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INFLUENCE OF FENTON OXIDATION AND BIODEGRADATION ON TOXICITY OF TANNERY INDUSTRIAL EFFLUENT

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ABSTRACT

The main objective of this study was to treat the common effluent treatment plant (Tannery effluent) and to evaluate the pollutant removal efficiency in terms of COD reduction by both Advanced Oxidation Process and Biological treatment using bacterial consortium. More efficient Fenton's oxidation process was carried out as a pre-treatment and tried to reduce COD at different pH (2-10) with varying Fe_2SO_4 doses (100-300 mg/L) and H_2O_2 doses (400-800 mg/L). This pre-treated effluent sample was then biologically treated with monoculture and mixed culture of bacterial strains. The efficiency of mixed consortium achieves 82% of COD removal in 6% saline tannery effluent. Monod, Moser and Logistic models were used to fit the microbial growth in which the experimental data fits better in Logistic equations. Phytotoxicity studies show that the seeds of *Vigna radiata* grown on biologically treated effluent experience no adverse effects and evident 95% germination rate. Thus this treated effluent could also be utilized for agricultural irrigation purposes.

KEY WORDS: Tannery effluent, Advanced Oxidation Process, Mixed consortium, Growth models, Phytotoxicity.

INTRODUCTION

Leather industries are so far creating obvious impacts on socioeconomics through various employment establishment and export earnings. As the industry operates with plentiful of water sources and chemicals, it attains a negative image with an unresolved environmental issue concerning the safe disposal and treatment of this toxic effluent containing acids, salts, surfactants, dyes, and tanning agents characterized by its dark brown appearance and highly odorous nature. This comprise both inorganic as well as organic environmental toxicants which harms the significant process of food web by influencing potential risks in the activities of flora and fauna (Pandi *et al.,* 2019; Ramesh Raja and Suresh, 2011). This likely deserves an exclusive attention as it persists for longer period of time in environment and poses inherent risks to human health (Chowdhary *et al.,* 2017). A huge number of researchers has been striving to treating and converting this tannery sludge in to a valuable resource.

The tannery sludge was disposed by landfills till 1998 and was then banned by international convention (Tizaoui *et al.,* 2007). Alternatively, other methods such as burning, composting and solidification were employed as potential disposal methods. The effective detoxification of tannery effluent was achieved by burning by reducing the amount of sludge formation yet toxic gases are generated and released to the ambient atmosphere (Chowdhary *et al.,* 2018). Composting was then extensively employed in the past decades by transforming the organic compounds in waste water sludge into plant nutrients (Keluskar *et al.,* 2019). Unfortunately, this results in concentration of heavy metal accumulation in plants and again negatively impacts in human health. Although this heavy metal incorporation were stabilized by different clays, bricks or cements, lack of implementation knowledge and skills pose harmful effects to human and environment (Bhattacharya *et al.,* 2014; Oller *et al.,* 2011).

In our study, the suitable technology we have chosen for permanent solution to treat the tannery effluent was advanced oxidation process (AOP). This produces non reactive radicals which are non specific in nature and could adapt to extensive organic pollutants in different effluents (Punzi *et al.,* 2015; Aravind *et al.,* 2016). Among various AOP technologies, Fenton process was selected for the pretreatment step as it generates high reactive hydroxyls faster than ozone process. This offers partial mineralization that leads to generation of secondary pollutants that rely on successive treatment process (Tamal *et al.,* 2010). Therefore treatment of this effluent by a simple one step appropriate process is unrealistic and demands different combo techniques for an efficient and significant treatment process. For this we developed an eco friendly biological treatment process as a viable method with the seeded bacterial consortium to treat the tannery effluent. The effect of Fenton pretreatment process as well as biological treatment with halo-tolerant bacterial strains and their kinetic performance to best fit the model that suitably match with the experimental data was also investigated. The treatment was further endorsed by biochemical characterization of treated effluent and the treatment efficacy was validated by phytotoxicity study through seed germination (Lefebvre *et al.,* 2006).

MATERIAL AND METHODS

Chemicals and Reagents

The chemicals and solvents used in our experimental studies are analytical grade and were purchased from Loba Chemie Pvt. Ltd., Mumbai, India. The media used in the microbiological treatment process were purchased from Hi-Media Pvt. Ltd., Bangalore, India. Waste water effluent used in the study was collected from commercial tannery plant, Chennai, India. Characteristics of untreated tannery effluent were evaluated using standard methods and were given in Table 1.

Table 1. Raw composition of tannery effluent

Parameter	Composition
pH	10.26 ± 3.17
TDS (mg/L)	200 – 850
TSS (mg/L)	15000 – 28000
BOD (mg/L)	150-1600
COD (mg/L)	960- 3200
Chlorides (mg/L)	1270-1940
Sulphates (mg/L)	1225-1860
Sodium (mg/L)	3400-7450
Potassium (mg/L)	900-1180
Calcium (mg/L)	100-178
Magnesium (mg/L)	200-240

Fenton pre-treatment

Fenton oxidation was carried out as pre-treatment process in order to increase the biodegradability of tannery effluent. For Fenton process, 100 mL of effluent sample was treated with different concentration of Fe^{2+} ions (100-300 mg/L) with different molar ratios of H_2O_2 at various pH and COD was examined at different intervals. As the alkaline condition oxidizes the H_2O_2, the pH of the medium was maintained in the range of 3-4 by external addition of formic acid as the activity of Fenton's reagent is high at acidic pH (Kavitha and Palanivelu, 2005). After this step, the pH of the medium was maintained to neutral. These pre treatment experiments were carried out at 500 mL conical flasks for the period of 120 min. The treated effluent was then filtered through a fine filter and the obtained filtrate was transferred for biological treatment process.

Bacterial strain

In general, the tannery effluent contains high salt content that impedes the regular biological treatment. Therefore biological treatment of such adverse effluent needs the utilization of halo-tolerant microbial species. In our study, we choose three halo-tolerant bacterial strains namely *Pseudomonas putilda* (PP), *Bacillus flexus* (BF) and *Micrococcus luteus* (ML) obtained from MTCC, Chandigarh and were sub cultured from corresponding glycerol stock. The single pure colony was then isolated and incubated at 37 °C for 24 h at 100 rpm. For mixed culture reaction, exponential phase of the inoculum was further grown for a period of 36 h in an agar media at a temperature of 37 °C consisting of 20.50 g/L glucose, 10 g/L peptone, 1g/L yeast extract, 15g/L agar, 5g/L NaCl, 3 g/L $MgSO_4.7H_2O$, 1 g/L $CaCl_2.2H_2O$. The sub culture was grown in an orbital shaker incubated at 35 °C for 36 h at 120 rpm with nutrient broth in same media composition. In this study, the growth pattern of the mixed consortium was analyzed using spectrophotometer at 600 nm.

Biological Treatment

A loopfull of bacterial culture obtained from the above mentioned mother culture was inoculated to 100 mL of tannery effluent sample taken in 500 mL conical flask and incubated at 35 °C and 200 rpm for 48 h. In the mean time, aliquots of effluent sample were withdrawn periodically at the interval of 10 h for estimation of COD. A control system without addition of extraneous microbes was run parallel to examine the role of native microbes in the reduction of COD.

Growth kinetics model analysis

The rate of COD removal depends on the rate of microbial growth and so different mathematical models have been exploited in this study to identify the bacterial specific growth rate and substrate utilization. The Monod model, Moser model as well as Logistic model was applied to analyze the bacterial growth kinetics and their equations were as follow:

$$\mu = \frac{\mu_{max} S}{K_s + S} \quad .. (1)$$

$$\mu = \frac{\mu_{max} S^n}{K_s + S^n} \quad .. (2)$$

$$X = \frac{x_0\, e^{\kappa t}}{1 - x_0/x_c(1 - e^{kt})} \quad .. (3)$$

where, μ is the specific growth rate (h^{-1}), μ_{max} is the maximum specific growth rate (h^{-1}), K_S is the substrate saturation constant and S is the concentration of the substrate (g/L), n is the degree of inhibition, t is the time, X_o is the initial biomass concentration (g/L), k is the carrying capacity coefficient(h).

Phytotoxicity study

Pre-treated, biologically treated and untreated effluent samples were examined for phytotoxicity study against *Vigna radiate* collected from local market. 50 g of seeds were soaked in respective test samples as well as distilled water (control) for 5 h and then allowed to germinate on 1% agar in dark condition. After 1 week of incubation, the root and shoot length as well as % germination were evaluated and all the experiments were done in triplicates (Chattaraj *et al.,* 2016).

RESULTS AND DISCUSSION

Fenton Pre-treatment of tannery effluent

Fenton oxidation studies were done at room temperature with pH of 4 at different molar concentration of $FeSO_4$ and H_2O_2 (1:4, 1:8, 1:12). Also the effects of pH, concentration of $FeSO_4$ and H_2O_2 were optimized for efficient reduction of COD in the effluent sample. The COD reduction using Fenton's reagent was constrained to dosage of iron catalyst and H_2O_2 in the sample and the oxidation is highly effective in the effluent consisting COD of more than 500 mg/L (Lalwani and Devadasan, (2013). At optimal concentration of ferrous ion and H_2O_2, reduction in COD level is principally due to the formation of intermediate oxidizing by-products that traps the OH radicals (Lofrano *et al.,* 2013).

Effect of pH

As ferrous ions and H_2O_2 were the major speciation factors in the Fenton's process, the oxidation efficacy of hydroxyl radicals decreases with increase in pH (Iyyappan *et al.,* 2019). This was also due to the formation of FeOH precipitate and inactive iron oxo hydroxides (Wu *et al.,* 2010). At relatively low pH, the Fe complex species reacts at moderate rate with H_2O_2 and forms stable oxonium ions in the presence of huge concentration of H^+ ions. Therefore at both high and low pH, the efficacy of the Fenton's reagent to degrade the organic pollutants reduces significantly (Das *et al.,* 2018). The observation shows that the optimum pH for the Fenton pre-treatment process was found to be around 4 and this was maintained for further studies.

Effect of ferrous ion concentration

As the dosage of ferrous sulfate plays a crucial role in the oxidation process as well as COD reduction, the effect of Fe ions in the process was studied with varying concentration of $FeSO_4$ from 100 – 300 mg/L. It was observed that the rate of COD removal was significant with increase in ferrous ion concentration up to 250 mg/L. Further increase in the ferrous ion dosage results in precipitation of iron salts that increase additional total dissolved solid content of the effluent (Senthilkumar *et al.,* 2008). Thus the present study was done at optimal concentration of $FeSO_4$ (250 mg/L) at pH 4 (Fig. 1).

Effect of hydrogen peroxide dosage

The dosage of H_2O_2 determines the overall performance of Fenton degrading treatment. The

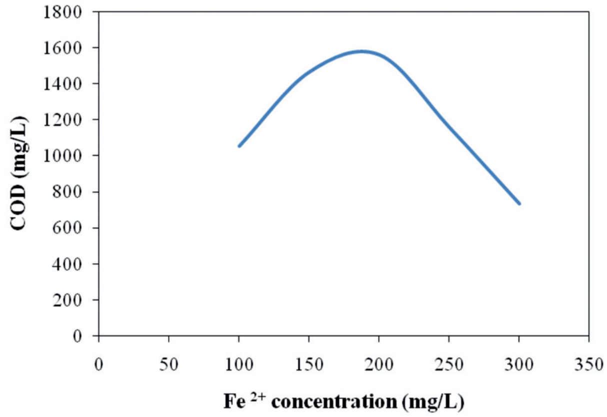

Fig. 1. Effect of ferrous ion concentration in treating tannery effluent (Fe^{2+} conc. =100–300 mg/L,H_2O_2 conc. =600 mg/L at pH 4.)

effect of H_2O_2 concentration was studied with varying concentration from 400-800 mg/L. The observed results shows that the rate of COD reduction increase with an increase in H_2O_2 concentration up to 600 mg/L and further increase leads to increase in COD due to the contribution of unused hydrogen peroxide in the reaction (Zhou *et al.*, 2018). Therefore the H_2O_2 concentration should be optimized in such a way that no H_2O_2 is left after the effluent treatment and optimized concentration of H_2O_2 in our study was found to be 600 mg/L. The effect of COD removal at different ratio of Fe ions with optimized H_2O_2 dosage was also carried out (1:4, 1:8, and 1:12).

Biological treatment of tannery effluent

Biological treatment of Fenton pre-treated tannery effluent were studied at batch experiments in shaking flask with pure monoculture of *Pseudomonas putilda* (PP), *Bacillus flexus* (BF), *Micrococcus luteus* (ML) and their mixed consortia at optimal conditions of 2% v/v inoculum dosage at 37 °C at 200 rpm in neutral pH. As tannery effluent is highly saline, batch experiments with varying salt concentration 2-12% (w/v) was studied. Experiments were carried out until 80% removal of COD was achieved and for this purpose samples were withdrawn at regular intervals to analyze COD level. Each set of experiments were replicated three times. Fig. 2 depicts the COD removal of tannery effluent with 2% salt concentration and *P. putilda* showed higher degree of biodegradation of low saline tannery effluent. The results of COD reduction obtained in 4% saline effluent also show *P. putilda* to be an ideal strain in biodegradation of tannery waste water. Higher COD removal of about 80% was recorded compared to other strains and mixed consortia (Fig. 3). In both 2 and 4% salt concentration, mixed consortia performs no significant removal of COD in effluent sample. This may be due to the competition for early adaptation at minimal saline concentration of effluent. The efficiency of mixed consortium increases in COD removal up to 82% in 6% saline effluent as shown in Fig. 4. This observation shows that the mixed consortia could be potentially utilized for bio augmentation of tannery effluent with moderate saline concentration. The result of the analysis reveals that *Micrococcus luteus* exhibits higher removal of COD (up to 86%) at 8% saline effluent whereas mixed consortium shows only 80% removal of COD under same condition (Fig. 5). The individual efficiency of *Micrococcus luteus* was inhibited if it was grown in mixed consortium due

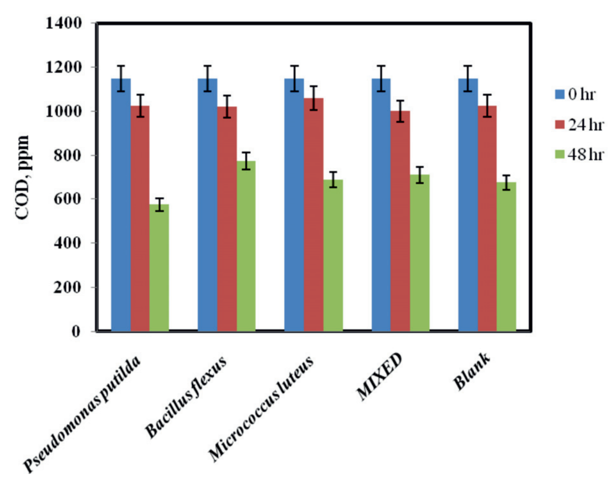

Fig. 2. COD reduction at 2% salinity ratio (T = 35 °C; pH = 7; RPM = 200)

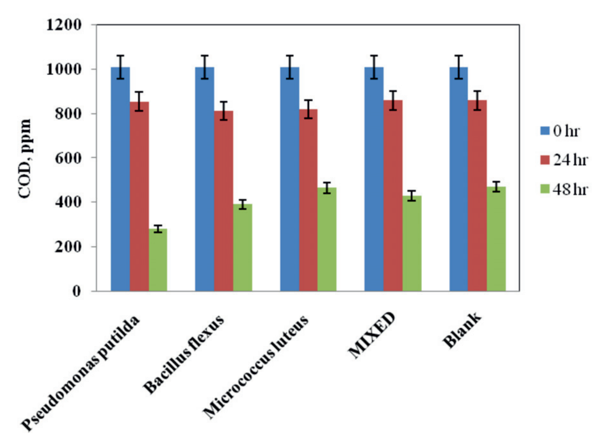

Fig. 3. COD reduction at 4% salinity ratio (T = 35 °C; pH = 7; RPM = 200)

to substrate competition. Fig. 6 shows a sudden fall (>55%) noticed in all pure as well a mixed cultures when the salinity increased up to 10% in sample. This indicates that very minute increment of salt dosage in the effluent sample could impact adverse effects on the treatment efficacy. Fig. 7 implies both *Micrococcus luteus* and mixed consortia shows similar percentage of COD removal i.e., up to 50% at 12 % salinity effluent sample. As per our study, the salt concentration of pre treated tannery effluent exhibits between 2 to 6% and rarely exceeds 6% and therefore the reported mixed consortia of our study could be potentially employed in treating the tannery effluent significantly. Fig. 2 - 7 shows that the effect of indigenous microbial strains in the effluent (blank) in COD removal was not a beneficial option.

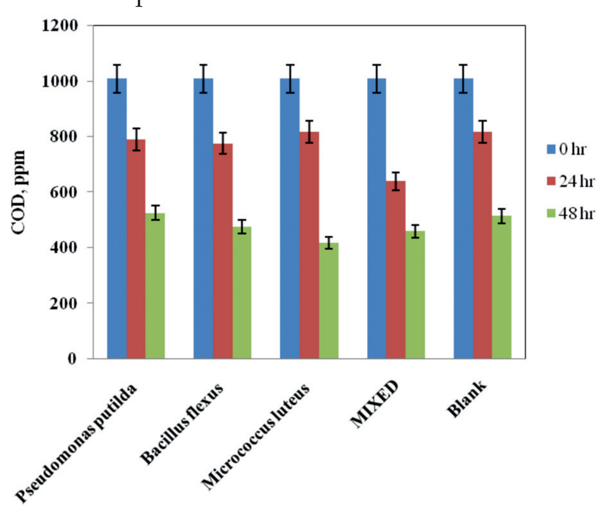

Fig. 4. COD reduction at 6% salinity ratio (T = 35 °C; pH = 7; RPM = 200)

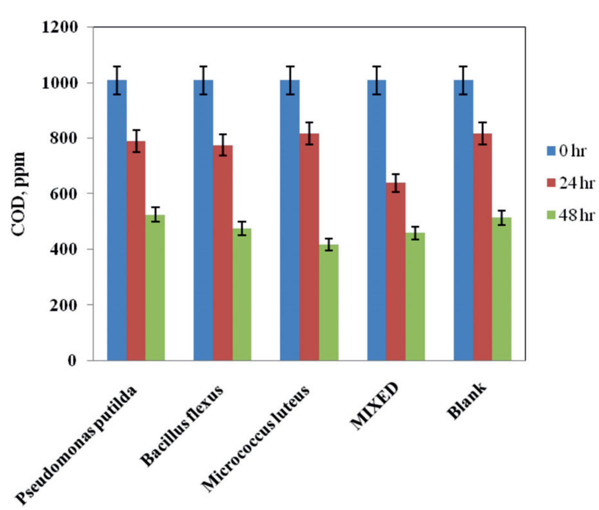

Fig. 5. COD reduction at 8% salinity ratio (T = 35 °C; pH = 7; RPM = 200)

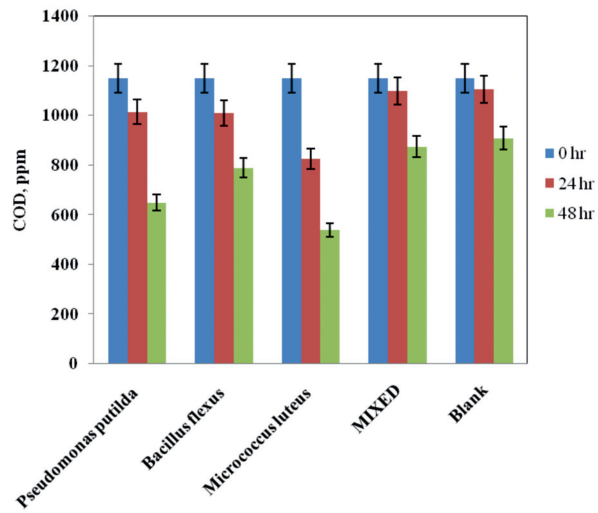

Fig. 6. COD reduction at 10% salinity ratio (T = 35 °C; pH = 7; RPM = 200)

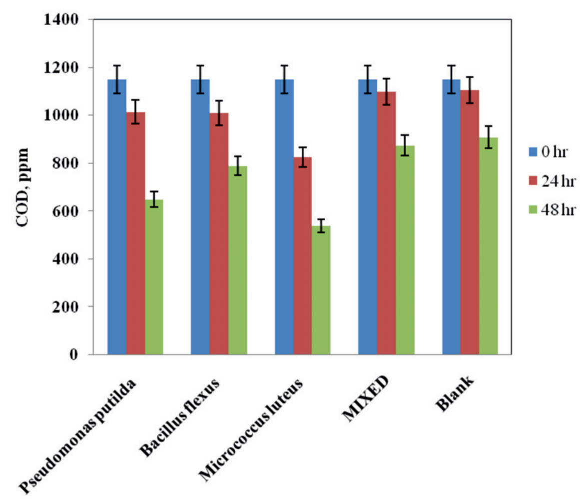

Fig. 7. COD reduction at 12% salinity ratio (T = 35 °C; pH = 7; RPM = 200)

Growth kinetics model analysis

A growth study of mixed consortium was performed in optimal growth conditions. The experimental data was fitted with the above three models according to equation 1, 2 and 3. The estimated kinetic parameters of the three growth models were tabulated in Table 2. The values achieved from Logistic model were observed to be precise to the experimental value and the plots were shown in Fig. 8. The Logistic model best fits with observed data and posses' high R^2 value (0.9682) and low root mean square error (RMSE) values. The maximal biomass concentration of mixed bacterial consortium was 3.45g/L with carrying capacity coefficient 0.173 h^{-1}.

Table 2. Growth kinetic parameters of mixed culture for efficient COD removal in tannery effluent

	μ_{max}	K_S	R^2	RMSE	% Error
Monod	0.163	29.26	0.825	0.0189	2.14
Moser	0.154	79.2	0.842	0.0196	-0.84
Logistic	0.173	-	0.968	0.0133	-

Table 3. Phytotoxicity study on *Vigna radiate* with treated and untreated tannery effluent by mixed consortia

	Distilled water	Untreated Effluent	Treated Effluent
Germination rate (%)	100	30	95
Height (cm)	24.2 ± 1.4	10.1 ± 0.2	20.5 ± 1.6
Root length (cm)	9.50 ± 0.3	3.74 ± 0.6	8.41 ± 0.4
Shoot length (cm)	14.6 ± 0.8	7.0 ± 0.7	12.2 ±0.2

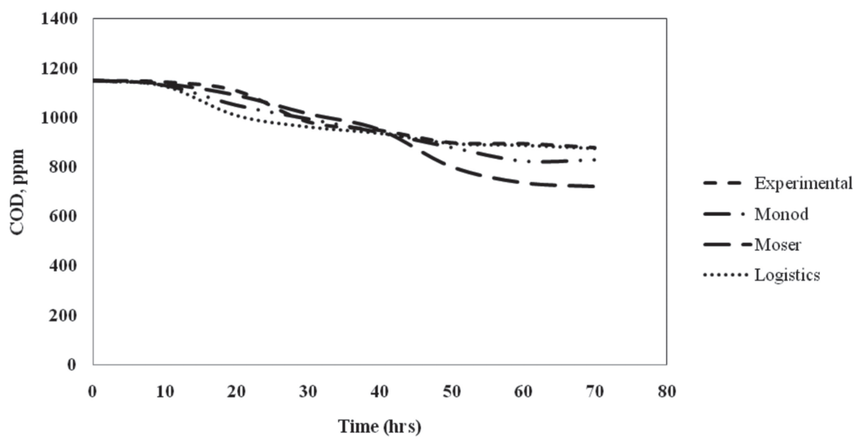

Fig. 8. Results of fitting of Monod, Mosser and Logistic model in determining the growth rate of mixed consortia.

Phytotoxicity study

The toxic quality of tannery effluent is due to the presence of various harmful chemical constituents and has been greatly reduced by both Fenton and biological treatment process. Thus the impact of treated waste water on plants to explore the reusability possibilities of this waste water for irrigation purpose was examined by seed germination process (Veeramalini *et al.,* 2019). In our study, the relative sensitivity of *Vigna radiate* was investigated against both untreated and treated waste water and was shown in Table 3. Exposure of *Vigna radiate* seeds in untreated effluent showed reduced growth with drastically affected root and shoot length whereas 95 % germination was observed in biologically treated tannery effluent.

CONCLUSION

Commercial tannery effluent sample was characterized and pretreated with Fenton's oxidation process and the effect of pH, ferrous ion concentration plus hydrogen peroxide concentration (Fenton's reagent) were optimized and examined for COD removal. The effluent was further biologically treated with bothmono and mixed halo tolerant bacterial strains namely *Pseudomonas putilda*, *Bacillus flexus* and *Micrococcus luteus*. The efficiency of mixed consortium increases in COD removal up to 82% in 6% saline tannery effluent. The kinetics of mixed biomass growth was also evaluated to determine the best fit model and the growth data were successfully fitted with Logistic growth equation. Exposure of *Vigna radiate* seeds in both untreated effluent and biologically treated effluent which shows about 95 % seed germination that allows the possibilitiesof reusing for irrigation purpose.

ACKNOWLEDGEMENTS

Compliance with Ethical Standards

Conflict of interest

There are no conflicts of interest to declare.

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