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BIOREMEDIATION OF CHROMIUM AND ORGANIC MATTER FROM CHROME TANNING EFFLUENT BY CHROMIUM TOLERANT BACTERIA

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ABSTRACT

Bacterial strains named BS-1, BS-2, BS-4 and BS-6 with the ability to remove both chromium and organic pollutants were isolated from tannery disposal site effluent. The strains were found to be resistant for chromium concentrations 7 g/L for BS-1 followed by 6 g/L for BS-2 and 5 g/L for both the BS-4 and BS-6. Through morphological and biochemical characterization, the isolated strains were identified as completely distinct with the different ability to degrade organic matter. Four different sets of aerobic batch experiments with the isolates were designed for the bioremediation of chromium and COD from chrome tannery effluent. In this treatment process maximum chromium removal was observed for BS-6 (89.66%) and the removal efficiencies for BS-1, BS-2 and BS-4 were 83.37%, 86.13% and 87.65% respectively. In addition, maximum COD removal was found for BS-2 (96.11%) and the values were 83.25%, 78.59% and 89.45% for BS-1, BS-4 and BS-6 accordingly.

INTRODUCTION

Tanning industry is a major source of both inorganic and organic pollutants produced from different chemical sub-processes of leather manufacturing Mwinyihija (2006). Chrome tanning is a unit process of leather making where basic chromium sulfate binds to the collagenous protein of raw hides and skins to convert it to leather Cassano (2001). Almost 90% of the leather is produced globally through chrome tanning process. In conventional chrome tanning process, hides and skins uptake only 50-70% of chromium where the rest of the huge amount of chromium gets exhausted to the effluent Saravanabhavan (2004). Hence wastewater from the chrome tanning process contains extensive chromium and chrome bearing organic loads those are responsible for the high concentration of BOD, COD and TDS in the effluent. The low-biodegradable complex nature of the effluent creates unfavorable environmental outcomes and technological difficulties in treating it to discharge UNEP (1994). More specifically at high concentrations chromium is very toxic and mutagenic to the human, aquatic life and microbial communities Altaf (2008). Therefore, chrome tannery effluent must be treated before discharge.

The green solution for managing the chrome tannery wastewater is the exercise of biological technologies using bacteria and it is also very cost effective Ganguli (2002). In this kind of bioremediation process bacteria fix the organic pollutants by converting it to inert substances through controlled technological processes Marcos von Sperling (2007). Chromium and other heavy metal can be treated and removed by bacteria in different mechanisms as bio-sorption, bio-accumulation and enzymatic reduction. Large extent of heavy metal can easily be absorbed by the bacteria due to their high surface to volume ratio caused by very small sizes Zouboulis (2004). Bio-accumulation of heavy metal occurs in bacteria through two phases as first the metal gets adsorbed on the cell surface later it is transported into the cell. The bioremediation of chromium and organic pollutants occur following the above-mentioned mechanisms Gadd (1990).

But the nature and composition of tannery wastewater makes it difficult to treat it through biological strategies. The major challenges are the inability of the bacteria to survive in the high chromium concentrations, non-biodegradable organic pollutants due to microbial growth inhibiting compounds and unfavorable environmental conditions for the growth and multiplications of the bacterial population Dilck (1992), UNEP (1994). Therefore, the proper selection of chrome resistant bacteria with the ability to degrade organic compounds can be the ideal solution of the problem. Many research works have already

been done regarding isolation of chrome tolerant bacteria and to test their performance to remove chromium from wastewater. Some of the study was focused on the chrome removal either by bio-sorption or by bio-accumulation where other works highlighted the reduction of chromium (VI) to chromium (III). Some other researches were aimed to design a specific biological treatment process of chrome removal Chaturvedi (2011), Farabegoli (2004), Srinath (2002).

The main aim of the research was to remove chromium and organic matter (COD) from chrome tanning effluent by chrome removable bacteria isolated from tannery disposal site effluent. To do so isolation of chrome resistant and chrome removable bacteria and their morphological and biochemical characterization were also highlighted. The disposal site effluent of any tannery having no ETP was chosen with the hypothesis of the availability of bacteria surviving in high chrome concentration. This paper also focused on the effectiveness of the isolated bacterial strains for chrome and COD removal in simply designed batch mode aerobic biological process. To find the relationship between the bacterial population densities and the removal performances was also tested in this study.

MATERIALS AND METHODS

Materials

Tannery disposal site effluent as a source of chrome resistant bacteria was collected in a pre cleaned plastic pot from a renowned tannery at Savar, Dhaka. Waste chrome liquor for treatment purposes was collected from a tanning industry at Jessore, Khulna. All the chemicals required for the culture and identification of bacteria and for the characterization of wastewater were of analytical grade and were purchased from a local scientific store in Khulna.

Culture and Isolation of Chrome Tolerant Bacteria

Tannery disposal site effluent with very high chrome concentration was considered as the source of chrome tolerant bacteria. The effluent was serially diluted to 6 folds with the dilution factor of 10^6 and was used for culturing the bacteria. Solid media of nutrient agar was prepared by dissolving calculated amount of nutrient agar in distilled water following the formula of 48 gm of agar per 1 L of water and the mixture was heated until boiling after that the media was cooled, sterilized by autoclave and were ready to use. Bacteria were cultured by pour plate method where 1 mL of serially diluted effluent was poured in a previously cleaned and sterilized Petri dish and required amount of solid nutrient agar media was poured onto it and then the system was mixed properly by swirling the dish and kept undisturbed for solidify the media. After that the Petri dish was incubated at 37 ºC for 48 hours. After incubation period number of bacterial colonies cultured in the Petri dish was counted by a colony counting machine. The colonies were also isolated in 10 different culture tubes having liquid broth medium. Liquid culture broth was prepared following the formula of peptone (10g), meat extract (10g), yeast extract (5g) and salt (5g) per 1000 mL of the media and the broth was sterilized by autoclave and was cooled to use.

Primary Screening for Chrome and COD Removal by Isolated Bacteria

The suitability of the isolated colonies for chrome and COD removal was performed in batch mode experiments. Ten plastic pots of 5 L sizes of each were taken for the test and 2 L waste chrome liquor was added in each pot. The ten different pots were inoculated with 50 mL of the ten different isolated bacterial colonies. The colonies were identified as BS-1, BS-2, BS-3, BS-4, BS-5, BS-6, BS-7, BS-8, BS-9 and BS-10. After inoculation all the batches were aerated using electrically driven surface aerators. In each batch 10 mL of newly prepared liquid media was added from the second day to sixth day for the proper growth and multiplication of bacteria. At day-7 samples were withdrawn from each batch and were tested for chromium content and COD value. From the initial values of the parameters in the waste chrome liquor to the final values after seven days of treatment the percentages removal was calculated. The four bacterial strains responsible for the maximum removal of chromium and COD were selected as chrome removable strains and all the further experiments were carried out with these four strains.

Estimation of Bacterial Chrome Tolerance Limit

The experiments for determining the tolerance limit of chromium for the selected four colonies were done by the method explained in a research article Parvekar (2020) with some modification. Four sets of ten different culture bottles of 100 mL of liquid media in each bottle were prepared for the four bacterial strains with the chrome concentrations of 500 mg/L and 1000 mg/L to 8000 mg/L with the interval of 1000

mg/L. After that the sets of bottles were inoculated with the four different colonies and the bottles were incubated at 37 ºC for 48 hours. After the incubation time absorbance of the culture were measured at 600 nm using a spectrophotometer, Boeco, Germany. Here for all the culture at different chrome concentrations, the same chrome concentrations without bacteria were considered as zero absorbance. The absorbance of zero or nearly zero was considered as no growth of bacteria. The four colonies were finally sub cultured in chromium containing solid media where maximum tolerate chrome concentrations were used for the respective colonies.

Morphological and Biochemical Characterization of the Selected Bacteria

The colony morphologies of the finally selected four bacterial colonies were studied from their culture in chrome bearing media by visual observation. The cellular morphology of the chrome removable bacteria was identified according to the Gram staining method using crystal violet as primary stain and safranin as counter stain. The strains were also tested for the identification of their biochemical nature. Different biochemical tests as indole, catalase, oxidase, urease, methyl red, starch and lipid hydrolysis, glucose and sucrose fermentation and gelatinase test were performed following the manual of Descriptive Bacteriology by Bergy Holt (1994).

Bioremediation Experiments for Chrome and COD removal

The finally selected four bacterial strains were taken for day wise chrome and COD removal experiments in bath mode. In these experiments four plastic container of 20 L sizes were collected and 10 L wastewater were added in each container. Broth culture of the selected four colonies was inoculated in four different containers with the wastewater to culture ratio as 100:5. All the systems were continuously aerated with mechanical aerator devices. As the previous manner newly prepared broth media was added in each container up to 4th day. Starting from the 5th day to 20th day in 2 days interval samples were collected from each container in a measuring cylinder, kept undisturbed for 1 hour to settle down the suspended solids and were tested for chromium content and COD values. The total volatile solids concentration (TVSS) which indicates the bacterial population densities of the treatment process were also measured from the collected samples from the treatment tank without sedimentation. After 20th day it was not possible to collect samples due to evaporation losses.

Analytical Techniques

Physico-Chemical characterization of both the tannery disposal site effluent and the chrome tannery wastewater were done by following the different standard methods for wastewater analysis. Wastewater parameters such as pH, Total Suspended solids (TSS), Total dissolved solids (TDS), Total volatile solids (TVSS), Sulphates, Chlorides, BOD5 and COD were measured by the methods 4500 H^+B, 2540 D, 2540 C, 2540 E, 4500 E, 4500 Cl^- B, 5210 B and 5220 C respectively according to the American Public Health Association (APHA) standard methods APHA (1998). Chromium and Sulfides were measured based on the official methods of the Society of Leather Technologists and Chemists (SLC) such as SLC 208 and SLC 202 respectively.

RESULTS AND DISCUSSION

Physico-Chemical Characteristics of Wastewater

The disposal site effluent was slightly alkaline and brownish in color. The wastewater from chrome tanning process was dark blue in color and highly acidic in nature. Due to the chromium concentration more than 1000 mg/L, disposal site effluent was selected as source of chrome tolerant bacteria. The Physico-chemical characteristics of the both disposal site effluent and chrome tannery wastewater was shown in the table 1. The value of different parameters explored that the wastewater from both the different sources exceeded the permissible discharge limits to the inland water set by the environmental conservation rules of Bangladesh, ECR (1997).

Table 1 Physical and chemical characteristics of wastewater

Parameters	Disposal Site Effluent	Chrome Tanning Wastewater	Discharge Standard ECR (1997)
Color	Brownish	Dark blue	-
pH	7.8	3.9	6-9
TDS (mg/L)	12436	62250	2100
TSS (mg/L)	5722	2800	500
Chlorides (mg/L)	3396	27500	600
Sulfides (mg/L)	128	-	0
Sulphates (mg/L)	2162	19350	-
Total Chromium (mg/L)	1032	3820	2.0
BOD_5 (mg/L)	1535	935	250
COD (mg/L)	6915	2885	450

Isolation and Screening of Chrome Tolerant Bacteria for Chromium and COD Removal

The total number of primary culture of the chrome resistant bacteria colonies was counted as 3.22×10^6 CFU/mL and is shown in the figure 1. Randomly isolated 10 colonies in liquid broth media is represented in figure 2.

Figure 1 Chrome tolerant bacterial colonies from source

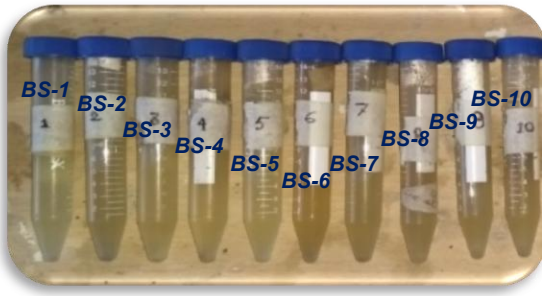


Figure 2 Randomly isolated 10 bacterial colonies

The isolated ten colonies were further tested to investigate their efficacy to remove chromium and COD from chrome tannery effluent. Table 2 represents the primary screening test results of the isolated bacterial strains for chrome and COD removal. From the table 2 it is observed that only four bacterial colonies were able to remove significant amount of chromium (around 50% or more). It is noticed that maximum removal was achieved by BS-6 (69.03%), followed by BS-4 (61.04%), BS-1 (58.74%) and BS-2 (48.35%). In case of COD removal six of the colonies were found to remove more the 50% of the organic loads. The highest removal was performed by BS-2 (68.36%) and the removal percentages were achieved by other five colonies was BS-6 (65.03%), BS-5 (62.78%), BS-8 (58.03%), BS-1 (56.11%) and BS-4 (53.91%). Based on the combined efficiency for chromium and COD removal BS-1, BS-2, BS-4 and BS-6 were finally selected as test bacteria and were used in bioremediation experiments of chrome tannery wastewater.

Table 2 Chromium and COD removal by isolated bacteria for seven days of treatment

Bacterial Stains	Residual Chromium (mg/L)	% Removal of Chromium	Residual COD (mg/L)	% Removal of COD
BS-1	1576	58.74	1266	56.11
BS-2	1973	48.35	913	68.36
BS-3	2496	34.67	1730	40.05
BS-4	1488	61.04	1330	53.91
BS-5	2624	31.32	1074	62.78
BS-6	1183	69.03	1009	65.03
BS-7	2352	38.42	1622	43.78
BS-8	2689	29.61	1211	58.03

BS-9	3364	11.93	1842	36.15
BS-10	2843	25.57	1747	39.45

Chrome Tolerance Limits of the Selected Bacteria

Wastewater generated from chrome tanning process contains very high chromium concentration and it varies from batches to batches. Heavy metals like chromium affects the growth and metabolism of bacteria and in high concentrations can denature the cellular integrity Tscherko (2000). But some bacteria adopted different resistance mechanisms to survive in the toxic conditions caused by different heavy metals and the mechanisms include metal sorption, enzymatic degradation, bioaccumulation, mineralization etc .Hrynkiewicz (2014). For the removal of chromium by bacteria, it is very important to find the bacteria that adopted the survival system against chromium. In this research the finally selected four strains were tested for the identification of their chrome tolerance limits by spectrophotometric method and the results is represented in the figure 3 and figure 4. It is noticed from the figure 3 that for all the bacteria the absorbance that indicates the growth of bacteria was decreased with the increase of chrome concentrations. The results signify that increased chrome concentrations affect the metabolic activity of the bacteria. For BS-1 for 8000 mg/L chromium concentration the absorbance was about to zero and for BS-2, BS-4 and BS-6 the near about zero absorbance was observed for 7000 mg/L, 6000 mg/L and 6000 mg/L of chromium respectively. It can be concluded from the results that for the above chrome concentrations the cellular structure of the respective bacteria was denatured and the cells got dead. Figure 4 shows the chrome tolerance limits of the selected colonies. It is seen from the figure that BS-1, BS-2, BS-4 and BS-6 can survive in chrome concentrations up to 7000 mg/L, 6000 mg/L, 5000 mg/L and 5000 mg/L respectively.

Morphological and Biochemical Characteristics of the Selected Bacteria

Solid media culture of the selected bacteria along with their colony morphology is given in the figure 5. It is seen from the figure that the shape and margin were completely different for all the four colonies. Here flat elevation was observed for BS-4 and the elevation of other three colonies was raised. BS-1 and BS-4 were noticed as rough surface where smooth surface was seen for BS-2 and BS-6. All the data of the figure represent that these four colonies are completely different from each other and they are different bacterial strains.

The microscopic cellular morphology of the bacteria was studied by gram's staining method and the colonies were identified as gram positive and gram-negative strains. The results of the experiment are shown in the figure 6. The figure represents that the BS-1 and BS-6 were rod shaped bacteria and were morphologically classified as bacillus bacteria. Here the shape of the BS-2 and BS-4 were observed as round and the category of the strains are coccus. It is also noticed from the figure 6 that the BS-1 and BS-4 retained primary stain by their cell wall and were categorized as gram positive bacteria. In addition, due to the retention of counter stain BS-2 and BS-6 were identified as gram negative bacteria.

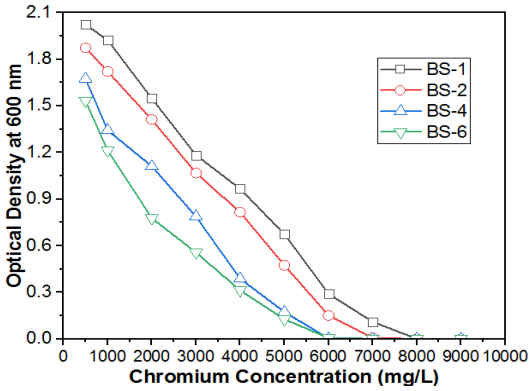


Figure 3 Growth response curve of the selected bacteria.

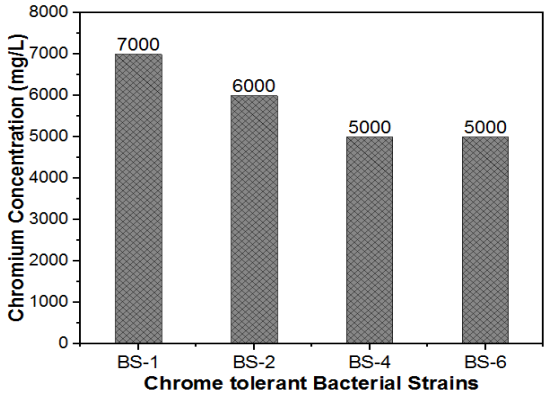
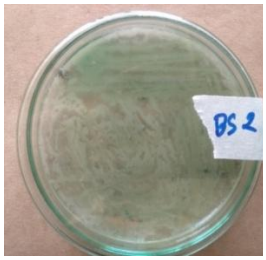
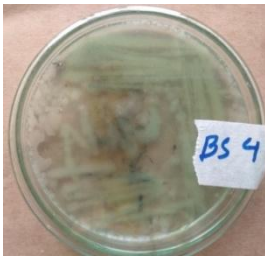


Figure 4 Maximum chrome concentrations for the survival of BS-1, BS-2, BS-4 and BS-6.

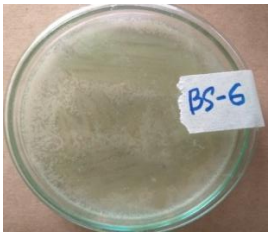
Shape: Circular
Surface: Rough
Elevation: Raised
Margin: Erose
Opacity: Opaque



Shape: Punctiform
Surface: Smooth
Elevation: Raised
Margin: Undulate
Opacity: Translucent

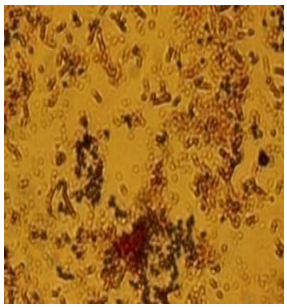


Shape: Irregular
Surface: Smooth
Elevation: Flat
Margin: Entire
Opacity: Translucent



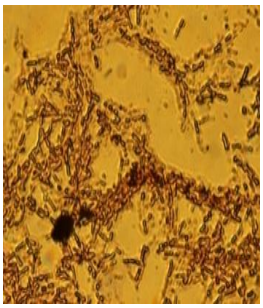
Shape: Spindle
Surface: Rough
Elevation: Raised
Margin: Lobate
Opacity: Opaque

Figure 5 Colony morphology of the BS-1, BS-2, BS-4 and BS-6.

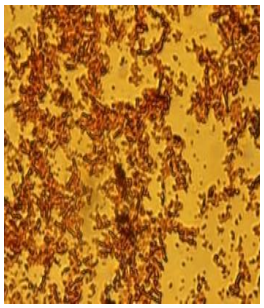


Bacillus
Gram Positive (+ve)

Coccus
Gram Negative (-ve)



Coccus
Gram Positive (+ve)



Bacillus
Gram Negative (-ve)

Figure 6 Gram staining morphology of BS-1, BS-2, BS-4 and BS-6

It is observed from the biochemical characterization represented in the table 3 that all the four colonies were exclusively different from each other. BS-2 and BS-6 were able to breakdown all type of carbohydrates as they showed positive results in starch hydrolysis test and fermentation test for both glucose and sucrose. In case of hydrolysis of lipids, strain BS-1 and BS-2 showed positive results. Positive results of the indole and gelatinase tests for BS-1, BS-2, BS-4 and BS-6 signify that all the strains have the ability to produce tryptophanase and gelatinase enzymes in ideal environment. The bacteria used oxygen as electron acceptor in aerobic respiration process was confirmed by the positive oxidase test result of the BS-1, BS-2, BS-4 and BS-6. Different test results were observed for catalase and uriase tests by different bacteria.

Table 3 Biochemical tests of the selected chrome resistant bacteria

Name of the tests	Bacterial Strains			
	BS-1	BS-2	BS-4	BS-6
Indole test	(+) ve	(+) ve	(+) ve	(+) ve
Oxidase test	(+) ve	(+) ve	(+) ve	(+) ve
Catalase test	(+) ve	(-) ve	(+) ve	(+) ve
Urease test	(+) ve	(+) ve	(-) ve	(+) ve
Starch hydrolysis test	(+) ve	(+) ve	(+) ve	(+) ve
Lipid hydrolysis test	(+) ve	(+) ve	(-) ve	(-) ve
Fermentation test (Glucose)	(-) ve	(+) ve	(-) ve	(+) ve
Fermentation test (Sucrose)	(+) ve	(+) ve	(+) ve	(+) ve

Gelatinase test	(+) ve	(+) ve	(+) ve	(+) ve

The positive (+) ve and negative (-) ve results indicated that targeted reactions didn't happen.

Bioremediation of Chrome Tannery Wastewater by Selected Bacterial Strains

In an aerobic biological treatment process growth and multiplication of bacteria occurs in two phases. Production of more cells by the breakdown of organic matter with the help of oxygen is called synthesis phase. These organic substances are known as food for the bacteria. In the endogenous respiration phase bacterial concentration starts to decrease due to the scarcity of foods. The bacterial population density in the biological process is determined by measuring the volatile suspended solids (VSS) of the system Marcos von Sperling (2007). The figure 7 represents the total volatile suspended solids (TVSS) concentration of the selected bacteria during the treatment period. It is noticed from the figure 7 that for all the bacteria TVSS content increased with time up to 13th day and after that the values started to decrease. This trend of the graph indicates that during 13 day period organic matter which was the food for the bacteria was available for the growth of bacteria. Hence TVSS continued to increase. Maximum TVSS was for BS-2 (4050 mg/L) followed by BS-6 (3850 mg/L), BS-1 (3720 mg/L) and BS-4 (3670 mg/L). At the end of 13th day due to low food supply TVSS decreased for all the strains.

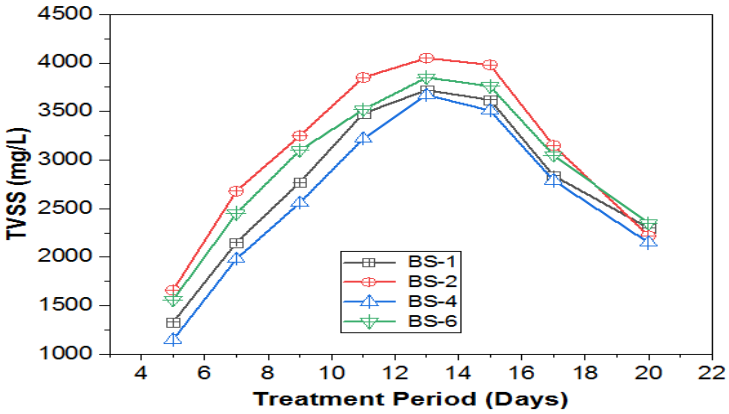


Figure 7 TVSS concentration for the selected bacterial strains

It is observed from the figure 8 that chromium removal percentages increased with times during the treatment period for all the strains. The quick increment of the Chromium removal efficiency of different bacteria was observed up to day 13.Chromium removal efficiencies of BS-1 were 58.74%, 68.82%, 74.11%, and 78.37% for day-7, day-9, day-11 and day-13 respectively where 19.63% increment of the removal of chromium was noticed from day-7 to day-13. For other strains from day-7 to day 13 same trends was marked. It raised 32.76% from 48.35% to 81.11% for BS-2, 24.07% from 61.04% to 85.11% for BS-4 and the increment was 17.96% from 69.03% to 86.99% for BS-6. After that the removal percentages raised very slowly such as the values were 80.74% (day-15), 81.92% (day-17) and 83.37% (day-20) for BS-1 followed by 84.04% (day-15), 84.89% (day-17) and 86.13% (day-20) for BS-2. The removal efficiency was 85.91% (day-15), 86.68% (day-17) and 87.65% (day-20) for BS-4 and 88.03% (day-15), 88.82% (day-17) and 89.66% (day-20) was for BS-6. It has already been mentioned in the previous section that main bioremediation mechanisms of heavy metal by bacteria are bio sorption and bioaccumulation. The prime factor for bioaccumulation is the number of cells and bio sorption depends on the surface area of the adsorbent. Figure 7 illustrated that bacterial population density increased up to day-13 and then decreased. Due to the high population density of bacteria during this period both the surface area and the cell number were very favorable for chromium removal by both the above mentioned mechanisms. Hence increased percentages of chrome removal were noticed. After that due to less food supply for bacteria, number of cells decreased but the removal efficiency was further increased in a slow rate. This may be due to that the surfaces of the remaining cells were not saturated with chromium and in this long contact time it was adsorbed slowly. The maximum chrome removal efficiency for BS-

1, BS-2, BS-4 and BS-6 were 83.37%, 86.13%, 87.65% and 89.66% respectively after 20 days of treatment.

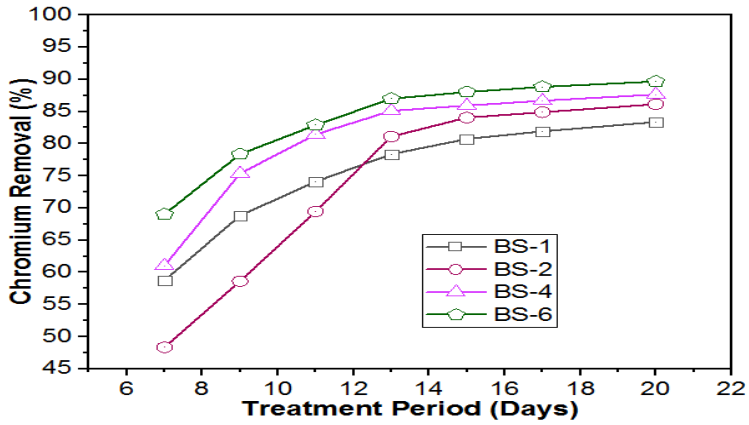


Figure 8 Chromium removal efficiency during the treatment period

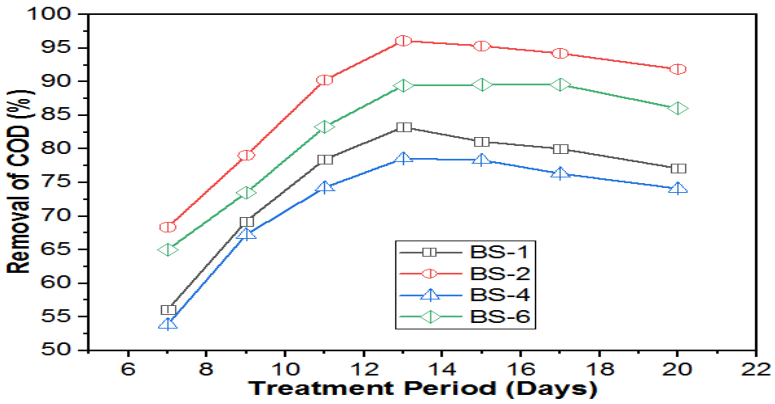


Figure 9 COD removal efficiency during the treatment period

For COD removal it is seen from the figure 7 and the figure 9 that highest COD removal percentage was for BS-2 (68.36%) at day-7 where the TVSS (bacterial population density) was also the highest (2680 mg/L) among all the strains. The same scenario was also noticed for BS-1, BS-4 and BS-6 where the removal efficiency along with the corresponding TVSS were 56.11% for 2150 mg/L, 53.91% for 1980 mg/L and 65.03% for 2450 mg/L accordingly. With times the removal efficiency of COD increased with the increase of TVSS and both the parameters reached at maximum in day 13. At this point maximum COD removal percentages were 83.25%, 96.11%, 78.59% and 89.45% for BS-1, BS-2, BS-3 and BS-4 where the corresponding TVSS were 3720 mg/L, 4050 mg/L, 3670 mg/L and 3850 mg/L respectively. Bacterial biodegradation increases with the increase of the number of cell. Hence the COD removal efficiencies of the strains were increased with the increase of TVSS up to day 13. After that organic loads were deficient and bacteria were started to decrease due to the endogenous decay that causes cell breakdown and cell death. At these stages the broken cell debris were soluble to the water and contributed to organic loads in water as a result of which COD values started to increase and removal percentages decreased. At the end of the study period the removal efficiencies of COD for all the bacteria were decreased and the final values were accordingly 77.12%, 91.88%, 74.11% and 86.03% for BS-1, BS-2, BS-4 and BS-6.

CONCLUSION

This study based on bioremediation of COD and chromium was planned with the hypothesis that chrome resistant bacteria may be available in the tannery disposal site effluent and some of which may be able

to detoxify chromium and degrade organic pollutants in chrome tannery effluent. After a series of experiments finally four bacterial strains (BS-1, BS-2, BS-4 and BS-6) were isolated with the benefits of the removal efficiency of both the COD and chromium. The isolates were selected through a primary screening procedure in wastewater discharged from chrome tanning process where removal efficiency of the strains for both the parameters in combination was considered. At this stage both COD and chromium removal performance were 56.11% and 58.74% for BS-1, 68.36% and 48.35% for BS-2. The efficiencies were 53.91% and 61.04% for BS-4 and 65.03% and 69.03% were for BS-6 accordingly. Various morphological and biochemical tests were done to identify the strains. Maximum chromium and COD removal efficiencies of the isolates were finally checked in chrome tannery effluent through the specially designed batch experiments. Among the four isolates highest chrome removal was observed for BS-6 (89.66%) where the concentration decreased from 3820 mg/L to 395 mg/L. The chrome removal efficiencies for BS-1, BS-2 and BS-4 were 83.37%, 86.13% and 87.65% where the residual chromium concentrations were 635 mg/l, 829 mg/L and 472 mg/L accordingly. Considering COD removal BS-2 was able to remove maximum percentage (96.11%) and the COD fell from 2885 mg/L to 112 mg/L. The residual COD values were 483 mg/L, 618 mg/L and 304 mg/L at the maximum removal efficiencies of BS-1 (83.25%), BS-4 (78.59%) and BS-6 (89.45%) respectively. Tanneries can easily practice this method of treatment for the detoxification of chrome bearing wastewater and to diminish organic loads after onsite tests.

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