of Cr(VI) to Cr(III) represents a potentially useful approach for the detoxification of chromate from wastewater and environment.

During leather tanning, PCP is used as biocide [2]. It is highly toxic and recalcitrant to biodegradation due to its polychlorinated aromatic ring structure. Pentachlorophenol easily bioaccumulates in various food chain of biological systems, and thus can cause profound problems to the human as it causes inhibition of oxidative phasphorylation, inactivation of respiratory enzymes and damage to mitochondrial structure (1,8). Reductive dechlorination has been suggested as the primary PCP bioremediation mechanism resulting in partially or fully dechlorinated product which is then more sensitive to ring cleavage [2]. Both the Cr(VI) and PCP are among the most hazardous classes of environmental pollutants. Owing to their toxicity, they are listed as priority pollutants by the United State Environmental Protection Agency [9,10]. Therefore, wastewater contaminated with Cr(VI) and PCP together should be treated carefully before being discharged into receiving water bodies and agricultural lands.

Conventional physicochemical method for the treatment of tannery effluent has been well documented [11,12]. However, these methods require large amounts and they also produce huge amount of toxic sludge and

hazardous by products. Further, the concentration of Cr(VI) and PCP obtained is still toxic to flora and fauna [13]. Microbial bioremediation offers an attractive treatment option, as it is cost effective and environmental compatible.

Moreover, even if the promising indigenous strains for bioremediation of Cr(VI) and PCP are identified, their usefulness for simultaneous bioremediation cannot be recommended unless the survival, multiplication and detoxification activity of the bacteria are evaluated in tannery wastewater. This could be achieved by performing bioreactor studies. However, for industrial purposes, using free bacterial cells is disadvantageous due to the difficulty of biomass effluent separation [14] and the Cr(VI) and PCP toxicity may cause free cell damage and loss of activity. These problems can be overcome by using immobilized bacterial cells [15,16]. Several support materials have been reported for cell immobilization, such as natural gels (e.g. agar, sodium alginate, carrageenan) and synthetic matrices (e.g. polyurethane, polyethylene glycol, polyacrylamide) [17]. Sodium alginate is widely used in immobilization of bacteria because it is non-toxic and very stable [18-21]. This will make the detoxifying system more promising and economical.

Despite widespread pollution caused by chromate and PCP concomitants, limited research was performed on indigenous bacteria that are capable of bioremediation dual pollutants [1,22,23]. However, all the above studies have been carried out with free cells at shake-flask level in laboratory media with low pollution load which do not directly explain the simultaneous detoxification of chromate and PCP in the treated tannery effluent. There is not even a single report on simultaneous removal of Cr(VI) and PCP from the tannery effluent using immobilized biomass in bioreactor. In our previous study, we reported the simultaneous detoxification of chromate and PCP by single native *Brevibacterium casei* strain. This strain was capable to detoxify 69% Cr(VI) and 72% PCP simultaneously in laboratory media (in shake flask) utilizing PCP as sole carbon source. This bacterium may potentially be useful for simultaneous bioremediation of Cr(VI) and PCP containing wastes in the environment after thorough investigation of its removal efficiency.

The present investigation was therefore, aimed to evaluate the simultaneous bioremediation potential of Cr(VI) and PCP from the treated tannery effluent using the immobilized cells of earlier isolated *Brevibacterium casei* strain in bioreactor for the efficient removal of binary contaminants from polluted site.

II. MATERIALS AND METHODS

2.1 Bacterial strain, growth media and culture conditions

A bacterial strain, *Brevibacterium casei* isolated previously in our laboratory from treated tannery effluent was employed in the present study [1]. This bacterium was able to grow in minimal salt medium (MSM) containing 850 mg/L Cr(VI) and 1000 mg/L PCP and utilized PCP as a sole carbon source. This strain concomitantly reduced 69% Cr(VI) and degraded 72% PCP in presence of other metals and pH (7.5), temperature (35°C) and aeration rate (120 rpm). The identity of this strain was authenticated from Institute of Microbial Technology (IMTECH) Chandigarh, India based on morphological, physiological and biochemical tests. This bacterium was grown medium which contained the following components (g/L) K_2HPO_4 (1.73), KH_2PO_4 (0.68), $(NH_4)_2SO_4$ (1.0), $MgSO_4{\cdot}7H_2O$ (0.1), $FeSO_4{\cdot}7H_2O$ (0.02), $CaCl_2{\cdot}7H_2O$ (0.03), $MnSO_4{\cdot}7H_2O$ (0.03). Appropriate volumes of PCP (Sigma Aldrich chemicals, USA) from stock solution of 8,000 mg/L as a carbon source and of Cr(VI) as $K_2Cr_2O_7$ (E-Merck, Mumbai, India) from 10,000 mg/L stock solution were added to the sterilized medium before inoculation to get the desired effective Cr(VI) and PCP concentration. Millipore membrane filters of 0.22 µm were used for filter sterilization of stock solutions prior to their use and were stored in brown glass bottles to avoid photo-oxidation. The solid medium contained 16.0 g/L agar (Hi-Media, India Ltd.) in addition to the components described above. The media components and all reagents used in the study were of analytical grade and purchased from Hi- Media, Merck, Qualigens, India Ltd. and Sigma Aldrich chemicals, USA.

2.2 Estimation of chromate

The concentration of Cr(VI), in the culture supernatant was determined spectrophotometrically using diphenylcarbazide (DPC) method [24]. Five hundred microlitre of 1, 5-diphenylcarbazide solution and 0.2 mL of 6M H_2SO_4 were added to 0.2 mL of the sample solution followed by addition of 9.1 mL deionized water in 2.5 mL of volumetric flask. The solution was allowed to stand for 10 minutes after which the absorbance of the purple colored solution was read at 540 nm using a UV-Vis spectrophotometer (Shimadzu 1601, Japan). Hexavalent chromium concentration was extrapolated from a standard curve prepared from standard solutions of $K_2Cr_2O_7$ (100–1000 mg/L) [25]. Total chromium and Cr(III) were determined using atomic absorption spectrophotometer (AAS) (Perkin-Elmer 5000, USA) at 357.9 nm, when the supernatant was treated with a mixture of nitric acid:perchloric acid (6:1, v/v) (36).

2.3 PCP and chloride ions estimation

The utilization of PCP was determined by spectrophotometer at 320 nm by the dichloromethane method [26]. The utilized PCP concentration was determined by the standard curve of PCP (100–1200 mg/L). Simultaneously, the PCP degradation was also determined by estimation of chloride ion released in culture supernatant using 0.4 mL of culture supernatant mixed in 1.6 mL distilled water. A further 0.2 mL of reagent A was added fallowed by reagent B and finally the volume was making up to 2.5 mL by distilled water [27]. The solution was allowed to stand for 10 minutes for color development and absorbance of solution was read at 460 nm using a UV-Vis spectrophotometer. The Chloride ion concentrations were quantified by standard curve of sodium chloride (100–1200 mg/L). Chloride ion concentrations of culture supernatant were also quantified by ion analyzer (Elico LI 126, India) with calibrated selective chloride ion electrode.

2.4 Effluent collection

The treated tannery effluent samples were collected in sterile glass bottles from the release point of common effluent treatment plant (CETP) of tanneries located at Jazmau, Kanpur, India and transported on ice to the laboratory within 6 h of collection. The effluent was filtered using Whatmann filter paper and stored in a refrigerator at 4°C for further use.

2.5 Physicochemical analysis of treated tannery effluent

Physiochemical parameters of the treated tannery effluent such as pH, electrical conductivity (EC), total solids (TS), suspended solids (SS), dissolved solids (DS), dissolved oxygen (DO), biological oxygen demand (BOD), chemical oxygen demand (COD) and other chemical constituents were estimated following the standard methods of APHA [28]. Heavy metals were determined according to Elith Garwood [29].

2.6 Bacterial immobilization

The bacterial strain of logarithmic phase grown in minimal salt medium was harvested by centrifugation (10,000 rpm for 10 min at 4°C). The sodium alginate entrapment of cells was performed according to Wang *et al.* [30]. The cell pellets (1.0 g) were then suspended in sterile sodium alginate solution and mixed by stirring on a magnetic stirrer. Calcium alginate beads of about 3 mm diameter were obtained by dropping the alginate cell mixture into a cold, sterile calcium chloride (200 mM) solution using a sterile syringe to get even sized beads. The gel beads formed were left in the solution for 24 h. The beads were washed with sterile distilled water repeatedly and used for experiments.

Agar entrapment of cells was carried out in sterile 2% (w/v) agar saline solution [31]. The bacterial cell pellets (1.0 g biomass weight) was mixed into sterile agar-saline solution to obtain cell-entrapped beads. These agar beads were washed successively with distilled water for further applications.

Agarose entrapment of cells was carried out following the method [32]. A solution containing 4.0% bacteriological agarose (20 mL) in a 100 mL Erlenmeyer flask was sterilized and cooled to 40-45°C. The 1.0 g wet biomass was mixed with the prepared agarose solution, poured in a sterilized petridish and allowed to solidify for 10 minutes. The solidified agar block was cut into equal size cubes and added into an oil phase (paraffin oil) and the cubes were washed with sterile distilled water 3 to 4 times before use.

2.7 Simultaneous Cr(VI) and PCP removal by *Brevibacterium casei* embedded with different matrices

Cells immobilized in Ca-alginate, agarose and agar-agar beads were tested for simultaneous Cr(VI) reduction and PCP degradation. Simultaneous reduction of Cr(VI) and PCP degradation experiment was carried out at 35°C with shaking at 120 rpm in 250 mL Erlenmeyer flasks, which contained 100 mL of mineral salt medium (pH 7.5), immobilized beads of *Brevibacterium casei*, 850 mg/L of Cr(VI) and 1000 mg/L of PCP concentration. Three sets of the above said flasks were taken. The experiment was initiated by the addition of 2 g immobilized beads (Ca-alginate, agarose and agar-agar) of *Brevibacterium casei* per flask. Samples (2 mL) were aseptically removed at every 24 h interval up to 168 h, centrifuged at 7,000 rpm for 10 min and the Cr(VI) reduction was monitored over time by measuring the disappearance of Cr(VI) in the culture supernatant by using DPC method and simultaneously PCP and chloride ion concentration was also estimated in the supernatant fluids described above [26,27]. Control flasks were incubated in parallel under the same conditions to ascertain the residual Cr(VI) and PCP concentration. Simultaneous experiments with free cells equivalent to those used in immobilized cultures were also conducted for comparative studies.

2.8 Bench-scale bioreactor level bioremediation of raw treated tannery effluent using immobilized bacterial cells

The simultaneous bioremediation potential of calcium alginate immobilized bacterial cells was assessed in the raw treated tannery effluent which was stored at 4°C. The experiment was performed using a bioreactor (capacity 5 L, BIOFLO 110, New Brunswick Scientific, USA) fitted with air sparger aseptically. The filter sterilized tannery effluent was added in the bioreactor and the pH was maintained at 7.5. Calcium alginate

immobilized bacterial cells (2%, w/v) was aseptically transferred in filtered 3 L raw treated tannery effluent and the bioreactor was shaken at 120 rpm at 35°C for 24 h. After 24 h, the bioremediated treated effluent was drawn under aseptic condition and collected in a sterile conical flask. Physicochemical parameters of effluent were estimated after treatment. Two parallel experiments were performed for each experimental condition and the arithmetic mean was used for data evaluation.

III. RESULTS AND DISCUSSION

3.1 Bacterial strain

The bacterium *Brevibacterium casei* was tolerant to 850 mg/L Cr(VI) and 1000 mg/L PCP concentration and concomitantly reduced 69% Cr(VI) and degraded 72% PCP in 168 h at 7.5 pH, 35°C temperature in the minimal salt medium using PCP as sole carbon source. At 168 h of growth, bacterium showing maximum PCP utilization (720 mg/L) and released 900 mg/L chloride ion.

3.2 Simultaneous Cr(VI) and PCP removal by *Brevibacterium casei* embedded with different matrices

Simultaneous chromate reduction and PCP degradation by *Brevibacterium casei* was evaluated with free and immobilized cells in batch culture. The performance of three inert matrices, *viz.* Ca-alginate, agarose and agar-agar were screened. Figure 1 shows that the free cells reduced 586.5 mg/L (69 %) of 850 mg/L Cr(VI) and simultaneously degraded 720 mg/L (72 %) of 1000 mg/L PCP in 168 h while the Ca-alginate immobilized cells (2%, w/v) showed better concomitant bioremediation efficiency of Cr(VI) and PCP (74% + 80%) under similar conditions. But complete reduction of Cr(VI) could not be achieved by *Brevibacterium casei* cells in any of the matrices tested. The significant detoxification in media Cr(VI) and PCP concentration following presence of immobilized beads of *Brevibacterium casei* suggest that the bacterial cells embedded in these natural carriers are capable of removing these toxic metals in solutions. Agarose entrapped cells showed considerably maximum Cr(VI) reduction and PCP degradation compared to that of free cells within same incubation period.

However, Agar entrapped cells showed significantly lower Cr(VI) reduction and PCP degradation values compared to that of free cells within 168 h of incubation, suggesting that it is less efficient than the other two natural carriers used for immobilization of *Brevibacterium casei.* Results as shown in Table 1 revealed that the agar immobilized cells reduced concomitantly 544 mg/L (64%) of Cr(VI) from 850 mg/L as well as degraded 670 mg/L (67%) PCP from 1000 mg/L concentration, respectively in 168 h at pH 7.5 and temperature 35°C at 120 rpm. The agarose immobilized cells reduced concomitantly 603.5 mg/L (71%) of Cr(VI) from 850 mg/L as well as degraded 750 mg/L (75%) PCP from 1000 mg/L, respectively in 168 h at pH 7.5 and temperature 35°C at 120 rpm. Chromate and PCP concentration in un-inoculated broth (negative control) remained unchanged and the total chromium was almost the same throughout the experiment (data not shown). Table 1 also reveals that the Ca-alginate immobilized beads of *Brevibacterium casei* were more efficient for simultaneous bioremediation of chromate and PCP than other natural carriers. Hence, calcium alginate immobilized bacterial cells were selected for further experiments.

The PCP utilization with correspondence to liberation of chloride ion by free and Ca-alginate immobilized beads of *Brevibacteium casei* as shown in Fig. 2, revealed that the Ca-alginate immobilized beads achieved good efficiency with simultaneous liberation of chloride ion. The free cells released 900 mg/L chloride ion in medium but during the presence of Ca-alginate immobilized beads the liberation of inorganic chloride ion in the culture medium increased up to 1000 mg/L at the 168 h of incubation. The release of chloride ion in culture medium is may be due to mineralization of PCP and also due to the activity to aromatic ring oxidation enzymes [33-35].

Few researchers have also reported Cr(VI) reduction and PCP degradation by immobilized bacterial cells in separate studies [14,15,36,37]. Freely suspended microbial biomass has disadvantages that include small particle size and low mechanical strength [14]. Ganguli and Tripathi [38] reported that *Pseudomonas aeruginosa* A2Chr cells immobilized in an agarose-alginate film in a rotating biological contactor exhibited significantly higher rates of chromate reduction than did planktonic cells. Pal *et al.* [39] reported that PVA-alginate immobilized cells of *Bacillus sphaericus* AND 303 could be used as a continuous bioprocess in treating Cr(VI) contaminated effluents. Polyvinyl alcohol alginate immobilized cells (*Paenibacillus xylanilyticus* MR12) had the highest Cr(VI) removal efficiency than that of free cells and could also be used in effluent treatment for Cr(VI) removal. Complete reduction of chromate in simulated effluent containing Cu, Mg, Mn and Zn by immobilized cells has been reported by Rawat *et al.* [40]. The use of immobilized bacterial cells offers a multitude of advantages, such as high bio-mass, high metabolic activity and strong resistance to toxic chemicals [41-43] and hence the immobilized cells will be useful to treat the industrial waste for its conversion in to less toxic form [17-19,41,44].

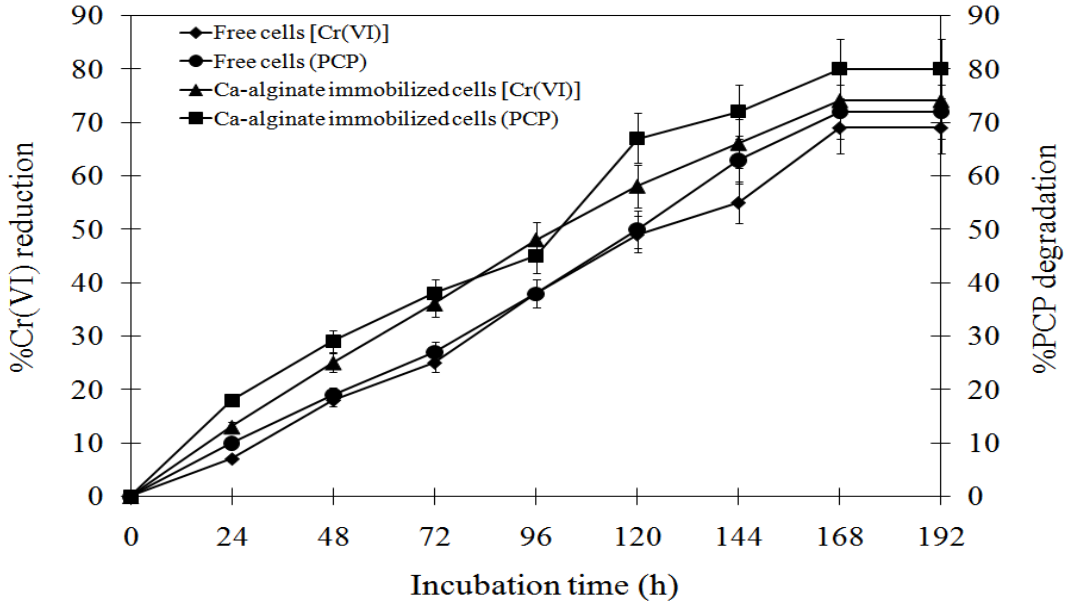

Fig 1. Chromate reduction and PCP degradation by free and immobilized *Brevibacterium casei* cells in MSM broth containing 850 mg/L of Cr(VI) and 1000 mg/L of PCP at pH 7.5, temperature 35°C and 120 rpm. Error bars represent mean ± standard deviation. Each point is the mean of three independent experiments.

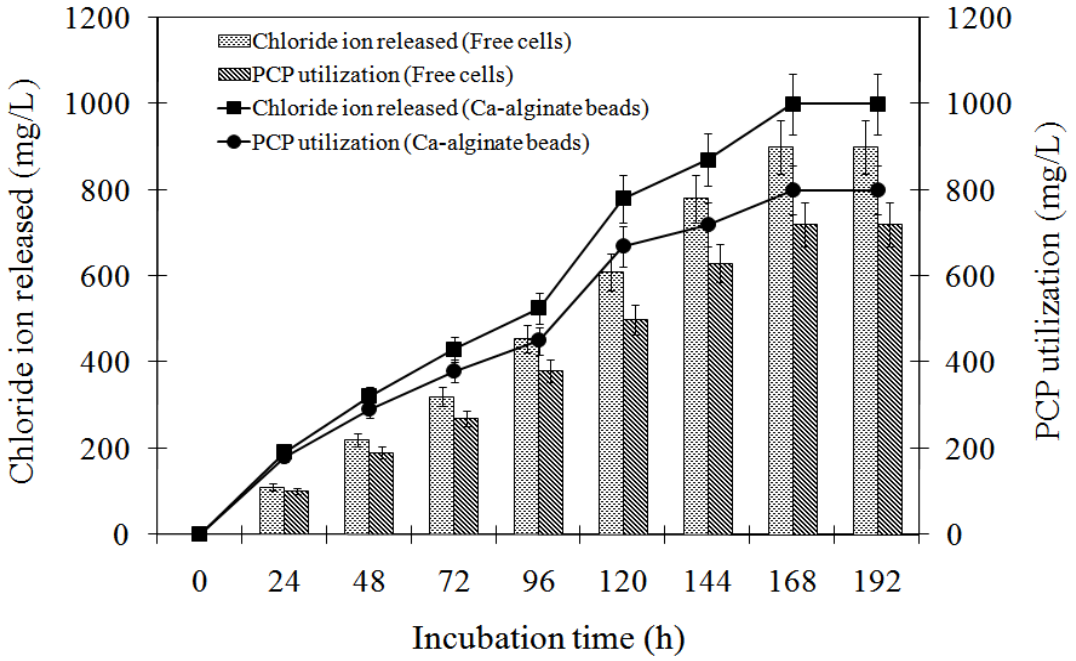

Fig 2. Release of chloride ion in the medium at different time intervals due to degradation of PCP (1000 mg/L) by free and immobilized *Brevibacterium casei* cells. Error bars represent mean ± standard deviation. Each point is the mean of three independent experiments.

Table 1. Simultaneous bioremediation of Cr(VI) and PCP by free and immobilized *Brevibacterium casei* cells embedded within different matrices

S. No.	Free and immobilized *Brevibacterium casei* cells	Initial Cr((VI) concentration (mg/L)	Cr(VI) concentration after 168 h (mg/L)	Chromate reduction efficiency (%)	Initial PCP concentration (mg/L)	PCP concentration after 168 h (mg/L)	PCP degradation efficiency (%)
1.	**Agar immobilized *Brevibacterium casei* cells**	850	306	64	1000	330	67
2.	**Agarose immobilized *Brevibacterium casei* cells**	850	246.5	71	1000	250	75
3.	**Calcium alginate immobilized *Brevibacterium casei* cells**	850	221	74	1000	200	80
4	**Free *Brevibacterium casei* cells**	850	263.5	69	1000	280	72

3.3 Physicochemical analysis of treated tannery effluent and its bioremediation by immobilized bacterial cells

The physicochemical characteristics of treated tannery effluent before and after treatment with immobilized *Brevibacterium casei* (in bioreactor) are presented in Table 2. The results of treated tannery effluent analysis are in accordance with the findings of Verma and Singh [1]. Analytical data of treated tanneries wastewater of Jajmau, Kanpur city, U. P. (India) showed that it can pose ecological threat when discharged into the receiving river or soil (Table 2). The raw treated tannery effluent was dark brown in color, deficit in dissolved oxygen, rich in total solids, high value of BOD and COD. It had nearly all hazardous metals, minerals and constituents in excess amount compared with Central Pollution Control Board (CPCB), New Delhi, India [45]. Dissolved oxygen was also increased from 2.9 to 4.6 mg/L which is one of the essential requirements of aquatic life. The colored effluents prevent the sun light penetration through contaminated waterways. This decreases the photosynthetic activity of aquatic plants, creating depletion of dissolved oxygen, ultimately causing death and deterioration of aquatic animals [34,46]. The BOD is an important indicator of organic matter which indicates the amount of biodegradable compounds present in the effluent. A large amount of inorganic compounds are present which remain unaffected by microorganism, and hence results in higher COD [47]. A high BOD and COD values shows that the effluent have highly oxygen demanding waste which has great implications in the biological and physical waste water treatment processes [48]. Along with BOD, COD and TDS gives valuable information about the pollution potential and their treatment.

The pH of dark brownish colored raw treated effluent was 8.3, which was well within the permissible range of 6.0 to 8.0. After treatment by immobilized *Brevibacterium casei* there was a decrease in pH from 8.3 to 6.9. The pH is an essential factor in the formation of algal blooms [49]. Low pH or high pH makes the water in poor condition for irrigation resulting in reduced crop, growth and yield. The value of total solids (TS) (2350 mg/L), BOD (258 mg/L) and COD (451 mg/L) were very high in the treated tannery effluent but in the bacterially treated tannery effluent the TS was (2290 mg/L), BOD (72 mg/L) and COD (310 mg/L). Similar observations have been reported by Mythili and Karthikeyan [50] and Indira and Mycin [51]. It suggests that the higher amount of TDS were also decreased within 72 h after bacterial treatment. However, the amount of total suspended solids (TSS) (260 mg/L) was found to be higher in effluent before treatment whereas after bacterial treatment the amount of suspended solids was reduced to 180 mg/L. Further, the electrical conductivity and total dissolved solids of the bacterial treated tannery effluent was highly reduced when compared with the raw treated effluent. The potassium salts were mainly responsible for increasing the EC of the effluent.

Table 2 depicts that the level of total chromium, hexavalent chromium and other heavy metal such as Ni(II), MgII), Fe(II) and As(III) in the raw treated tannery effluent (without treatment with immobilized *Brevibacterium casei* cells) exceeded the permissible limit. However, heavy metals like Cd(II), Zn(II), Co(II), Cu(II) and Mn(II) were present in the raw effluent within the permissible limits. The concentration of arsenic was 0.4 mg/L in raw effluent which was reduced to 0.26 mg/L after treatment with Ca-alginate beads of *Brevibacterium casei* after 24 h of incubation. Further, the concentration of iron and zinc was around 3.2 mg/L in raw effluent which shows maximum reduction in their values after treatment by *Brevibacterium casei.* Chatterjee *et al.* [52] has reported 55% zinc biosorption from wastewater by *Geobacillus thermodenitrificans*. The concentration of lead and cadmium was very less (0.1 mg/L) and remain unchanged (0.04 mg/L) after bacterial treatment. Other bacterial strains have been reported for its possible adsorption efficiency for lead, chromium and nickel [53,54]. The bacterium not only showed the tolerance capacity to the heavy metals toxicity but also potency of single strain with greater adaptability to absorb the different heavy metals have a little impact on the absorption of one meticulous metal.

The total concentration of chromium and PCP initially in raw treated tannery effluents was 3.82 and 14.2 mg/L, respectively. The removal of 14.2 mg/L PCP and 3.82 mg/L chromium using Ca-alginate beads of *Brevibacterium casei* grown in suspended form in the raw treated tannery effluent is shown in Table 2. The results indicated that ~60% simultaneous detoxification of 14.2 mg/L of PCP and 3.82 mg/L of Cr(VI) in bioreactor within 24 h under aerobic condition. From the results mentioned above, PCP could be degraded in the raw treated tannery effluent without adding supplementary carbohydrate (glucose) source. In addition, PCP degradation occurred simultaneously with reduction of Cr(VI) in to Cr(III). Both pollutants were removed approximately 60% from the raw treated effluent *via* Ca-alginate beads of *Brevibacterium casei* within 24 h. The initial concentration of chloride ion was 362 mg/L in tannery effluent but during the course of bacterial treatment, the liberation of inorganic chloride ion in culture medium increased up to 780 mg/L. The ability of bacterium to dechlorinate and mineralize chlorinated phenols was related to chlorine ring substitution patterns of specific compounds. Available data of earlier studies indicated that chlorinated phenols are mineralized to chlorine free end products [33].

Chromate in a stimulated effluent containing Cu(II), Mg(II), Mn(II) and Zn(II) was completely reduced by PVA-alginate immobilized cells within 9 h [14]. In the present investigation, high extent of effluent bioremediation at bioreactor level was evident under optimized cultural and nutritional conditions. Hence, the wastewater of raw tannery effluent treated with immobilized *Brevibacterium casei* in large scale can be used for irrigation which will not create any health problem in the living biota due to the absence of hazardous pollutants.

Table 2. Physico-chemical properties of raw treated tannery effluent and of effluent treated with calcium alginate immobilized *Brevibacterium casei* cells (mg/L)

Parameters	**Treated Tannery effluent without treatment with immobilized *Brevibacterium casei* cells**	**Treated Tannery effluent after treatment with immobilized *Brevibacterium casei* cells**	**Maximum permissible level of CPCB for different parameters for waste waters**
Color	Dark brown	Light brown	colorless
Temperature	30±1°C	37°C	-
pH	8.3	6.9	6.0 - 8.0
Electrical conductivity (moles/cm)	11,300	6750	850
Total dissolved solids (mg/L)	2,090	1975	2,100
Total suspended solids (mg/L)	260	180	100
Total solids (mg/L)	2350	2290	2200

Alkalinity (mg/L)	740	680	500
Dissolved oxygen (mg/L)	2.9	4.6	4 – 6
BOD (mg/L)	258	72	30
COD (mg/L)	451	310	250
Oil and grease	16.3	14.4	10
Phenolic compound (mg/L)	10.2	6.8	1.0
PCP (mg/L)	14.2	6.4	0.1
Mg(II) (mg/L)	240	210	200
Chloride (mg/L)	362	780	600
Sulphate (mg/L)	2,390	1850	1000
Fluoride (mg/L)	3.8	2.5	2.0
Nitrate (mg/L)	12	10.6	10
Total chromium (mg/L)	20.21	5.83	2.0
Cr(VI) (mg/L)	3.82	1.9	0.1
Fe(II) (mg/L)	3.2	3.02	3.0
Ni(II) (mg/L)	3.0	2.8	2.5
Mn(II) (mg/L)	1.65	1.63	2.0
As(II) (mg/L)	0.4	0.32	0.2
Pb(II) (mg/L)	0.1	0.1	0.1
Cu(II) (mg/L)	1.78	1.05	3.0
Zn(II) (mg/L)	3.2	2.43	5.0
Co(II) (mg/L)	0.35	0.22	1.5
Cd(II) (mg/L)	0.1	0.1	2.0

IV. CONCLUSION

The results obtained in this study indicated that the Ca-alginate immobilized *Brevibacterium casei* was able to mineralize high concentrations (1000 mg/L) of PCP and simultaneously reduce the maximum concentration (850 mg/L) of Cr(VI). Immobilization experiments demonstrated that *Brevibacterium casei* in Ca-alginate matrix is more effective as compared to that of the agar-agar and agarose matrix and free cells for the simultaneous bioremediation of chromate and PCP. Almost 60% removal of chromate and PCP were achieved when the raw tannery effluent was treated by immobilized *Brevibacterium casei* in the bioreactor. However, a significant decrease in the physicochemical parameters of raw treated tannery effluent after treatment with immobilized *Brevibacterium* cells in bioreactor studies indicates its possible use for eco-friendly *in situ* bioremediation of tannery and other industrial wastewater.

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