Desalination and Water Treatment
www.deswater.com

doi: 10.1080/19443994.2012.751155

51 (2013) 3189–3195
April

Taylor & Francis
Taylor & Francis Group

A comparison of the efficacy of two strains of *Bacillus subtilis* and *Pseudomonas fragii* in the treatment of tannery wastewater

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Received 4 September 2012; Accepted 11 November 2012

ABSTRACT

Tannery wastewater treatment using two isolated micro-organisms (*Bacillus subtilis* and *Pseudomonas fragii*) has been investigated in the present study. The growth patterns, pH normalisation and the abilities of these micro-organisms to reduce the concentrations of chemical oxygen demand (COD), total suspended solids (TSS) and chloride were studied using the shake flask apparatus. The specific growth rate and biomass concentration of *B. subtilis* and *P. fragii* were observed 0.138 h^{-1} and 3.01 mg/l and 0.051 h^{-1} and 2.34 mg/l, respectively, after 84 h. Both micro-organisms normalised pH of the wastewater. COD removal efficiency for *B. subtilis* was 87.6% while that of *P. fragii* was 85.2%. For TSS, *B. subtilis* caused a reduction of 91.7% (from 876 mg/l to 73 mg/l), while *P. fragii* reduced the solid concentration from 876 mg/l to 98 mg/l (88.8%). *B. subtilis* could only achieve 48.5% reduction in chloride concentration (from 127.08 mg/l to 65.39 mg/l compared to a reduction of 54.6% (from 127.08 mg/l to 57.72 mg/l) for *P. fragii*. From the results, it can be said that the bacteria present in tannery effluents have significant potential in treatment of tannery wastewater.

Keywords: Micro-organisms; Dissolved oxygen; Tannery wastewater; Growth kinetics; Shake flask; Microbial growth

1. Introduction

Increasing rate of industrialisation leads to the generation of various forms of hazardous wastes. These wastes are composed of organics, inorganics, heavy metals and refractory materials that have to be safely disposed of [1]. The harmful effects of effluents and waste products from different industries have been reported [2,3]. The specific water use per unit of production for each industry varies from one place to another within the same country. These variations depend on the technology chosen, the climate, the water availability and many other factors [4].

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The manufacturing of animal products for human consumption (meat and dairy products) or for other human needs (leather) results in the production of waste. For the leather industry, tanning is the key operation that transforms raw skins into leather [5]. The tanning industry is said to be one of those industries with the highest toxic intensity per unit of output [6]. For every tonne of hide produced, about 300kg of different chemicals are added [7]. Some of the chemicals include surfactants, tannins, sulphonated oils, chromium salts, biocides, acrylic resins, auxiliaries, organic acids, solvents, including ammonium salts, dyes, chloride, and sulphide salts, bases and organic and inorganic compounds like natural and/or synthetic tannins acids and polyphenolic compounds [8]. Water consumption is about 30–40 m^3/tonne of processed hide [9] while some authors reported that about 30–35 l/kg hide of wastewater is generated [10].

Tannery effluent contains high concentrations of suphonated polyphenols, fatty acids, aromatic and aliphatic ethoxylates, chlorides, dyes, sodium sulphide, aliphatic sulphonates, soluble hydrocarbons, sulphates, chromium, proteins and acrylic acids condensates [3,11–14]. The wastewater, therefore, has complex characteristics [15]. In 2004, it was reported that global processing capacity of hides and skins was 9×10^9kg and effluent generated was estimated to be $30–40 \times 10^{10}$l [16]. Similarly, coloured effluent discharge into the environment, apart from being aesthetically unpleasant, also impedes light penetration, reduces the quality of the receiving streams in terms of reduced dissolve oxygen and may be toxic to the treatment processes, to food chain organisms and to aquatic organisms [17].

Different conventional treatment methods for tannery wastewater have been investigated and proposed. These include wet air oxidation [18], coagulation [19], electrocoagulation [13,20], coagulation–electrocoagulation [11], electrochemical oxidation [21–24] and coagulation–flocculation [25]. The use of lime, bittern and activated carbon in tannery wastewater post-treatment has been investigated [26] while the optimisation of coagulant dosage in tannery effluent treatment has also been explored [5]. These conventional methods of tannery wastewater treatment are being gradually replaced by biochemical and biotechnological systems because conventional systems are considered very expensive in terms of energy and reagents consumption [27]. In contrast, biological wastewater treatment has the advantage of low capital and operating costs, operational flexibility, reduction of aquatic toxicity, ability to oxidise wide variety of organic compounds, energy saving and are environmentally safe [1,15]. Biological treatments include mixed culture [15], sequential batch reactor (SBR) [28,29], activated sludge process (ASP) [30] and upflow anaerobic sludge blanket (UASB) [31].

Combination treatment includes aerobic biological–Fenton oxidation process [32], SBR–chemical oxidation [33], activated sludge process–reverse osmosis [34], advanced oxidation–sequential batch [14], membrane bioreactor–conventional activated sludge process (MBR/CASP) [35], oxidative–biological treatment [36] and advanced oxidation–biotreatment [37]. As part of the investigations in biological treatment of tannery effluent, this study compared the effectiveness of *Bacillus subtilis* and *Pseudomonas fragii* isolated from a tannery wastewater in reducing the concentrations of four key parameters; pH, chemical oxygen demand (COD), chloride and total suspended solids (TSS).

2. Materials and methods

Tannery wastewater was collected at ambient conditions from the storage pond of a leather tanning industry in Kano, Nigeria. Part of the effluent was immediately analysed for pH using the portable pH metre and the remainder was stored as recommended [36,37]. Part of the effluent was analysed in the laboratory to determine the baseline parameters such as colour, biological oxygen demand (BOD), COD, etc. using standard methods [37] while another part was taken to the microbiology laboratory for the isolation of surviving bacterial isolates (micro-organisms) using the serial dilution method [39].

2.1. Isolation of micro-organisms

Ten test tubes were prepared with each test tube filled with 9 ml of deionised water. Each was tightly covered with cotton wool and foil, and was then autoclaved. One millilitre of the tannery effluent suspension was aseptically taken in the incubating room using syringe and added to the first flask. This was lightly shaken and 1 ml was withdrawn and added to the second tube. This process of withdrawal of 1 ml from a tube and putting it in the subsequent test tube continued until the tenth test tube was reached.

The medium was prepared using nutrient agar. The nutrient agar was also sterilised in the autoclave. It was then inoculated with the sample from the tenth tube using the pour plate method. The whole sample was then incubated at 37°C for 24 h. Subculturing to pure culture was carried out and further incubation was also carried out at 37°C for 24 h. Nutrient agar slants were then prepared and were inoculated with the pure culture. The slants were incubated and stored at 37°C for 24 h.

2.2. Biochemical characterisation

The biochemical characterisation of the micro-organisms was carried out by the following methods. All the micro-organisms were cultured on the prepared medium in duplicates and incubated aerobically at 37°C. The colonies were observed on the agar medium plates while the cell morphology was observed microscopically after staining. Various biochemical tests were carried out on the bacterial isolates for possible identification. Seven bacterial organisms were identified and two of them (*B. subtilis* and *P. fragii*) were used in this investigation. About 1 ml of broth culture of each micro-organism was used for all the tests.

2.3. Nutrient preparation

The mineral salts (nutrient) medium for the bacterial isolates (micro-organisms) was prepared by pouring 5 l of distilled water into a *Pyrex* glass bottle of 10 l capacity [40]. Salts to make the nutrient medium were measured into the glass bottle in the proportions stated in Table 1. The glass bottle was sterilised in the autoclave at 121°C and 1.05 kg cm^{-3} for 15 min.

About 50 ml was added to each flask as carbon source (substrate). Each conical flask was put on the gyratory shaker maintained at 30°C and 120 rpm for 32 h. About 20 ml of this medium was then withdrawn from each flask. These flasks were then transferred to the gyratory shaker again for another 16 h to make a total of 48 h.

Table 1
Concentrations of mineral salts for nutrient medium

Material	Concentration (g/l)
Mineral salts	
KH_2PO_4	1.6
$NaHPO_4{\cdot}2H_2O$	3.1
NH_4NO_3	0.5
KNO_3	2.0
$MgSO_4{\cdot}7H_2O$	0.1
$CaCl_2{\cdot}2H_2O$	0.02
Trace elements	
Boric acid	0.1
$ZnSO_4{\cdot}7H_2O$	0.04
Mo salt	0.02
$MnSO_4$	0.04
$CuSO_4$	0.04
$FeSO_4{\cdot}7H_2O$	0.003

2.4. Experimental procedure

The shake flask experiment was conducted in a 250 ml Erlenmeyer flask on a gyratory shaker at 200–250 rpm (New Brunswick Scientific, model G-25R) at 30°C. About 200 ml of fresh broth from the secondary culture was added to each marked flask along with 1% v/v of the wastewater was added. Using an inoculating wire loop that has been sterilised by flaming to redness and cooled by oscillating briefly in the air, three loopfulls of *B. subtilis* and *P. fragii* were streaked into each of the marked flasks. The flasks were then placed on the gyratory incubator shaker at 30°C and 120 rpm for 84 h. Samples were withdrawn every 6 h from each flask for the analysis of pH, chloride and total suspended solid and COD.

2.5. Analytical methods

Biomass was evaluated by dry weight measurements. Medium samples (10 ml) were washed (NaCl, 0.9% w/v) and centrifuged (Beckman J2HS centrifuge) three times for *n*-hexadecane removal. Afterwards, the samples were filtered though a 0.45 mm millipore membrane and were oven-dried to a constant weight at 85°C. Specific growth rate was determined from the measured biomass concentrations. The abilities of the micro-organisms to reduce pH of the tannery wastewater were evaluated. The COD of the wastewater was determined by titrimetric method. A reflux apparatus consisting of 400 ml Erlenmeyer flask with quick fit ground glass necks fitted with a sizeable Leihbig condenser was used. The reduction in COD concentration was recorded at six-hourly time intervals and reported in terms of mg/l and % COD reduction. The total suspended solids (TSS) were determined by filtering a well-mixed sample through a 0.2 μm pore size and 24 mm diameter membrane. The membrane filter was placed in a Gooch crucible, and the residue retained on the filter was dried in an oven for at least 1 h to a constant weight at 103–105°C. This was also at six-hourly intervals. Chloride was determined by the use of HACH spectrophotometer (DR 2010) at six-hourly intervals.

3. Results and discussion

3.1. Analysis of the tannery effluent

Table 2 showed the result of the tannery effluent sample analysis for certain baseline parameters. Measurement for each parameter was made in duplicate and the average values were determined. The result showed that the pollution levels for some of the

measured parameters in the effluent were very high with the exception of temperature and pH. The pattern of the characterisation showed similar trends with earlier works [5,38].

3.2. Growth pattern of the micro-organisms

Biomass growth of *B. subtilis* and *P. fragii* were initially very slow until after 42 h (Fig. 1). At that period, the growth of *B. subtilis* was 0.27 mg/l while that of *P. fragii* was 0.24 mg/l. This was their lag and acceleration phases where they were adjusting and acclimatising to their environment. The growths picked up rapidly (log phase) and continued till when the nutrients were exhausted or probably when the metabolic products formed inhibited further multiplication. This was after 60 h for *P. fragii* when the growth reached 1.99 mg/g and 72 h for *B. subtilis* with a growth of 2.80 mg/l. The growths of the micro-organisms slowed down significantly after these times and were almost constant with marginal increases to 2.34 mg/l and 3.01 mg/l, respectively, for *P. fragii* and *B. subtilis*. These growth patterns followed the well-accepted pattern of microbial growth kinetics. The pattern could be identified as the lag, acceleration, exponential or log, deceleration and stationary phases.

3.3. Specific growth rates

The specific growth rates of the micro-organisms, μ, were determined by using the relationship

$$\mu = \frac{1}{X}\frac{dX}{dT}$$

where μ is the specific growth rate (h^{-1}), X is the cell concentration (g/l) and $\frac{dX}{dT}$ is the change in cell concentration with time.

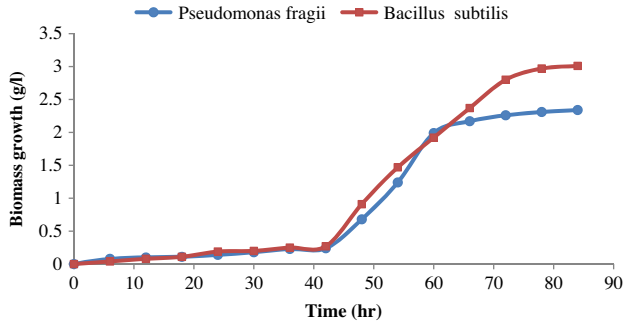

Fig. 1. Biomass growth of *B. subtilis* and *P. fragii*.

The specific growth rate for *B. subtilis* was 0.01378 h^{-1} while that of *P. fragii* was 0.01448 h^{-1}. This is an indication that both micro-organisms were able to take advantage of the nutrient medium to increase their growth rate.

3.4. pH of the wastewater

The abilities of the micro-organisms to reduce the pH of the wastewater are depicted in Fig. 2. The pH of the effluent was initially in the basic region (8.67). By the third day of bioremediation, *P. fragii* was able to bring the effluent to near-neutral condition of 7.13 with the pH remaining constant for the remaining period of investigation while *B. subtilis* brought down the pH value to 7.2 which was almost similar to that of *P. fragii*. Highly alkaline waters, apart from being corrosive, give bad taste and are undesirable. In addition, if pH is not within acceptable range will lead to the loss of more sensitive fish and plant life if such effluent is discharged. The two micro-organisms therefore demonstrated excellent abilities in normalising the pH of the tannery effluent. These were achieved by the micro-organisms breaking down the organic

Table 2
Tannery effluent analysis

Parameter	Measurement A	Measurement B	Average	SD
Temperature (°C)	29	24	26.5	3.535534
pH	8.76	9.78	9.27	0.721249
Total solids	3520	3670	3595	106.066
Total dissolved solids	2718	2720	2719	1.414214
Total suspended solids	872	880	876	5.656854
Sulphide	170	175	172.5	3.535534
BOD_5	2163	2117	2140	32.52691
COD	4373	4397	4385	16.97056
Chromium	42.3	42.7	42.5	0.282843
Chloride	129.12	125.04	127.08	2.884996

All values except pH and temperature are in mg/l.

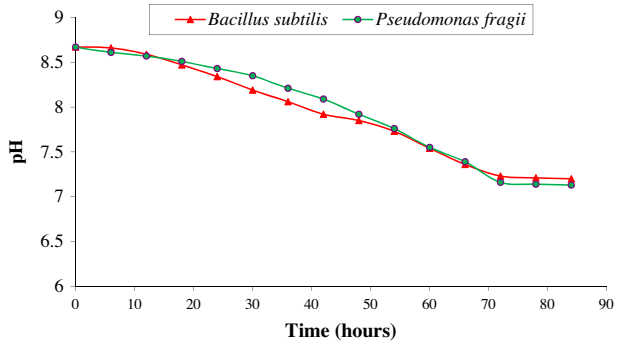

Fig. 2. Variaation in pH of wastewater.

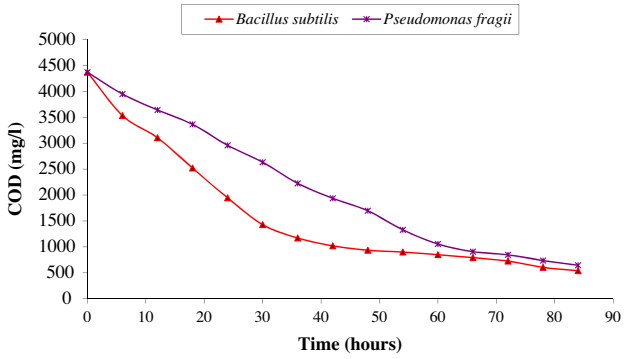

Fig. 3. Variation in the concentration of COD.

constituents of the wastewater into smaller molecules through the constant supply of oxygen.

3.5. COD reduction

The changes in the concentration of COD due to the actions of the micro-organisms are shown in Fig. 3. The COD values obtained at the end of the investigation by the action of *P. fragii* on the effluent was 646 mg/l (85.2%) while that of *B. subtilis* was 541 mg/l (87.6%). These percentage reductions are highly significant although the final values were far above the acceptable limits for COD discharge. Prolonged exposure of the effluent to these micro-organisms above 84 h could produce the expected results. The positive aspect was that there was a significant reduction in the COD value and this will reduce the pollution load of the receiving water body although the water cannot be used for irrigation as it may restrict plant growth, especially on poorly drained soils. This high concentration of COD is an indication of toxic condition that will cause a reduction of dissolved oxygen (DO) levels in water, and will affect the survival of aquatic organisms with objectionable water quality.

3.6. Effect on TSS

Fig. 4 showed the abilities of *B. subtilis* and *P. fragii* to bring down the concentration of TSS. The TSS concentration reduced from 876 mg/l to 73 mg/l after 84 h (91.7%) for *B. subtilis* and from 876 mg/l to 98 mg/l (88.8%) for *P. fragii*. Although the percentage reductions were appreciable, the concentrations obtained were still higher than the acceptable limits bearing in mind that extended exposure above 84 h could achieve the desired results. Waters with high solids concentration (>500 mg/l) often have a laxative effect on people. Solids such as TSS affect the operation and sizing of treatment units. High solids will lead to hindered oxygen transfer, reduced light penetration which will adversely affect the natural aquatic environment. If these solids should settle on the river bottom, they will cover flora and fauna and result in an anaerobic sludge layer.

3.7. Effect on chloride concentration

Fig. 5 showed the changes in chloride concentration with time using *B. subtilis* and *P. fragii*. The chloride values obtained after 84 h of remediation were 65.4 mg/l (48.5%) for *B. subtilis* and 57.72 mg/l (54.6%)

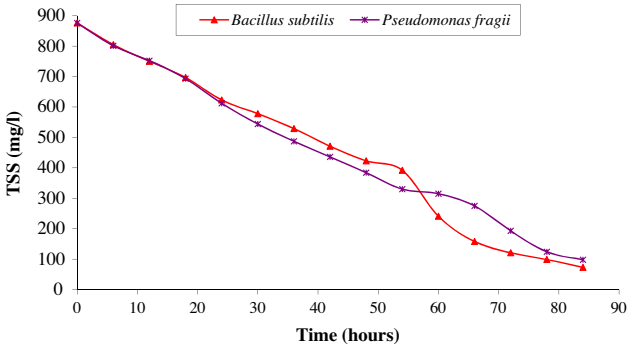

Fig. 4. Variation of the total suspended solids with time.

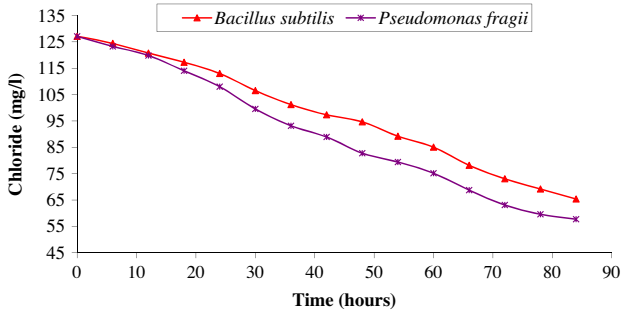

Fig. 5. Variation of chloride concentration with time.

for *P. fragii*. This is evident that both micro-organisms could be used to reduce the concentration of chloride in wastewater with prolonged exposure. Since a large amount of salt is used in the tannery industry, effective removal of chloride from the effluent will be beneficial to the receiving water and will increase the potential for water reuse and recycling. The maximum allowable chloride concentration in drinking water was established for reasons of taste rather than as a safeguard against physical hazard as no toxic effect of high chloride concentration in water has been reported.

4. Conclusions

The effectiveness of *B. subtilis* and *P. fragii* in the bioremediation of tannery wastewater has been evaluated. *B. subtilis* and *P. fragii* could be used to normalise the pH as both micro-organisms brought the pH value within the acceptable limit of 6–9. For the reduction of chloride concentration, *P. fragii* was able to bring the chloride concentration to 57.72 mg/l, which is close to the acceptable limit of 50 mg/l whereas *B. subtilis* only reduced the concentration to 65.4 mg/l, which was a bit on the higher side. The study clearly established the possibility of using *P. fragii* as a suitable alternative management strategy for the removal of chlorides as it is a psychrophilic, gram-negative bacterium and is responsible for dairy spoilage [41]. *P. fragii* does not produce siderophores and this makes it different from other members of the *P.* genus [42]. It is placed in the *P. chlororaphis* group based on 16S rRNA analysis [43]. *B. subtilis* is a gram-positive, catalase-positive bacterium and is found in soil [44]. It is rod-shaped, and has the ability to form a tough, protective endospore which allows it to tolerate extreme environmental conditions. It has historically been classified as an obligate aerobe unlike several other well-known species but of recent, research has shown that this is not totally correct [45]. From the results, it can be said that the bacteria present in the tannery effluents have significant potential in treatment of leather industrial wastewater.

References

[1] T. Mandal, D. Dasgupta, S. Datta, A biotechnological thrive on COD and chromium removal from leather industrial wastewater by the isolated microorganisms, Desalin. Water Treat. 13 (2010) 382–392.
[2] A.S. Naidu. Indian leather Industry in 21st Century: Challenges and Opportunities, Yojana, 2000.
[3] S. Yadav, O.P. Shukla, U.N. Rai, Chromium pollution and bioremediation, Arch. Enviro. News lett. ISEB India 11 (2005) 3–4.
[4] J.B. Carmichael, K.M. Strezepek, Industrial Water uses and Treatment Practices, United Nations Industrial Development Organisation (UNIDO), Cassel Tycooly, Philadelphia, 1987.
[5] M.A. Aboulhassan, S. Souabi, A. Yaacoubi, Pollution reduction and biodegradability index improvement of tannery effluents, Int. J. Environ. Sci. Tech. 5 (2008) 11–16.
[6] D.H. Ahn, Y.C. Chung, Y.J. Yoo, D.W. Pak, W.S. Chang, Improved treatment of tannery wastewater using *Zoogloea ranigera* and its extracellular polymer in an activated sludge process, Biotechnol. Lett. 18 (1996) 917–922.
[7] N.K. Uberoi, Environmental Management, Excel Books, New Delhi, 2003.
[8] P. Thanikaivelan, J.R. Rao, B.U. Nair, T. Ramasami, Recent trends in leather making: Processes, problems, and pathways, Crit. Rev. Environ. Sci. Technol. 35 (2005) 37–79.
[9] R. Suthanthararajan, E. Ravindranath, K. Chits, B. Umamaheswari, T. Ramesh, S. Rajamam, Membrane application for recovery and reuse of water from treated tannery wastewater, Desalination 164 (2004) 151–156.
[10] M. Wiemann, H. Schenk, W. Hegemann, Anaerobic treatment of tannery wastewater with simultaneous sulphide elimination, Water Res. 32 (1998) 774–780.
[11] J. Naumczyk, M. Rusiniak, Physicochemical and chemical purification of tannery wastewaters, Pol. J. Environ. Stud. 14 (2005) 789–797.
[12] I.A. Oke, J.A. Otun, C.A. Okuofu, N.O. Olarinoye, Characteristics of tanning industries in Nigeria for aquatic animals and plants, Res. J. Agric. Biol. Sci. 2 (2006) 209–217.
[13] R.R. Babu, N.S. Bhadrinarayana, K.M.M.S. Begum, N. Anantharaman, Treatment of tannery wastewater by electrocoagulation, J. Univ. Chem. Technol. Metall. 42 (2007) 201–206.
[14] S. Srinivasan, G. Mary, C. Kalyanaraman, P. Sureshkumar, K. Sri Balakameswari, R. Suthanthararajan, E. Ravindranath, Combined advanced oxidation and biological treatment of tannery effluent, Clean Technol. Environ. Policy 14 (2012) 251–256.
[15] G. Durai, N. Rajamohan, C. Karthikeyan, M. Rajasimman, Kinetics studies on biological treatment of tannery wastewater using mixed culture, Int. J. Chem. Biol. Eng. 3 (2010) 105–109.
[16] P. Thanikaivelan, J.R. Rao, B.U. Nair, T. Ramasami, Progress and recent trends in biotechnological methods for leather processing, Trends Biotechnol. 22 (2004) 181–188.
[17] A.M. Talarposhti, T. Donnelly, G.K. Anderson, Colour removal from a simulated dye wastewater using a two-phase anaerobic packed bed reactor, Water Res. 35 (2001) 425–432.
[18] G. Sekaran, K. Chitra, K. Mariappan, K.V. Raghavan, Removal of sulphide in anaerobically treated tannery wastewater by wet air oxidation, J. Environ. Sci. Health 31 (1996) 579–598.
[19] Z. Song, C.J. Williams, R.G.J. Edyvean, Treatment of tannery wastewater by chemical coagulation, Desalination 164 (2004) 249–259.
[20] Ö. Apaydin, U. Kurt, M.T. Gönüllü, An investigation on the treatment of tannery wastewater by electrocoagulation, Global NEST J.3 11 (2009) 546–555.
[21] L. Szpyrkowicz, S.N. Kaul, R.N. Neti, Tannery wastewater treatment by electrooxidation coupled with a biological process, J. Appl. Electrochem. 35 (2005) 381–390.
[22] K.S. Min, J.J. Yu, Y.J. Kim, Z. Yun, Removal of ammonium from tannery wastewater by electrochemical treatment, J. Environ. Sci. Health. Part A Toxic/Hazard. Subst. Environ. Eng. A39 (2004) 1867–1879.
[23] S.G. Schrank, H.J. Jose, R.F.P.M. Moreira, H. Schröder, Applicability of Fenton and H_2O_2/UV reactions in the treatment of tannery wastewaters, Chemosphere 60 (2005) 644–655.
[24] C.R. Carla, C.M.R. Botta, E.L.G. Espindola, P. Olivi, Electrochemical treatment of tannery wastewater using DSA electrodes, J. Hazard. Mater. 153 (2008) 616–627.
[25] L. Zhang, W. Li, K. Wang, Enhancement of tannery wastewater treatment at low temperature by coagulation coupled with cationic polyacrylamide, in: International Conference on Energy and Environment Technology, 2009.

[26] G.M. Ayoub, A. Hamzeh, L. Semerjian, Post treatment of tannery wastewater using lime/bittern coagulation and activated carbon adsorption, Desalination 273 (2011) 359–365.
[27] S.R. Stern, I. Azpyrkowicz, Rodighiro Aerobic treatment of textile dyeing wastewater, Water Sci. Technol. 47 (2003) 55–59.
[28] C. Di Iaconi, A. Lopez, R. Ramadori, R. Passino, Tannery wastewater treatment by sequencing batch biofilm reactor, Environ. Sci. Technol. 37 (2003) 3199–3205.
[29] O. Lefebvre, N. Vasudevan, M. Torrijos, K. Thanasekaran, R. Moletta, Halophilic biological treatment of tannery soak liquor in a sequencing batch reactor, Water Res. 39 (2005) 1471–1480.
[30] G. Farabegoli, A. Carucci, M. Majone, E. Rolle, Biological treatment of tannery wastewater in the presence of chromium, J. Environ. Manage. 71 (2004) 345–349.
[31] S.G. Schrank, H.J. Jos, R.F.M.P. Moreira, H.F. Schroder, Fentons oxidation of various-based tanning materials, Desalination 50 (2003) 411–423.
[32] T. Mandal, D. Dasgupta, S. Mandal, S. Datta, Treatment of leather industry wastewater by aerobic biological and Fenton oxidation process, J. Hazard. Mater. 180(1–3) (2010) 204–211.
[33] C. Di Iaconi, F. Bonemazzi, A. Lopez, R. Ramadori, Integration of chemical and biological oxidation in a SBBR for tannery wastewater treatment, Water Sci. Technol. 50 (2004) 107–114.
[34] S. De Gisi, M. Galasso, G. De Feo, Treatment of tannery wastewater through the combination of a conventional activated sludge process and reverse osmosis with a plane membrane, Desalination 249 (2009) 337–342.
[35] G. Munz, D. De Angelis, R. Gori, G. Mori, M. Casarci, C. Lubello, The role of tannins in conventional and membrane treatment of tannery wastewater, J. Hazard. Mater. 164 (2009) 733–739.
[36] G. Vidal, J. Nieto, H.D. Mansilla, C. Bornhardt, Combined oxidative and biological treatment of separated streams of tannery wastewater, Water Sci. Technol. 49 (2004) 287–292.
[37] T. Mandal, S. Maity, D. Dasgupta, S. Datta, Advanced oxidation process and biotreatment: Their roles in combined industrial wastewater treatment, Desalination 250(1) (2010) 87–94.
[38] HACH, Water Analysis Handbook, third ed., HACH Company, Loveland, Colorado, USA, 1997.
[39] APHA, Standard Methods for the Examination of Water and Wastewater, 20th ed., American Public Health Association (APHA), Water Environment Federation (WEF) and American Water Works Association (AWWA), Washington, DC, USA, 1998.
[40] V. Tare, S. Gupta, P. Bose, Case studies on biological treatment of tannery effluents in India, J. Air Waste Manage. Assoc. 53 (2003) 976–982.
[41] K.R. Aneja, Experiments in Microbiology, Plant Pathology and Biotechnology, Plant Pathology and Biotechnology, New Age Publishers, 2003.
[42] D.C. Old, J.P. Duguid, Selective outgrowth of fimbriate bacteria in static liquid medium, J. Biotechnol. 103 (1970) 447–456.
[43] J.N. Pereira, M.E. Morgan, Nutrition and physiology of *Pseudomonas fragi*, J. Bacteriol. 74 (1957) 710–713.
[44] M.-C. Champomier-Verges, A. Stintzi, J.-M. Meyer, Acquisition of iron by the non-siderophore-producing *Pseudomonas fragi*, Microbiology 142(1996) (1996) 1191–1199.
[45] Y. Anzai, H. Kim, J.Y. Park, H. Wakabayashi, H. Oyaizu, Phylogenetic affiliation of the pseudomonads based on 16S rRNA sequence, Int. J. Syst. Evol. Microbiol. 50(Pt 4) (2000) 1563–1589.