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Treatment of leather industry wastewater by aerobic biological and Fenton oxidation process

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ABSTRACT

Degradation of leather industry wastewater by sole aerobic treatment incorporating *Thiobacillus ferrooxidans*, Fenton's reagents, and combined treatment was investigated in this study. The sole treatment by Fenton's oxidation involving the introduction of 6 g $FeSO_4$ and 266 g H_2O_2 in a liter of wastewater at pH of 3.5 and 30 °C for 30 min at batch conditions reduced COD, BOD_5, sulfide, total chromium and color up to 69%, 72%, 88%, 5%, 100% and *T. ferrooxidans* alone showed maximum reduction to an extent of 77, 80, 85, 52, 89, respectively, in 21 d treatment at pH 2.5, $FeSO_4$ 16 g/L and temperature of 30 °C. The combined treatment at batch conditions involving 30 min chemical treatment by Fenton's oxidation followed by 72 h biochemical treatment by *T. ferrooxidans* at batch conditions gave rise up to 93%, 98%, 72%, 62% and 100% removal efficiencies of COD, BOD, sulfide, chromium and color at pH of 2.5 and 30 °C. Decrease in photo absorption of the Fenton's reagent treated samples, as compared to the banks, at 280, 350 and 470 nm wave lengths was observed. This may be the key factor for stimulating the biodegradation by *T. ferrooxidans*.

1. Introduction

Tannery industry is one of the important industries in India, which earns large foreign exchange through the leather export. The untreated release of tannery effluents containing high COD, BOD levels, trivalent chromium, sulfides, sodium chloride, Ca, Mg, organics and other toxic ingredients, to the natural water bodies effect flora and fauna of the ecosystem and increases the health risk of human beings [1,2]. In Kolkata (India), municipalities dump site (Dhapa) receives the wastewater from nearby tanneries, which affects the human beings through food chain [2]. Conventionally the effluent of tannery industry is treated by coagulation/flocculation [3], adsorption [4,5], nanofiltration [6,7] oxidation by Fenton's reagent [8]. But no treatment process has been identified to remove all sorts of waste present in the leather industrial wastewater.

Now-a-days biochemical/biotechnological approaches are being tried in many process industries to destroy or detoxify their generated waste aerobically [9–13]. The bacterial oxidation of ferrous iron using chemolithotrophic *Thiobacillus ferrooxidans* was initially assumed as the first stage of the indirect mechanism of metal leaching of low-grade sulfide ores [14]. Presently, this reaction of ferrous to ferric conversion has become the core of quite different industrial application starting from acid mine drainage wastewater treatment, chromium, zinc and aluminum recovery from various industrial waste sludge, Flue dust treatment of H_2S desulfurization, electroplating wastewater treatment and biodegradation of hydrocarbons in extreme condition [15–19]. The leather industrial wastewater contains a large amount of COD, BOD, chromium and sulfide compound which could be oxidized by these microbes. Although traditionally biological process has been considered to be the most potential for treatment of wastewater, Woolard and Irvine [20] have recently reviewed and expressed that, this method does not constitute a suitable choice even when using acclimatized seeds alone and the process is time consuming, with low removal efficiency of toxic pollutants [9,10]. The pretreatment with chemical oxidation processes seems to be the appropriate step to reduce the contaminant load of this type of effluent, and hence render them more biodegradable. Therefore a combination of physicochemical and biological treatment process has been tried in many cases [21–24]. Among the various physicochemical treatment processes, advanced oxidation processes (AOP) (Fenton's treatment) have become the chosen technology for waste treatment [8,25–28]. The organic waste present in the reaction system can be oxidized by the hydroxyl radical produced by Fenton's reagents. It has been reported by Tekin et al. that Fenton's treatment enhances the biodegradability

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Nomenclature

AFT	after Fenton's treatment
BT	represent biochemical treatment by *Thiobacillus ferrooxidans*
CT	chemical treatment by Fenton's oxidation
k'_1, T	rate constant at any temperature T
$k'_{1,T20}$	rate constant at a temperature 20 °C
K_d	first order endogenous decay coefficient (h^-)
K_m	Michaelis–Menten rate constant
K_S	saturation constant and is equal to the value of S at which $\mu = \mu_{max}/2$
L	value of the parameter at any time
LW	leather wastewater
L_0	value of the parameter concentration at $t = 0$,
n	constant
S	substrate concentration
t	time
V	reaction velocity,
V_{max}	maximum reaction velocity
X	biomass concentration (mg/L)
X_0	biomass concentration (mg/L) at $t = 0$
X	specific growth rate of biomass (h^-)
Y	COD at any time
Y_0	COD of the wastewater before any biochemical action occurred

Greek letters

α	any constant
μ	specific growth rate of the biomass
μ_{max}	maximum specific growth rate of the biomass on a particular substrate when $S \rangle\rangle K_S$
θ	E/RTT_0 assumed to be 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.

[29] and it is 100-fold faster than biological treatment [30]. This study, therefore, explores the potential of biological treatment with *T. ferrooxidans* and in combination with advanced oxidation process using Fenton's reagent in the degradation of wastewater of Kolkata (India) based leather industry.

2. Materials and methods

2.1. Materials

Hydrogen peroxide (30%, density 1.11 kg/L, Merck, India), ferrous sulfate, potassium chloride, ammonium biphosphate, potassium dichromate, sulfuric acid and other chemicals (E. Merck, India). *T. ferrooxidans* (ATCC19859), TCA [tri-carboxylic acid], BSA [bovine serum albumin], 9K medium containing ammonium sulfate 3 g/L, ferrous sulfate 14.2 g/L, potassium chloride 0.1 g/L, potassium biphosphate 0.5 g/L, magnesium sulfate 0.5 g/L, 1,5-diphenylcarbazide and zinc-acetate, NaOH, potassium iodide, sodium thiosulfate, etc.

2.2. Wastewater sample

The wastewater produced from leather industries in the area of Tangra, Tiljala and Topsia of Kolkata, India was collected at regular intervals, for sampling and characterization. The average values of the composition of wastewater are presented in Table 1.

2.3. Bacterial strain and culture medium

T. ferrooxidans (ATCC19859) was collected from Indian Institute of Chemical Biology [IICB, Kolkata, WB, India]. This strain was originally collected from the Department of Microbiology, University of Helsinki, Finland and was subcultured regularly in 9K medium. It was used for copper extraction from Indian ore and its genetic study in Indian Institute of Chemical Biology [IICB, Kolkata].

2.3.1. Acclimatization of *T. ferrooxidans* and inoculum development

T. ferrooxidans (ATCC 19859) was acclimatized in the modified 9K medium with 10% leather industrial wastewater; at 30 °C in a BOD incubator shaker (120 rpm) for 7 days and transferred to the flask with higher concentration of wastewater. This was continued for a certain period of time (7 d) by gradually increasing the wastewater concentration in the medium. So that *T. ferrooxidans* could adopt in original wastewater. This adopted *T. ferrooxidans* was used in the present study.

2.4. Research lay out

The laboratory scale experiment in this study involved two sequential stages depending on the type of treatment process applied: (a) biological treatment of leather industrial wastewater as a single process, (b) combination treatment: chemical oxidation using Fenton's reagent prior to biological treatment.

2.5. Treatment of wastewater by *T. ferrooxidans*

The 20 ml of acclimatized *T. ferrooxidans* culture sample was centrifuged at 10,000 rpm for 15 min to precipitate the microorganism. The microbial cells were added to 100 ml of wastewater in a 250 ml conical flask, along with 10% of 9K medium and incubated at pH of 2.5 and 30 °C in a BOD rotary shaker with 120 rpm, for different time periods. At each time point, the treated samples were collected, centrifuged and filtered. Filtered supernatant was analyzed for COD, BOD, salinity, conductivity, TDS and total chromium. The corresponding control sets (treated with deactivated microbes by boiling) were regularly maintained. All the results are the average of three experimental sets. The major operational condition like temperature, pH, $FeSO_4$ doses and inoculum size were investigated for the effective treatment of leather industrial wastewater keeping other parameters constant.

Table 1
The characteristics of leather industrial wastewater.

Parameters	Unit	Value	Indian standard for inland surface water
Odour	–	Obnoxious	–
pH	–	7.9–9.2	6.0–9.0
Temperature	°C	30	–
COD	mg/L	2533	250
BOD_5	mg/L	977	30 (3 d, 27 °C)
Salinity	–	49.80	
Suspended solid (TSS)	mg/L	1244	100
Total dissolved solid	mg/L	21,620	–
Ammonia-N	mg/L	118	15
Phosphorus	mg/L	62	5.0
Sulfide	mg/L	860	2.0
Chloride	mg/l	6528	1000
Conductivity	mS/cm	20,042 (at 25 °C)	–
Total chromium	mg/L	258	2
Total iron	mg/L	2.56	–

2.6. Treatment of wastewater by Fenton's reagents

The experiments were conducted in a batch reactor. The effects of pH, temperature, time of reaction, doses of H_2O_2 and $FeSO_4$ were studied. The role of pH in the reaction system is very important, since pH plays an important role in the mechanism of $OH^{\bullet}$ production [24,26,31]. In brief, to the 100 ml wastewater in 250 ml conical flask, required amount of H_2O_2 and $FeSO_4$ was added, pH was adjusted to 3.5 and incubated for 30 min in an incubator shaker at constant temperature of 30 °C and 180 rpm. After 30 min of treatment, pH was adjusted to 11.0. The treated samples were allowed to stand for 30 min to get the clear supernatant. The filtered supernatant was analyzed for COD, BOD, salinity, sulfide, chromium, conductivity and absorption at 200–400 nm (Tech comp. UV/vis-2300 China). Untreated wastewater was used as control.

2.7. Combined treatment procedures (Fenton's and biochemical)

This experiment was designed to see the combination effect of Fenton's treatment followed by biochemical treatment with *T. ferrooxidans*. The Fenton's reagent [H_2O_2 111 g/L and $FeSO_4$ 6 g/L] was 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 *T. ferrooxidans* cultures (20%) was added along with 10% of 9K medium and again incubated at 30 °C in BOD incubator shaker for 72 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, salinity and absorption at 200–400 nm.

2.8. Growth kinetics

Growth kinetics of *T. ferrooxidans* was studied in 9K medium at pH 2.5 by monitoring the following parameters (i) protein content (ii) ferrous oxidation. The medium was inoculated with 10% of previously grown culture, incubated at 30 °C for different time period. At each time point, the protein concentrations of the acclimatized *T. ferrooxidans* were estimated by a modified Lowry method [32].

The concentration of ferrous iron was determined by titration with 0.02 N $KMnO_4$. Total iron (both Ferrous and ferric) in a solution was determined by a colorimetric method using 1,10 phenanthroline [33].

2.9. COD, BOD, TDS, TSS, turbidity (NTU), NH_3, conductivity and salinity

The COD of untreated and treated sample with chemical (Fenton's reagent), biochemical (*T. ferrooxidans*) and in combination treatment were measured using the standard protocol of APHA [35] and reactor digestion method (for a COD range of 0–1500) using automatic COD analyzer of LoviBond, Germany. BOD, TDS, TSS, turbidity (NTU) were measured according to standard methods of APHA [35]. For NH_3, Orion 95-12 ammonia electrode, for pH, pH meter WTW, inoLab PH/Ion-735, Germany was used. The TDS, conductivity and salinity 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).

2.10. Cr (VI) analysis

The treated wastewaters with *T. ferrooxidans*, Fenton's reagent and in combination treatment were centrifuged at 10,000 rpm for 15 min. The supernatant was analyzed for the concentration of Cr (VI). Cr^{3+} has 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 determined using 1,5-diphenylcarbazide. The pink color developed was estimated at 540 nm by UV/vis spectrophotometer [35].

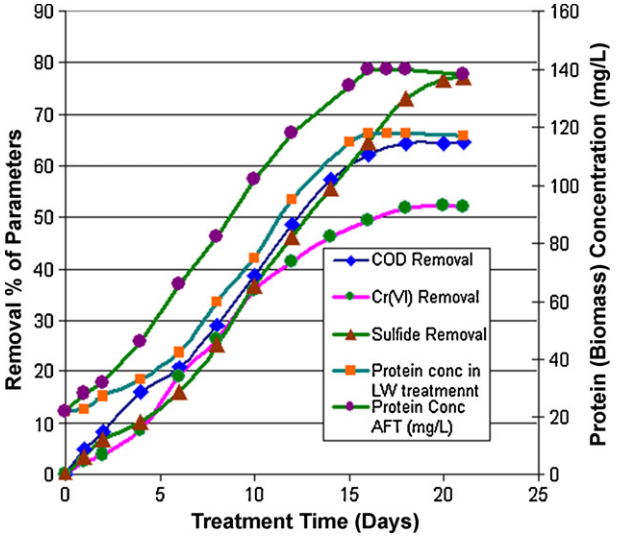

Fig. 1. Removal % of COD (mg/L), total chromium (mg/L) and Sulfide (mg/L) in aerobic batch treatment of leather industrial wastewater (Kolkata, WB, India) by *T. ferrooxidans* during different time intervals.

2.11. Sulfide measurement

Iodometric method was used [35] to measure the total sulfide (dissolved H_2S and HS^-, as well as metallic sulfides present in suspended matter) in untreated, *T. ferrooxidans,* Fenton's treated wastewater samples. Interference of sulfite, thiosulfate, iodine and any other soluble substances (but not ferrocyanides) was eliminated, using 22% zinc-acetate and 6 N NaOH.

3. Results and discussion

3.1. Effect of T. ferrooxidans on leather industrial wastewater treatment with respect to time

Degradation of leather industry wastewater by sole aerobic biochemical treatment has been studied. The removal of COD, sulfide and chromium (mg/L) from leather industry wastewater by *T. ferrooxidans* alone, at different time periods has been represented in Fig. 1. From the experimental results (Fig. 1) it is clear that *T. ferrooxidans* could reduce the COD level up to 64% in 21 d compared to about 10% in control. The chromium and sulfide removal are 52% and 78%, respectively. The degradation pattern followed the growth kinetics of *T. ferrooxidans* as presented in Fig. 1. This indicates that there is a direct involvement of *T. ferrooxidans* in tannery wastewater degradation. Initially, the degradation rate is high and with progress of time, it slows down and ultimately reaches to a constant value. The *T. ferrooxidans* induced COD, sulfide and chromium removal from the wastewater can be explained on the basis of the two facts, the bacteria *T. ferrooxidans* can oxidize the waste directly and indirectly. In indirect mechanism, the waste is oxidized by the acidic ferric sulfate produced by *T. ferrooxidans* in the system (Eq. (1))

$$2FeSO_4 + H_2SO_4 + O_2 + T.ferrooxidans \rightarrow Fe^{3+} + H_2O[36]. \quad (1)$$

Fe^{2+} oxidizing enzymes are present in *T. ferrooxidans*. These enzymes can rapidly reduce the *T. ferrooxidans* ferricytochrome [37] with Fe^{2+} ions at acidic pH and produce Fe^{3+} ions which then act as an oxidizer. Its coagulant nature also helps in total waste removal. A wide spectrum of substances, in tannery wastewater makes non-viable the development of reaction pathway for degradation of individual waste compounds. As a consequence, the COD of the effluent can be considered as a pseudo-component subject to oxidation [38]. *T. ferrooxidans* when oxidize the organic substrate, produce $COD^{\bullet}$ radical by the enzymatic action as proposed:

$$T.ferrooxidans + COD + O_2 \rightarrow COD^{\bullet} + H_2O. \quad (2)$$

Ferric sulfate thus produced in Eq. (1) act as an electron acceptor. This accepts an electron from the $COD^{\bullet}$ and the waste gets oxidized by the following reaction [39–41].

$$COD^{\bullet} + Fe^{3+} \rightarrow P + Fe^{2+} \quad (3)$$

$$COD^{\bullet} + Fe^{3+} \rightarrow COD^{+} + Fe^{2+} \quad (4)$$

$$COD^{+} + H_2O \rightarrow P + H^{+} \quad (5)$$

The direct mechanism, involves the enzymatic attack. The two enzymes cytochrome *C* and a blue copper protein rusticyanin are key factors responsible for the waste oxidation by absorbing electron from the waste present in leather industrial wastewater [42]. In this pathway, COD could be directly oxidized by the enzymatic attack of *T. ferrooxidans* into products

$$T.ferrooxidans + COD + O_2 \rightarrow P + H_2O + BP \quad (6)$$

Where P and BP are the products and byproducts were formed, due to microbial action on the waste.

3.1.1. Chromium removal by T. ferrooxidans from leather industry wastewater

The results presented in Fig. 1 showed that the maximum removal of total chromium is about observed 52% in 21 d of treatment by *T. ferrooxidans*. 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, sulfhydryl and phosphate groups differ in their affinity and specificity for metal binding [43]. In the present study, the total chromium removal has reached to 46% in 14 d and 52% in 21 d (Fig. 1), after that there is no significant Cr reduction. This pattern is quite similar to the growth kinetics of *T. ferrooxidans*. The reason for initial rapid phase of Cr removal may involve higher physical adsorption due to higher amount of functional groups as binding sites at the cell surface. The subsequent slower phase may involve other mechanism such as complexation, micro-precipitation or saturation of binding site. The *T. ferrooxidans* which grows on sulfur pyrites as substrate at acidic pH exhibit positive charge at their surface, which indicate the presence of NH_3 group on the surface [44]. The FTIR spectra showed the presence of NH_3, NH_2, NH, CONH, CO, CH_3, CH_2, CH and COOH groups on the surface of sulfur grown cells. At acidic pH, the predominant species of chromium are $Cr_2O_7^{2-}$, $HCrO_4^{-}$ and Cr_2O_4. Thus the surface of protonated microbial cells may play a predominant role in attracting anionic species of chromium and lead the chromium removal in present case [45,46].

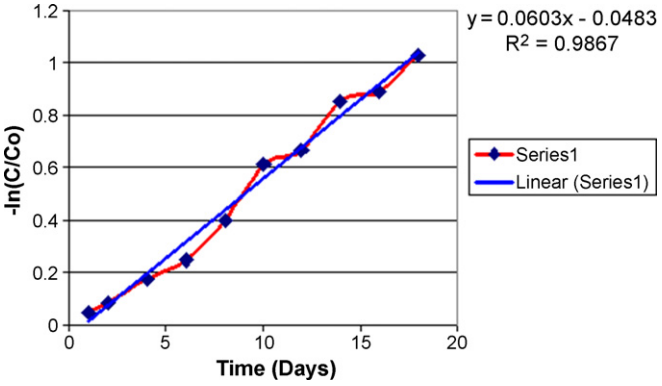

Fig. 2. plot of $\ln(C/C_0)$ vs. *t*, where *C* is remaining COD at any treatment time *t* and C_0 is the value COD at time $t = 0$.

3.1.2. Sulfur removal by T. ferrooxidans from leather industry wastewater

As the *T. ferrooxidans* have the capability to oxidize the sulfur and reduced sulfur compounds [14], the removal of sulfide from this wastewater has been investigated. Maximum 78% of sulfide has been removed within 21 d of treatment, Fig. 1 indicate the *T. ferrooxidans* has potential role to remove the sulfide present in leather industrial wastewater. The initial rate of sulfide oxidation is slow and after 72 h of treatment, the rate has been enhanced. This can be due to the fact that, at the initial phase of reaction microorganism *T. ferrooxidans* oxidize $FeSO_4$ as an energy source until the total $FeSO_4$ gets converted into $Fe_2(SO_4)_3$. After that the sulfur compounds of tannery wastewater has been oxidized. The % removal of sulfide thus enhanced from 8% in 3rd d to 64% on 16th d and finally reached to 78% in 21 d of treatment compared to the control. The sulfur removal pattern is again correlating with the growth of *T. ferrooxidans*, considering protein concentration (mg/L) as biomass (Fig. 1). The electron has been absorbed by the cytochrome C_4 and rusticyanin of *T. ferrooxidans* [42] resulting in oxidation of reduced waste compound remarkably modified redox properties. H_2S gas might be produced in this process, which could be further oxidized by $Fe_2(SO_4)_3$ produced by *T. ferrooxidans*, as presented by

$$H_2S + Fe_2(SO_4)_3 \rightarrow S^0 + 2FeSO_4 + H_2SO_4 \quad (7)$$

$$2FeSO_4 + H_2SO_4 + O_2 \xrightarrow{\text{bacteria}} Fe_2(SO_4)_3 + H_2O. \quad (8)$$

3.2. Growth kinetics of biomass and waste degradation

The tannery industrial waste degradation pattern by *T. ferrooxidans* as observed from Fig. 1 is following the first-order kinetics which can be represented by $Y = Y_0 e^{-\alpha t}$. Initially the rate of degradation is high with time and then reaches to a constant value. The pattern of the waste degradation by *T. ferrooxidans* closely resembles with the growth kinetics of the bacterial cells and it follows the first order kinetics as the plot $-\ln(c/c_0)t$ vs. *t* gives a straight line (Fig. 2).

3.3. Effect of temperature in waste degradation

Various studies have shown the role of a specific temperature in micro-organism induced wastewater treatments, such as in Cr (VI) biosorption [47], COD reduction and color removal by aerobic biological treatment [48]. Temperature exceeding 60 °C has shown to reduce the activity of thermophilic bacteria [49]. In this study, the optimum temperature for COD, sulfide and total chromium removal from leather industry wastewater by *T. ferrooxidans* has been examined. The experiments were conducted at different temperatures ranging from 25 to 40 °C, keeping all other

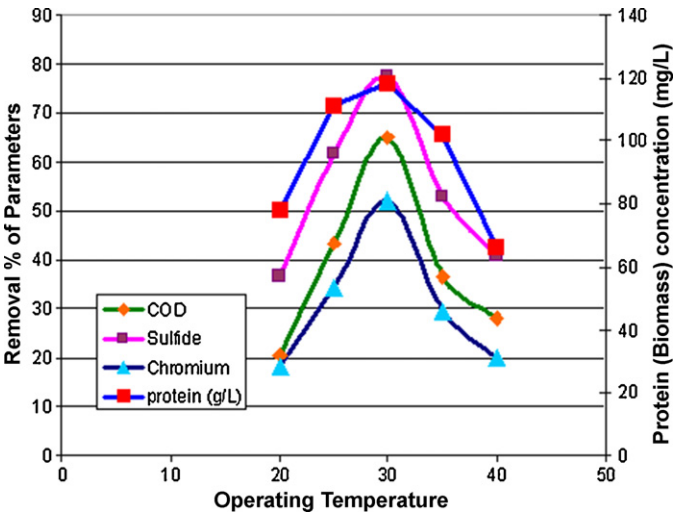

Fig. 3. *T. ferrooxidans* treatment of leather industrial wastewater (Kolkata, WB, India). Evolution of COD (mg/L), total chromium (mg/L) and sulfide (mg/L) removal in % at different pH. temperature = 30 °C, $FeSO_4$ = 6 g/L, inoculum vol. = 20%, initial COD = 2533 (mg/L).

experimental parameters at constant value (pH 2.5, $FeSO_4$ = 7 g/L, inoculum vol. = 20%, initial COD = 2533 (mg/L)).

It is observed from results (Fig. 3), the maximum percentage of COD, sulfide and chromium reduction was about 64%, 76% and 52%, respectively, at 30 °C in 21 d. At the temperature range of 25–30 °C, the removal of COD, sulfide and chromium has been increased. Further increase in temperature, i.e., 35 °C or so, showed negative effect, which is attributed to the effect on microbial growth as above 35 °C the growth is observed to decline. The protein concentration is considered for microbial growth in different temperature. It has been observed that, with increasing temperature from 20 to 30 °C, the biomass concentration increased and reached a maximum concentration of 118 mg/L at 30 °C, after that, the microbial growth has been observed to decline.

The calculation of reaction rate constant at different temperature has been tried using Hoff–Arrhenius equation. The equation is $k'_1, T = k'_{1,20}\theta^{(T-20)}$, the value of $k'_{1,20}$ was calculated from Thomas equation and $\theta = 1.135$ for temperature range 4–20 °C and 1.056 for the temperature range 20–40 °C. The results obtained from the model equation were not following the results obtained from experiments beyond 30 °C. This is probably due to the thermal shock, the micro-organisms were not capable to degrade the waste effectively. Thus Thomas model could be used for kinetic study at any temperature.

Following published report in Refs. [25,27], 30 °C temperature has been considered the optimum temperature for Fenton's treatment.

3.4. Effect of pH in wastewater treatment

Highly acidic and basic environment in the reaction medium increases the toxicity to the microorganisms [50,51]. An optimum pH is essential for any microbe to grow and show its maximum activity. Most of the microorganism cannot tolerate pH levels above 9.5 or below 4.0. However, the optimum pH for different organisms lies in different range. It is reported that *T. ferrooxidans* normally grow in acidic pH from 2.5 to 3.5 and it can be adopted to pH 1–4. Thus the effect of pH on COD, sulfide and chromium removals by *T. ferrooxidans* has been investigated at the pH range of 1.5–4.0 at a constant temperature of 30 °C. The results, presented in Fig. 4, showed that the removal of COD, sulfide and total chromium is maximum at the pH of 2.5, below and above which, the waste degradation is not significant. The optimum pH for COD, sulfide and chromium removal from this leather industrial wastewater is thus 2.5.

In Fenton's treatment maximum waste removal has been observed in the pH range of 3–4. At the pH below 2.0, the waste degradation has been reduced, probably due to the formation of $[Fe(H_2O)_6]^{2+}$ and $[H_3O_2]^{2+}$ [52]. On the other hand at pH above 4.0, the insignificant waste degradation observed could be due to the formation of Fe $(OH)_4$ which is known to reduce the production of hydroxyl radical.

3.5. Effect of $FeSO_4$ concentration in wastewater treatment by *Thiobacillus ferrooxidans* and Fenton's treatment

The role of $FeSO_4$ in *T. ferrooxidans* induced leather industry wastewater degradation in terms of COD, conductivity and salinity removal were examined, by varying the $FeSO_4$ concentration from 4 to 20 g/L in the reaction mixture. The results show that %COD reduction with 4 g/L $FeSO_4$ is about 55% and it is increasing with increasing $FeSO_4$ concentration of up to 14 g/L (75% COD reduction). The increase Fe^{2+} ion concentrations influenced the oxidation of the waste by *T. ferrooxidans*, because of the generation of more Fe^{3+} which can act as an oxidizer as well as coagulant to the waste [25,26]. Above 14 g/L $FeSO_4$, there is no significant change in %COD reduction. In case of conductivity removal the reverse trend has been found at and above 10 g/L of $FeSO_4$ concentration. This can be due to the presence of high amount of un-reacted $FeSO_4$ and/or excess ferrous to ferric conversion by *T. ferrooxidans,* which lead to increase the conductivity and salinity in the reaction medium.

In Fenton's treatment with increasing $FeSO_4$ concentration from 1.5 to 6 g/L, the %COD removal has been found to increase at pH 3.5, but at above 6.0 g/L, there is no significant change in the %COD removal. At more than 10 g/L $FeSO_4$ the %COD removal has been found to decrease. Therefore 6 g/L $FeSO_4$ has been considered as an optimum concentration for Fenton's treatment in the present study. The inorganic sludge production is known to increase with increasing $FeSO_4$ concentration. Thus an optimum $FeSO_4$ concentration should be chosen for minimum sludge production. It has been observed that, Fenton's oxidation can remove 78% salt and conductivity 50% in 30 min at pH 3.5 with H_2O_2 111 g/L and $FeSO_4$ 6 g/L. But pH lower than 2.0 and above 4.5, the conductivity removal has been observed insignificant due to the formation of other complex species, hindering hydroxyl radical generation and subsequently interfering in waste removal.

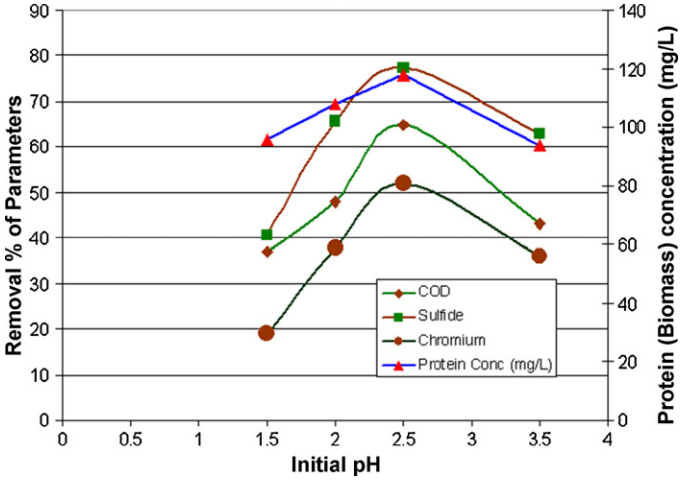

Fig. 4. Treatment of leather industrial wastewater (Kolkata, WB, India) by *T. ferrooxidans*. Computed kinetic parameters (K_1, L_u) from Thomas model. *Conditions*: $FeSO_4$ = 7 g/L, pH 2.5, temperature = 30 °C, inoculums vol. = 20%, initial COD = 2533.

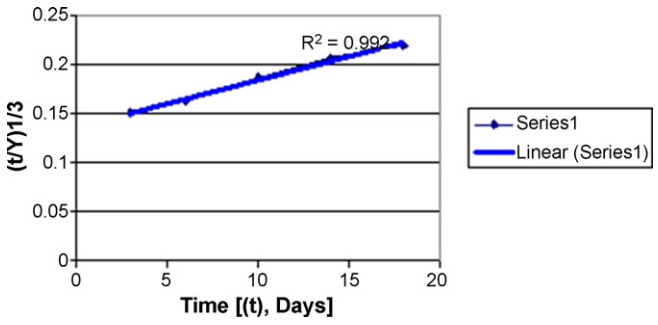

Fig. 5. *T. ferrooxidans* treatment of leather industrial wastewater (Kolkata, WB, India). Evolution of kinetic parameters from Monod equation and Michaelis–Menten equation. *Conditions*: $FeSO_4$ = 7 mg/L, pH 2.5, temperature = 30 °C, inoculums vol. = 20%, initial COD = 2533.

3.6. Effect of inoculums size of *T. ferrooxidans* in wastewater treatment

Microbial inoculum's volumes play a key role in process industry [53,54]. To find out the optimum volume of inoculums for economic treatment, five inoculums sizes in the range of around 10–50% were used for the wastewater treatment. The results indicate that with 10% and 20% inoculum's size, the COD removals were 50% and 64.80%, respectively and above 20% inoculums size there is no significant change in COD removal. So 20% inoculums size can be considered optimum for maximum treatment efficiency.

3.7. Kinetic study on wastewater treatment by *T. ferrooxidans*

3.7.1. Calculation of kinetic parameters

Thomas equation is used to calculate the kinetic parameters for wastewater treatment by *T. ferrooxidans*. The COD removal experimental data has followed the first order kinetics, thus Thomas model seems to be the appropriate one for calculation of kinetic parameters.

A straight line was obtained when $(t/Y)^{1/3}$ vs. t is plotted following the Thomas equation [55]

$$\left(\frac{t}{Y}\right)^{1/3} = (2.3K_1'L_u)^{-1/3} + \left[\frac{(K_1')^{2/3}}{3.43(L_u)^{1/3}}\right] t \quad (09)$$

From the slope and intercept of the straight line (Fig. 5), the kinetic parameter K_1', the reaction rate constant and L_u, the original concentration of the organic material (before any biological action has occurred) are found 0.093/d, 2540 mg/L, respectively.

In the present study Monod equation is tried to calculate the kinetic parameters in the *T. ferrooxidans* induced wastewater treatment, as has been used in many bioremediation cases [31]. Considering protein concentration and COD as microbial and substrate concentration respectively, the experimental data were putted in Eq. (11) to construct the straight line (Fig. 6).Monod equation:

$$\mu = \frac{\mu_{max}S}{K_S + S}, \quad (10)$$

This equation can be presented as

$$\frac{1}{\mu} = \frac{K_S}{\mu_{max}S} + \frac{1}{\mu_{max}} \quad (11)$$

As *T. ferrooxidans* mediated waste degradation is not fitting properly with Monod kinetics in this case. The use of Michaelis–Menten type rate model equation for kinetic study of substrate conversion by enzymes as well as living cells to understand the system function has been tried [56]. In the present study, cytochrome *C* and enzyme rusticyanin of *T. ferrooxidans* play a key role in the ferrous and waste oxidation, so Michaelis–Menten equation has been tried to calculate the kinetic parameters using Eq. (13).

Michaelis–Menten equation:

$$V = \frac{V_{max}S}{K_m + S}, \quad (12)$$

Rearranging the equation [14] we have,

$$\frac{1}{V} = \frac{K_{max}}{V_{max}S} + \frac{1}{V_{max}}. \quad (13)$$

The result in Fig. 6 is indicating that it is not following Michaelis–Menten kinetics too. From this it can be concluded that *T. ferrooxidans* mediated waste degradation is operating not only through biooxidation pathway but also via chemical oxidation through Fe^{3+} ions generated by the microbes. Probably due to this interference, the experimental data could not fit with Monod equation or Michaelis–Menten type kinetic model. Thus the rate constant obtained from Thomas model can only be accepted in this system.

3.8. Efficiency of Fenton's oxidation in wastewater treatment

The sole aerobic bio-treatment of leather industrial wastewater by *T. ferrooxidans* is an efficient process but it is a time consuming, as it took 21 days for 64% COD reduction as revealed from Fig. 1. The reasons could be presence of highly toxic and complex structures of chemicals like tannins present in the tannery wastewater with chromium, sulfide and ammonia, thus requires a longer period of time for oxidation [57]. So attempts have been made to see the efficiency of the Fenton treatment as a pretreatment step to reduce the toxicity in leather industrial wastewater by transforming constituents to by-products that are more readily biodegradable and reducing overall toxicity to micro-organism [58], so that, time could be reduced in *T. ferrooxidans* induced biodegradation.

The %COD, BOD, salinity and color removal by Fenton's reagent with $FeSO_4$ (6 g/L) and varying concentration of H_2O_2 from 44.40 g/L to 266.40 g/L has been studied at pH 3.5, temperature 30 °C and treatment time of 30 min. The % removal of COD, BOD, sulfide and color has been found to increase with increasing H_2O_2 concentrations (Fig. 7). Fenton's reagents with 6 g/L $FeSO_4$ and 111 g/L H_2O_2 has removed BOD, COD, color and salinity up to 46%, 40.5%, 65%, 75%, respectively. Increase in H_2O_2 dose at 266.6 g/L attained maximum 68.40% COD removal at the same experimental condition. This indicates that, there is only 1.7 time increase in %COD reduction by increasing the H_2O_2 dose about 2.3 times. So,

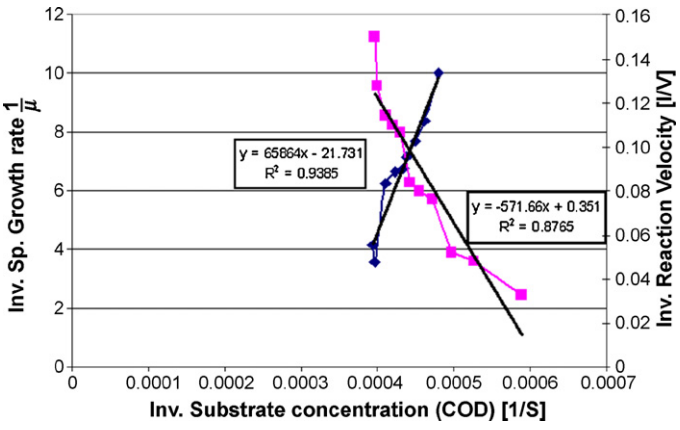

Fig. 6. Removal % of COD, color, and BOD in Fenton's treatment of leather industrial wastewater (Kolkata, WB, India), influence of H_2O_2 concentration. *Conditions*: pH 3.5, temperature = 30 °C, conc. $FeSO_4$ = 6 g/L, initial COD = 2533 mg/L and time of reaction = 30 min.

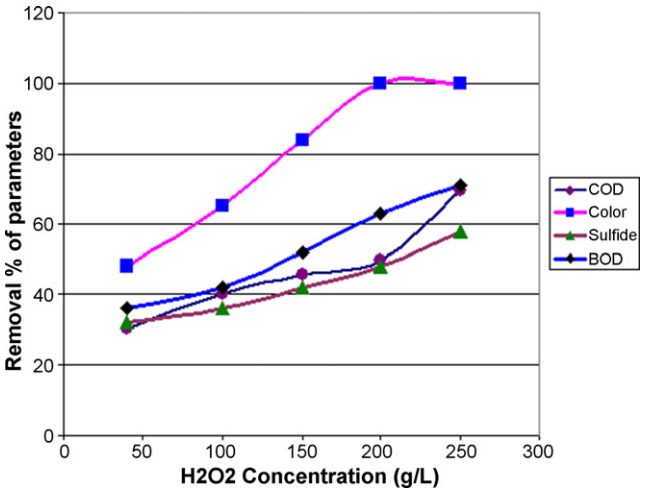

Fig. 7. Removal % of COD, BOD, sulfide, color, salinity and total chromium in different treatment process, biochemical treatment by *T. ferrooxidans*, chemical treatment of Fenton's treatment and conjunction of Fenton's treatment followed by biochemical treatment to leather industrial wastewater (Kolkata, WB, India). *Conditions*: pH 2.5–3.5, temperature = 30 °C, concentration of H_2O_2 = 111.10 g/L, $FeSO_4$ = 6 g/L, initial COD = 2533 g/L and time of reaction = 30 min for Fenton's treatment and three d (72 h) for biochemical treatment.

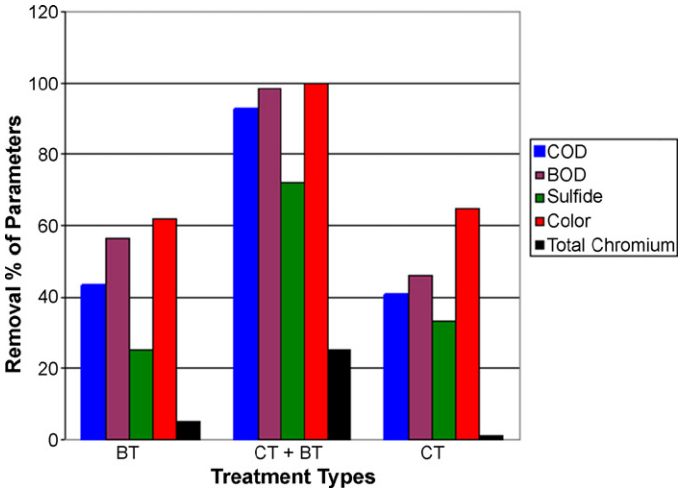

Fig. 8. Removal % of COD, BOD, Sulfide, Color, and total chromium in different treatment process, biochemical treatment(BT) by *T. ferrooxidans*, chemical treatment(CT) by Fenton's treatment and conjunction of Fenton's treatment followed by biochemical treatment(CT+BT) to leather industrial wastewater (Kolkata,WB, India).

keeping the treatment cost in mind Fenton's treatment with 111 g/L H_2O_2 and 6 g/L $FeSO_4$ has been selected for combination treatment of this wastewater.

3.9. Spectrophotometric analysis for removal of organic compounds by Fenton's reagent

It is seen that original wastewater have high absorbance at three particular wavelength of 280, 350 and 470 nm. Among these the absorbance is maximum at 350 nm, signifying the high load of organic matter. After Fenton's oxidation there is a continuous decrease in absorbance at 280, 350 and 470 nm. This indicates the Fenton's oxidations strength in removing the organics present in the leather industry wastewater. After coagulation stage, the absorbance is less comparing to oxidation stage, suggesting that the remaining organics are removed during coagulation stage too.

3.10. Combined treatment of Fenton's reagent and T. ferrooxidans

Fenton's reagent and *T. ferrooxidans* when used separately showed the potential in removing waste efficiently from the leather industry wastewater but both the treatment has some limitations. The advanced oxidation process by Fenton's reagents is costly and the biochemical treatment by *T. ferrooxidans* is too much time consuming. So the next experiment was designed as a combination treatment to overcome the limitations of both the processes. The Fenton's treatment with a particular concentration of H_2O_2 111 g/L and $FeSO_4$ 6 g/L at 30 °C and pH 3.5 for 30 min, followed by *T. Ferrooxidans* treatment for 3 d (72 h) at pH 2.5 and temperature of 30 °C showed a synergistic effect in waste degradation (Fig. 8). The COD, BOD, color, salinity, sulfide and chromium has been removed up to 40.44%, 46%, 65%, 78%, 33% and 5%, respectively, in 30 min by Fenton's treatment alone. Probably the biodegradability has been improved in the system [29], thus microbial growth has been enhanced, as a result within 3 d of *T. ferrooxidans* induced treatment, the COD, BOD, color, salinity, sulfide, and total chromium removal has reached to 92%, 98%, 100%, 88%, 72% and 23%, respectively. Compared to the 14% COD and 7.0% Cr removal in 72 h of *T. ferrooxidans* mediated treatment alone, the combined treatment seems to be more efficient in removing the pollution load in leather industry wastewater. It can be said that, Fenton's pretreatment to the wastewater is stimulating %COD reduction by *T. ferrooxidans*, 6.5 times more compared to the *T. ferrooxidans* treatment alone in 3 d. Compared to the Fenton's treatment alone, the combined treatment showed synergistic effect on overall pollutants removal. Although this combined treatment appears the effective one in this study, it produce huge inorganic sludge containing ferrous ferric oxides, jarosite, which is a porous inorganic substance with a general chemical formula $AFe_3(SO_4)_2(OH)_6$ [where A can be K^+, Na^+, NH_4^+ or H_3O^+] [46]. The amount of sludge production depends on the dose of Fenton's reagents used for the treatment. About 4.56 g (dry wt.) sludge was produced, when 1 L wastewater was treated with Fenton's reagents with H_2O_2 111 g/L and $FeSO_4$ 6 g/L. In this study diluted leather industrial wastewater was used, thus, the treatment of original wastewater, more amount of Fenton's reagents will be required to make it biodegradable, which may lead more sludge production. In combination treatment, sludge production can be minimized by reusing the $FeSO_4$ during biochemical treatment with *T. ferrooxidans*. The cost of the treatment with Fenton's reagents alone to achieve the 92% COD removal can be reduced by using lower dose of H_2O_2 in combined process. In combined treatment about half dose of H_2O_2 is enough for attaining the same percentage COD removal (92%). From the results obtained, it can be said that, the combined treatment can be considered as a better method for tannery wastewater treatment.

4. Conclusions

The present study provided significant information regarding the degradation of tannery wastewater in terms of COD, BOD, color, salinity, sulfide and total chromium by microbial treatment with *T. ferrooxidans* alone, AOP (Fenton's reagents) alone and in combination treatment. The combination treatment (AOP followed by *T. ferrooxidans*) showed synergistic effect in reduction of different pollution parameters studied. *T. ferrooxidans* showed maximum 77% COD removal in 21 d treatment, whereas in combination treatment, the %COD removal has enhanced up to 93% in 3 d only. This indicates the pretreatment with Fenton's reagent is stimulating the biodegradability and reducing the treatment time by *T. ferrooxidans*. Another important finding in combined treatment is the reduction of use of H_2O_2 comparing to Fenton's treatment alone with enhanced efficiency in degradation of waste. The sludge production by Fenton's treatment alone can also be reduced in combination

treatment. The overall results indicate that the combined treatment has the potential to be used as an efficient, economic and partial ecofriendly method for the removal of pollution load from leather industry wastewater.

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