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A biotechnological thrive on COD and chromium removal from leather industrial wastewater by the isolated microorganisms

Tamal Mandal[a*], Dalia Dasgupta[b], Siddhartha Datta[c]

[a]*Department of Chemical Engineering, NIT, Durgapur, India*
Tel.: +91-9474533097; Fax: +91-343-2547375; email: tamal_mandal@yahoo.com
[b]*Department of Biotechnology, NIT, Durgapur, India*
[c]*Department of Chemical Engineering, Jadavpur University, Kolkata-32, India*
Tel.: 91-33-2335-9345; Fax.: +91-33-2413-7121; email: sdatta_che@rediffmail.com, provc@admin.jdvu.ac.in

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ABSTRACT

Tannery wastewater treatment using three isolated bacteria has been studied separately and in combination in the present study. The removal of COD, BOD and Chromium were evaluated. The high salt and Cr tolerant bacteria designated as strain-I showed maximum efficiency in COD and Cr removal from the tannery wastewater of Kolkata, India compared to other two strain-II and strain-III. In combination treatment by all three bacteria showed additive effect in waste degradation. From the results it can be said that the bacteria present in the tannery effluents and nearby soil have significant potential in treatment of leather industrial wastewater.

Keywords: Leather industrial wastewater; COD; BOD; Chromium; *Halophiles*; Fenton's reagent; Combined treatment process

1. Introduction

Intensive industrialization generates hazardous wastes composing organics, inorganic, heavy metals, refractory etc that need to be tackled in a safe manner. Technologies have paved the way to eco-friendly bioremediation process [1,2]. Bioremediation uses natural as well as recombinant microorganisms to break down toxic and hazardous substances by aerobic and anaerobic means [3,4]. Among industrial wastes leather industrial waste is an important one as the COD, BOD, suspended solid, dissolved solid, sulphide and salinity level is very high, which further contaminants receiving water bodies as well as fertile land [5,6] and ultimately effects our food chain [7]. Chemicals such as sulfides, acids, alkalis and chromium are used to process and manufacture the leather. They and have deleterious effects on the environment if not handled properly. Tannery wastewater treatment presents a serious environmental and technological problem. In fact after conventional treatment the effluent do not have the quality to meet the required limits. The COD, chromium salinity, ammonia and surfactant are present higher than the required limit [8]. Now a days biochemical and biotechnological approaches are continuously being applied to many process industries to destroy and detoxify their waste. This approach is gaining importance because of low cost, energy savings and environmentally safe operations. Use of microorganisms has been proved to be useful in removal of color [9] COD from various specific industrial effluents [10,11]. Aerobic and anaerobic treatment by specific meso and thermophilic bacteria has been found to reduce the effective COD [12,13]. Recent

*Corresponding authors

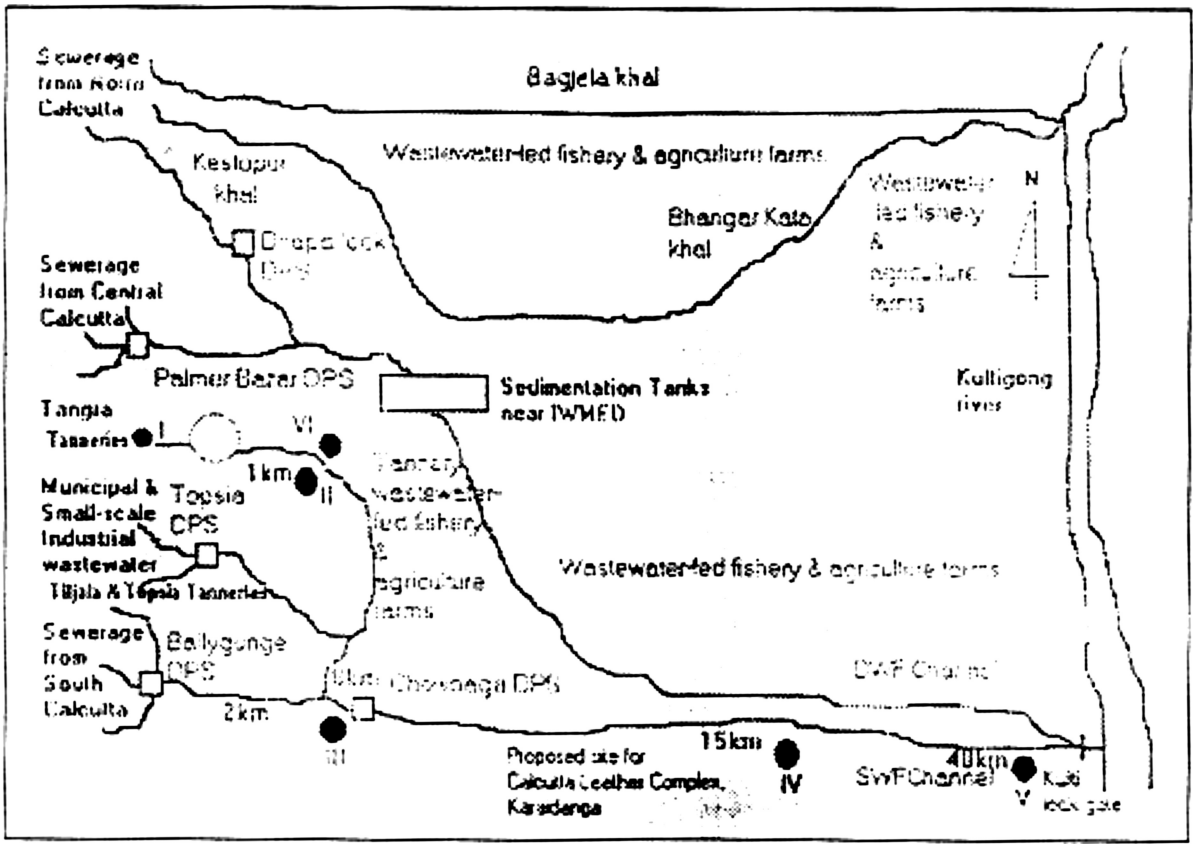

Fig. 1. Leather industrial wastewater collection site Tangra, Kolkata, West Bengal India. Spot of collection is marked with black circle.

studies have shown the effective role of fungus and bacteria in two stage anaerobic process in reducing the COD level [14]. Besides the high COD, BOD level in leather industry wastewater, the agents that mess this wastewater potentially hazardous is chromium. Chrome tanning is the most common type of tanning in the world and leather industrial wastewater normally carries the chromium (III). But there is a chance to transform chromium (III) to chromium (VI) and Cr(VI) is known to be toxic to plants, animals, a strong oxidizing agent and potential carcinogen [15,16]. The conventional methods used for removing metals from wastewaters are generally expensive and have many limitations [17]. Alternative methods of metal removal using biological resources like bacteria, algae, fungi are in progress [18–21]. The metal removal by suitable microorganisms from different industrial wastewater has important perspective as suggested by various researchers [18]. The aims of the present study are to isolation and selection of bacteria from tannery industrial effluents to remove the COD, BOD and chromium from the leather industrial wastewater, which are the main constituents of this wastewater.

2. Materials and methods

Materials: Wastewater sample collected from tanneries located in the Tangra region of Kolkata, India. Peptone, Beef extract, Sodium chloride, $FeSO_4$, Potassium Chloride, Ammonium Bi-Phosphate, Potassium Dichromate, Sulphuric Acid, silver sulphate, mercury sulfate, 1, 5-diphenylcarbazide, Acetone and other analytical grade chemicals (E.Merck, India). The purity of all reagents was more than 98%.

2.1. Wastewater sample

The tanneries in the area of Tangra, Tiljala and Topsia of Kolkata, W.B. India discharge their wastewater to nearby channel (Fig. 1). Usually the wastewater is draining out to the channel from the leather industry in a regular basis, reaches fishery and agricultural farms, and severely affects the food chain [7]. In a regular interval, sampling was carried out from this channel and characterized. The average values of parameters of wastewater was as follows; obnoxious, pH 6.4–7.6, 30 °C, COD 2,533 °mg/L, BOD_5 977 °mg/L, TDS 21,620 °mg/L, Ammonia-N 118 °mg/L, Phosphorus

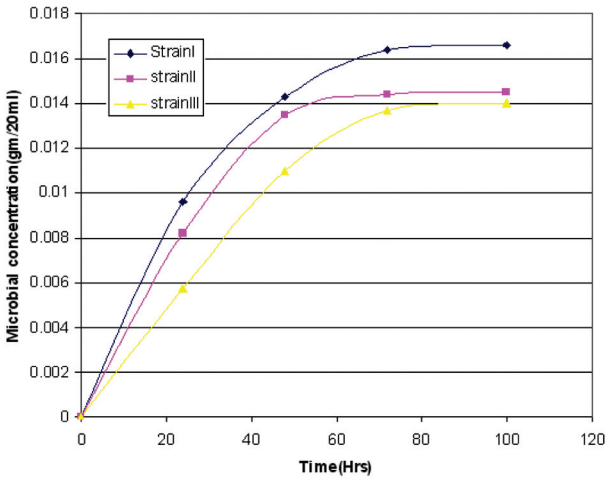

Fig. 2. Cellular growth of three isolated microorganisms (Strain1, Strain2 and Strain3) with time at pH 7.0 and temperature 30 °C.

62 mg/L, Sulphide 860 mg/L, Chloride 6,528 mg/L, Conductivity 20.04 mS/cm at 25 °C, Total chromium 58 mg/L, Total iron 2.56 mg/L.

2.2. Isolation of Bacteria from soil collected from waste site

The leather industrial wastewater and soil were collected from wastewater discharge channel Tangra, Kolkata, India. The microorganisms were isolated using the general procedure of dilution plate method. In brief, the different dilution of wastewater samples and soil samples were added to the nutrient agar media and kept at 30 °C for 24–48 h. After 48 h, the culture plates were checked for growth of bacterial colony. The morphological characteristics were detected using Gram staining. Selected bacteria were maintained in solid and liquid media for further work.

2.3. Culture medium

Isolation of bacterial colonies from wastewater samples was done using general serial dilution method. For this nutrient agar (Hi-media) media with the following composition peptone—10 gm/L, NaCl—5 gm/L, Beef Extract—5 gm/L and Agar 15 gm/L were used. Solid NaCl was added to the agar to obtain the desired salt concentration and pH was adjusted to 7.0. Isolated bacteria were then acclimatized with wastewater again.

2.4. Inoculums preparation

The isolated bacteria were transferred from solid to liquid nutrient media at pH 7.0 and for 24 h. After this, inoculated broth was incubated in an arbitrary shaker at 150 rpm and 37 °C for 24 h in about 100 mL nutrient liquid media. Well-grown culture suspensions with uniform concentration (absorbance at 660 nm) were used for the treatment as well as optimization.

2.5. Experiments

Experiments were carried out in shaking flasks for optimization of several growth parameters (Temp, pH, NaCl concentration etc) in synthetic nutrient media. In all optimization studies, the samples were analysed for biomass growth by monitoring the absorbance in UV/VIS spectrophotometer at 660 nm. Growth curves were made for each growth condition and optimum value of each parameter was fixed by comparing the growth for higher biomass yield. The growth rate of biomass was studied by the change of dry weight of the microbial cell with time. In brief the micro-organisms were cultured in a BOD incubator shaker at 30 °C and pH 7.0. At each time point 20 mL of culture fluid were taken and centrifuged [Sigma, 3K30, Laboratory Centrifuge] for 10 min at 12,000 rpm. The supernatant was removed and the cell debris were dried at 45 °C in an hot air oven. The dry weight was measured and presented in Fig. 2 with respect to time.

2.6. Treatment of waste water by isolated bacteria

The acclimatized isolated micro-organisms were cultured in a BOD incubator shaker at 30 °C and pH 7.0. 20 mL of culture sample was taken and centrifuged at 12,000 rpm to precipitate the microbial cell. The residue were added to 100 mL of wastewater in a 250 mL conical flask along with 10% of nutrient agar medium. It was kept in a BOD incubator at 30 °C on rotary shaker with 120 rpm for different time periods. The treated samples were collected at each time point, allowed to stand for 10–15 min, and centrifuged. Supernatant was taken for COD, BOD, Conductivity, TDS, TSS and DO and total chromium analysis. The corresponding control sets (treated with deactivated microbes by boiling) were regularly maintained. The pH of the treated samples were checked and found unchanged. All the results are the average of three experimental sets. The major operational condition temperature (20–40 °C) and pH (6–9) were investigated for the treatment of leather industrial wastewater.

2.7. Combination treatment

This experiment was designed to see the combination effect of Fenton's treatment followed by

biochemical treatment with isolated bacteria. The Fenton's reagents [required amount of H_2O_2 and $FeSO_4$] were added to the 100 mL of wastewater sample, and kept for 30 min at 30 °C and pH 3.5. After 30 min of Fenton's treatment, pH of sample was adjusted to 2.5 by 1(N) H_2SO_4 and bacterial cultures (20%) was added and incubated at 30 °C in BOD incubator shaker for 48 h. The treated samples were neutralized to a pH of 7–8, by strong ammonium liquor and filtered. The filtrate was analyzed for BOD, COD, color, sulfide, total chromium.

2.8. Cr tolerance test of isolated bacteria

Three types of bacteria isolated from leather industrial wastewater and were grown in liquid media (Nutrient broth) at pH 7.0 and temperature of 32 °C for 24 h in presence of different concentration of $K_2Cr_2O_7$ solution ranging from 100 to 700 mg/L. After 48 h, the growth rate was measured by taking absorbance at 660 nm at each case. The presences of the specific bacterial colonies in each experimental case were further confirmed by transferring the inoculum from liquid media to solid media.

2.9. Analysis

2.9.1. λ_{max} analysis of the red colour pigment produced by isolated bacteria

Solid culture (red colonies) were grown in nutrient agar media, cells were harvested by scraping and then centrifuged at 12,000 rpm for 25 min. The cell pellet was resuspended in a mixture of acetone:methanol (7:2 v/v) incubated at 4 °C overnight in the dark. It was again centrifuged to remove cell debris, and the absorption spectra were determined between 220 and 800 nm using UV/VIS spectrophotometer [22].

2.9.2. Analysis of physical and chemical parameters

(i) COD, BOD, TDS, TSS, Turbidity (NTU) and DO Test

The COD of untreated and treated sample were measured according to standard protocol of APHA [23] and reactor digestion method for a COD range of 0–1,500 using automatic COD analyser of LoviBond Germany. BOD, TDS, TSS, Turbidity (NTU) were measured according to standard methods of APHA [23]. The following electrode were used to measure the different parameters for different ions Orion four star Ion analyser (pH .ISE Benchtop) [Thermo electron corporation, USA (SN012820)], For NH_3 Orion 95-12 Ammonia electrode, For Cyanide Orion 94-06 & Orion 96-06 ionplus]. For pH (pH meter from WTW, Ino Lab PH/Ion-735,Germany) and TDS, conductivity were measured by inoLab Cond 720, with electrode TetraCon 325, WTW, Germany. The absorbances of the samples were determined with spectrophotometer (Tech Comp, UV/VIS-2300) at required wavelength. Dissolved oxygen was measured throughout the study by DO meter, Oxi-330i /SET (DurOx 325-3).

Table 1
Microscopic analysis

Isolated strains	Gram staining	Shape	Pigment produced
Strain 1	Positive	Coccus	Red
Strain 2	Negative	Bacillus	Brown
Strain 3	Positive	Bacillus	White

(ii) Cr(VI) analysis

The treated and untreated samples were centrifuged at 12,000 rpm for 10 min and the supernatant was used to determine the concentration of Cr(VI). Cr^{+3} is the lion share of leather industrial wastewater, thus potassium permanganate was used to oxidize Cr^{+3} to Cr^{+6}. The concentration of Cr(VI) in solution was then determined by UV spectrophotometers at 540 nm using 1,5-diphenylcarbazide [23,24].

2.10. Microscopic analysis

The morphological and gram staining study was done using light microscopy. The strain-1 red pigment producing organisms were gram-positive and coccus and they appeared as tetrad. Strain-2 was gram-negative and rod shaped and strain-3 gram-positive and rod shaped i.e bacillus. All these three microbes were subcultured in both solid and liquid media at regular intervals for maintenance of the strains and for wastewater treatment.

Table 2
Growth of three isolated micro-organisms at different % of NaCl in NA medium

Nacl (%) in nutrient agar	Strain-1 OD_{660} (halophyle)	Strain-2	Strain-3
5	0.125	0.112	0.130
10	0.280	0.022	0.027
20	0.430	0.008	0.009
25	1.237	–	–
30	0.878	–	–
35	0.325	–	–

Table 3
Growth of three isolated micro-organisms at different temperature

Incubation temperature (°C)	Strain-1 (OD_{660})/ pigment colour	Strain-2 (OD_{660})/ pigment colour	Strain-3 (OD_{660})/ (pigment colour)
4	0.013 (yellow)	0.001 (brown)	0.008 (white)
25	0.017 (red)	0.027 (brown)	0.036 (white)
30	1.573 (red)	0.620 (brown)	0.821 (white)
38	0.325 (red)	0.245 (brown)	0.220 (white)
54	0.120 (red)	0.009 (brown)	0.050 (white)

3. Results

Three different bacterial colonies distinguished by their pigment production were isolated using dilution plate method from the tannery wastewater collected from the nearby tannery industrial area of Kolkata, W.B. India.

3.1. Colony characteristics

Three organisms grew optimally at 30 °C and pH 6.8 in nutrient agar media. Each of the three organisms produced a specific colour in the nutrient media as shown in Table 1.

The organisms designated as strain—1, 2, 3 are red pigment producing colonies, light brown pigment producing colonies and white pigment producing colonies respectively. The surface of the colony is smooth in all three isolated bacteria.

3.2. Effect of salinity on the growth of isolated micro-organisms

The concentration of NaCl was high in the in the wastewater and soil of the tannery industrial area. The effects of different concentration of salt in the growth of all these three selected bacteria were investigated, by culturing them in both nutrient agar media and nutrient broths containing different concentration of NaCl at the range of 1–25%. Among the three, only red pigment producing bacteria were found to grow in the media with increasing NaCl concentration. In fact, the growth was found to be increased with NaCl for strain-I. The other two bacteria (brown strain II) and (White, strain III) did not grow in the said medium containing more than 5% NaCl. From the observation (Table 2) it seems that red pigment producing bacteria is within the family of halobacteria as it requires higher NaCl concentration of its proper growth [25]. The Extreme halophilic bacteria are defined as microorganisms that grow best in media containing 2.5–5.2 M NaCl [26]. Microscopic analysis and gram staining studies showed that, the red pigment producing bacteria to be gram-positive and coccus. The 16S rRNA profile of the isolated red pigment producing halophile could not be done due to lack of facility so it was not possible to specify the species of this halophile. The red pigment produced by this halophiles were analysed at different wavelength at 400—600 nm as reported in various work to test the λ_{max} of this pigment. The λ_{max} observed in this study is around 473 nm and 525 nm. Which seems to be similar to the carotene related pigment produced by halo-coccus in nutrient agar media at 30° C [27]. To check the colour of the pigment at different temperature as reported in the previous work [27], we have also grown the strain-I in nutrient agar at 4, 18, 38 and 58 °C. At 4 °C the colour of the pigment was found to be yellow rather than red. Thus, we may conclude that, strain-I can produce yellow pigment at 4 °C as some species of halococcus do. The λ_{max} of yellow pigment is at 365 nm.

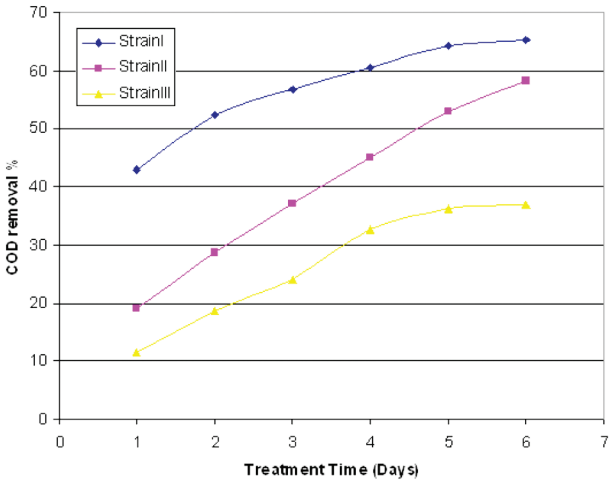

Fig. 3. COD (mg/L) removal by three isolated microorganisms in aerobic batch treatment of leather industrial wastewater (Kolkata, WB, India) with time (days) at pH 7.0 and temperature 30 °C.

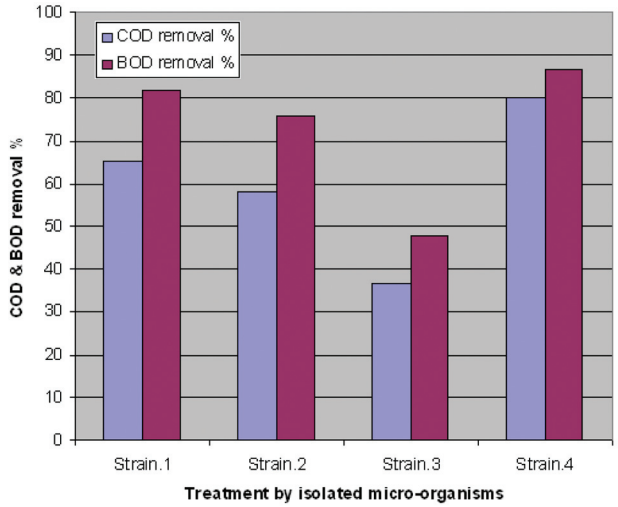

Fig. 4. COD and BOD (mg/L) removal by three isolated microorganisms and in combination in aerobic batch treatment of leather industrial wastewater (Kolkata, WB, India) for 5 days at pH 7.0 and temperature 30 °C.

3.3. Optimization of growth parameters for isolated bacteria (temperature and pH)

The growth parameters were analysed for the three isolated organisms I, II and III. For this, all the three strains were grown in liquid media at different temperature of 4, 25, 30, 38 and 54 °C for 24 h and optical density or absorbance at 660 nm were measured and presented in Table 3. The optimum temperature for maximum growth has been found to be at the range of 30–37 °C for all these strain I, II, III. The optimum pH has been found to be around 7.0 for all the isolated bacteria. Another interesting observation was that the red pigment production by strain-I varies with temperature. At 30 °C the colour of pigment is red absorbing maximum at 490 and 525 nm, whereas at 4 °C the color of pigment was observed yellow with maximum absorption at 372 and 365 nm.

3.4. COD reduction

The degradation of highly toxic wastewater from leather industries have been examined using the microorganisms isolated from the outlet channel of leather industrial wastewater. The treatment was done at an initial pH of 7.0 and temperature at 30 °C by all the three micro-organisms for different time periods. At each time point the treated waste water samples were centrifuged at 12,000 rpm [Sigma, 3K30, laboratory centrifuge], filtered with Wattman-42 and COD was measured by automatic COD analyser (LoviBond, Germany). Proper control sets were maintained where deactivated (by boiling) microbes were used for the

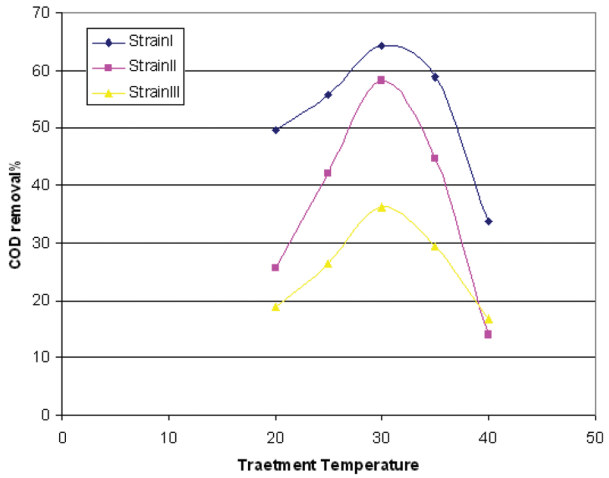

Fig. 5. COD (mg/L) removal by three isolated microorganisms with changing temperature from 20 to 40 °C in aerobic batch treatment of leather industrial wastewater (Kolkata, WB, India) for five days time at pH 7.0.

treatment to compare with the experimental set. From the results (Fig. 3), it can be said that the isolated microorganisms showed effective role in reducing the COD level of the leather industry wastewater compared to the control sets. It is also observed that, all the microorganisms initially need some time to degrade the waste and then there is a gradual increase in % COD reduction with time. Compared to the control set, day one treatment showed 42%, 19% and 11% COD reduction with strain I, II, III respectively. The % COD reduction was observed to be increased with time and reached up to 65, 52 and 36% with strain-I, Strain-II and Strain-III

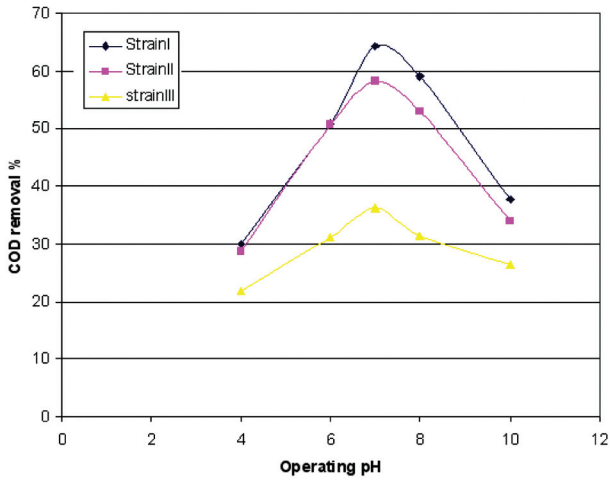

Fig. 6. COD (mg/L) removal by three isolated microorganisms with changing pH from 4 to 10 in aerobic batch treatment of leather industrial wastewater (Kolkata, WB, India) for 5 days time at temperature 30 °C.

respectively in 5 days. When all these isolated bacteria were used in combination (1:1:1 v/v), very prominent effect in COD reduction was observed (Fig. 4), which was around 80% in 5 days. Thus the isolated bacteria have the significant effect in waste degradation individually and even higher with combination to the control set (around 10% COD degradation) .The results are the averages of three different experimental sets and conclusively establish the efficacy of microbial waste degradation.

3.5. BOD removal by the micro-organisms

The initial BOD of the wastewater was 945 mg/L. The sample were treated for 7 days with specific isolated micro-organism at 30 °C and pH 7.2. The results presented in the Fig. 4, showed the isolated organisms were very much effective in BOD removal. The strain I, II, and III have removed 82, 76 and 48% of BOD respectively in 5 days. In combination treatment, by all three strain of bacteria the BOD removal has been observed to be increased (Fig. 4).

3.6. Temperature and pH optimization for the wastewater treatment process

The isolated microorganisms showed their potential role in waste degradation, in terms of COD reduction at pH 7.0 and temperature 30 °C. The optimum temperature and pH range for the treatment process were further investigated for waste degradation. For these the wastewater was treated at different temperature range of 20–40 °C and at different pH range of 4–10 as these micro-organisms have been observed to grow in all these temperature and pH range in the nutrient agar media. The results were presented in the Figs. 5 and 6. From the experimental results, it was found that the optimum temperature and pH ranges were 28–32 °C and 6.8–7.4 respectively. In this study, it reveals that biochemical.

The wastewater was treated with Fenton's reagents with dose of 4.5–266 g/L H_2O_2 and 1.5–6 g/L prior to biological treatment at pH 3.5 and temperature 30 °C (ambient) for 30 min. It was observed that with increasing the concentration of Fenton's reagents the waste removal in terms of COD, BOD, color, sulfide has been increased but sludge production is increased and cost too. The Fenton's treatment is an exothermic reaction. Thus after cooling the reaction mixture at to ambient condition and adjusting pH 7.0 biological treatments was performed with three isolated microorganisms. Most effective and economic condition was observed that pretreatment with Fenton's reagents with a dose of 4.5 g/L H_2O_2 and 3 g/L $FeSO_4$ and subsequently

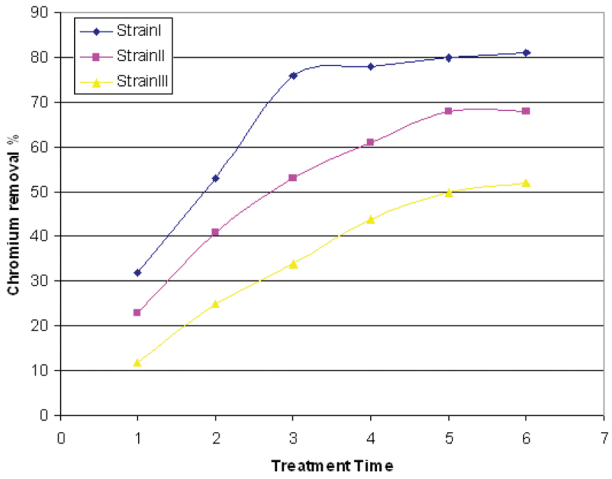

Fig. 7. Total chromium (mg/L) removal by three isolated microorganisms in aerobic batch treatment of leather industrial wastewater (Kolkata, WB, India) with time (days) at pH 7.0 and temperature 30 °C.

bio-treatment with isolated microorganisms for 96 h batch condition. In this combination treatment COD, BOD, color and chromium removal percentage were rise up to 88, 93, 96, 95 and 82, 86, 82, 82 and 52, 60, 70, 68 by the strain-I, strain-II and strain-III respectively.

3.7. The effect of microorganisms in reducing the Cr level in leather industry wastewater

The presence of chromium in the leather effluent is of major concern for the tanning industry [15]. Chemical precipitation, coagulation, electrodialysis are the common practice for removal of chromium. However, it leads to generate chrome bearing solid wastes. Membrane separation and ion exchange methods are very costly. Different studies are going on to reduce Cr level using microorganisms. From the various studies it appears that microbes can have a role in reduction of these toxic heavy metals [32,33]. Therefore, the potential role of isolated microorganisms in reduction of chromium level has been studied. The initial chromium concentration was 58 mg/L in the wastewater collected for treatment. The concentration of chromium in the untreated and treated tannery industrial effluent has been analysed spectophotometrically using DPC [23,24].The treatment was done by the three isolated micro-organisms (with 10% inoculation) separately for different time periods at 30 °C and pH.7. From the Fig. 7, it has been observed that, the high salt tolerant bacteria [Strain-1] has the maximum Cr reduction efficiency compared to strain-II and strain-III. The reduction of Cr was found about 81% by strain-I compared to 68 and 52% by strain-II and strain-III respectively.

The magnitude of metal ion removal by microorganisms from the effluent differs from strain to strain due to the properties of the metal as well as the properties of the microorganism (like. structure, functional groups on surface area). The cell wall capsules and slime layers of various micro-organisms contain polysaccharides as basic building blocks, which have an ion exchange property helping in removal of specific metals. They also contain proteins and lipids and therefore offer a host of functional groups capable of binding to heavy metals. These functional groups especially amino, carboxylic, sulphydryl and phosphate groups differ in their affinity and specificity for metal binding. Fig. 7 also depicts that initially the rate of removal percentage was low by all the microorganisms. However, the rate of removal increased with increasing time and ultimately reaches to a stationary phase. This is probably due to the fact that, the biosorption was affected by the microbial growth phase. Initially the rate of removal of chromium was slow as the microbes were in lag phase and its concentration was sufficiently low in the reaction medium. Thus, the ratio of available surface area for adsorption to the initial moles of chromium was also very much lower and subsequently the removal of chromium concentration was not significant. With increasing time, the microbes reached to its exponential growth phase and the available surface area for biosorption increased effectively as a result the percentage removal chromium also increased. The maximum potency of Cr reduction by strain-I (halophiles) compared to other two strain-II & strain)-III, were further confirmed by checking the Cr tolerance by the three strains. Isolated microorganisms were inoculated in nutrient media containing different Cr concentration of 100–700 mg/L and kept for 24 h at 30 °C. The growth was checked by measuring the absorbance in liquid media at 660 nm. The growth of strain-I was observed in each nutrient agar plates with varying concentration of chromium up to 600 mg/L, while strain-II and strain-III had shown insignificant growth at above 250 mg/L and 200 mg/L of chromium concentration respectively. These suggest that among the three isolated strains, strain-I halophiles, which has been found to reduce Cr significantly can tolerate towards high chromium concentration compared to the other two isolated strain. So it may conclude that the strain-I (halophiles), which can sustain at high salt concentration, tolerate high Cr concentration and can remove Cr and COD effectively from leather industry effluents.

3.8. TDS, TSS and Turbidity (NTU) in the reaction system

These parameters were measured regularly in the reaction system by centrifuging the samples at 12,000 rpm. The supernatant were filtered with wattman-42 for measurement of TDS, TSS and Turbidity. Initially these parameters were increasing in all cases. This is probably due to the effect of microbial population (exponential phase). However, after three days the TDS, TSS, Turbidity (NTU) values were gradually decreased showing the degradation of waste by the microbial activity.

3.9. DO and Conductivity in the reaction system

The wastewater treatment was performed in a BOD incubator with shaker. Shaking helps to supply air (oxygen) in the reaction medium, this is used by the microorganism for its cellular growth and reproduction. Oxygen helps in direct waste treatment too. In the reaction system the DO percentage was initially 23 and with the time it increased due to shaking (150 rpm) and reached up to 93%. The conductivity of the reaction systems were measured at regular intervals. The initial conductivity of the reaction system was 3.78 mho/cm, which linearly increases up to 5.78 mho/cm. The reason could be due to the enhanced microbial population and degraded waste product concentration.

3.10. Calculation of kinetic parameters

The removal of COD with time by the three isolated micro-organisms were observed to follow the first order kinetics. Thus, Thomas model [13] was used to calculate the kinetic parameters for wastewater treatment. When $(t/Y)^{1/3}$ vs. t was plotted, a straight line was obtained following the Thomas equation:

$$\left(\frac{t}{Y}\right)^{1/3} = (2.3\,K_1^{'}\,L_u)^{-1/3} + \left[\frac{(K_1^{'})^{2/3}}{3.43\,(L_u)^{1/3}}\right] t \qquad (1)$$

where $k = 2.303 \times K_1{}'$.(t—time, Y—remaining COD, L_u—the original concentration of the organic material and $K_1{}'$—the reaction rate constant).

From the slope and intercept of straight line the kinetic parameters $K_1{}'$—the reaction rate constant and L_u—the original concentration of the organic material before any biological action has occurred) has been calculated. The Hoff–Arrhenius equation was used to calculate the values of $K_1{}'$ for different temperatures. The equation is

$$k_{1,T}^{'} = k_1^{'},\ T_0\theta^{(T-T_0)} \qquad (2)$$

Normally the value of $k_1{}'_{,T0}$ is determined at 20 °C, then the reaction-rate constant $k_1{}'_{,T}$ at temperature T °C can be obtained by modifying Eq. (2)

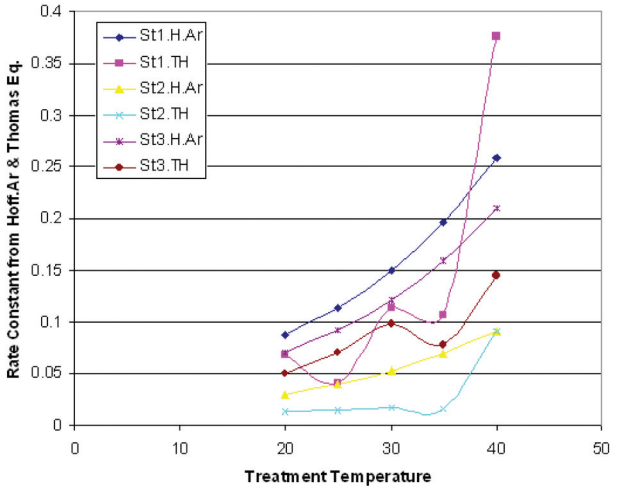

Fig. 8. Calculation of rate constant by Thomas Model and Hoff–Arrhenius equation at different temperature for wastewater treatment by the isolated microorganisms.

$$k'_{1,T} = k'_{1,20}\theta^{(T-20)} \quad (3)$$

Although θ was assumed to be a constant, it varies slightly with temperature and the following values are recommended: $\theta = 1.135$ for temperature range 4–20 °C and 1.056 for the temperature range 20–30 °C.

Using the data presented in this study the reaction rate constant by Thomas equation and Hoff–Arrhenius equation has been calculated and presented in the Fig. 8. It indicates that the values obtained from Hoff–Arrhenius equation are increasing gradually with the operating temperature. But the values generated from Thomas equation are different in nature. Fig. 8 depicts that the rate constants calculated by Thomas model are increasing initially with temperature but after a certain range it is decreasing. The values of reaction rate constant generated from Thomas equation deviates drastically from the Hoff–Arrhenius equation. This is due to the fact that microbial growth was considered for the development of Thomas equation but it was not incorporated in the Hoff–Arrhenius equation. So the results obtained from Thomas equation is more practical and useful in this case.

4. Discussion

The experiments conducted in the present study, provides some significant information regarding isolated bacteria mediated degradation of waste in terms of COD and BOD and reduction of chromium level of leather industry wastewater of Tangra region (Kolkata), India. The waste streams are loaded with high BOD, COD, chromium and sulphur, which has a drastic effect on the environment. In this present study, it is observed that all the three isolated bacterial species can reduce COD and BOD level significantly within five days of treatment. Among these three strains, strain-I, which is highly salt tolerant bacteria (Gram +ve, coccus) showed maximum potential to degrade the waste [26,27]. The percent reduction in COD and BOD has been found to be about 65% and 82% respectively compared to the other two strains (58% COD, 76% BOD reduction by strain II and 36% COD and 48% BOD reduction by strain-III). The experimental results for all the cases indicate that the rate of waste degradation (COD level decrease) is reasonably high initially and gradually decreasing and then drops quite sharply. This may be due to the non-oxidisable nature of the waste. The waste are oxidized by the micro-organisms to collect the energy as they are the energy-processing machine, tearing down molecules and commenting their bond energy into biologically useful energy to derive the energy required for growth, maintenance and reproduction [28,29]. From the results it appears that, the micro-organisms are playing an important role in wastewater treatment, as studied by other research group also. The mesophilic and thermophilic organisms can be used in aerobic treatment of wastewater [12]. Specific thermophilic bacteria can reduce COD level anaerobically [30]. Combination of treatment by fungus and bacteria has also been reported to be a useful technology for COD reduction [14]. Halophiles are already reported to be effective in removing COD from leather industry effluents [26] and treatment of saline wastewater [27,31]. The present study also emphasize the maximum efficacy of halophiles isolated [Gram+ve, coccus red) from specific area in the reduction of COD and BOD [results shown in Fig. 4] compared to the other two bacterial species (Strain-II and stain-III).

Conventional physicochemical methods for heavy metal removal from waste streams are not cost effective [32] and hence biological approach is the promising developing technology today. Recently microbial systems like fungus, bacteria and algae have been successfully used as an adsorbing agents for removal of heavy metals [33–36]. In the present study, the chromium reduction, by the isolated bacteria also showed promising results. As reported, the conventional methods applied to remove excessive heavy metals from aqueous solution appears inefficient and expensive [32], consequently it is urgent to find new technologies or biomaterials for removing heavy metals ions from waste water. Biosorption [17] is coming up as an alternative method to treat industrial effluents, mainly because of its low cost, high metal binding capacity, high efficiency in dilute effluents and ecofriendly

[20,37,38]. The present paper also used isolated bacteria for the reduction of chromium in leather industry effluents. From the results (Fig. 7) it appears that all the three bacteria's have some role in the reduction of chromium level compared to the control (treated with well boiled, deactivated micro-organisms). Among the three, Strain-I (Gram-ve, coccus red) halophiles shows maximum effect in the chromium reduction about 81% in 5 days. The other two strains removed the chromium around 68 and 54% respectively in the same time. The basic mechanisms, for this may involve intracellular uptake, surface binding or some unknown mechanism. Micro-organisms have the high surface to volume ratio, they can provide the large contact interface, which would interact with the metals from the sample solution [39]. So with time, the reduction of chromium removal has been increased in this study, suggesting that growth kinetics or the concentration of micro-organisms in the reaction medium (Fig. 2) have direct role in reducing chromium level. In spite of providing the high surface area (large contact surface) for metal binding, the structural polymer of bacterial cell wall provide acidic functional groups that are specifically reactive towards the dissolved metals [40]. Halophiles [Strain-I] have different cell wall structure [41] than the standard bacterial cell. A thick layer of polysaccharides which may be sulphated are commonly found in the halophiles groups may be responsible for higher Cr biosorption (81%) than the other two strains-II, and III. Strain-I halophiles has also shown greater Cr. tolerance that the strain-II and Starin-III. The results obtained showed that the maximum removal occurs at a particular time period and then it declined, indicates that growing cells can continuously detoxify the heavy metals from the solution due to some internal mechanisms too. Like other microorganism, these three strains have also showed their activity in a range of temperature from 28 to 32 °C and pH from 6.8 to 7.2. Although the Strain-I (halophiles) showed that it can survive in wide range of temperature from 4 to 58 °C and pH from 1 to 9, due to its cellular diversity. But, it showed the maximum activity in wastewater treatment and chromium removal at pH .7 and temperature at around 30 °C. The kinetics study using Thomas model and Arrhenius–Hoffman equation was done. The values of rate constant from Thomas model were found best fitting with the experimental results.

5. Conclusions

The isolated three bacteria from leather industrial wastewater are capable to remove COD and chromium efficiently from this wastewater. Within these three strain-I has shown maximum efficiency in COD and chromium removal. Although satisfactory removal was observed in bacterial waste treatment but the process was time-consuming, i.e. several days were required for satisfactory waste removal. To reduce the treatment time, a low dose Fenton's oxidation treatment was allowed prior to biochemical treatment. This combination treatment enhanced the COD and chromium removal above 90% within 4 days.

Thus it may be concluded that the combined process an efficient, energy saving, low cost and time saving process.

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