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Application of Bacterial Isolation Technology with Biological Treatment Method on Tannery Wastewater for reuse in irrigation

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Abstract

Leathering tanning wastewater contains high concentration of organics and other pollutants. the current study was dole out to analyze the biodegradation process of Leathering tanning process effluents using biological treatment such as activated sludge. A different aeration cycles were applied such as 6, 8 and 12 hours. In addition some locally isolated bacteria from the collected wastewater samples. Five different Leathering tanning effluent samples were collected from Leathering tanning factory at Elfostat city, Egypt. Two bacterial species (Bacillus and Staphylococcus) were separated from the raw industrial wastewater samples, identified and used for biodegradation process. Bacterial isolation process was prepared in the laboratory of national research center. Two-stage (aeration and sedimentation) laboratory scale model was designed. Results indicated the great ability of Bacillus bacteria for improving of the physicochemical parameters. The removal percentage of COD, BOD, TSS, NH3-N, TN and TP are 97.57, 96, 91.35, 96.15, 98.59 and 62.79 respectively. Biodegradation of leather tanning wastewater counting on local microorganisms is an efficient, cheap and eco-friendly technology. The final results of the treated industrial wastewater by using Bacillus bacteria with a 12-hour aeration followed by 2 hours settling period were less than the Egyptian standards of treated water for irrigation purpose.

Keywords: Bacteria, biodegradation, leather tanning effluent, tanning process.

INTRODUCTION

Water is an essential source of life, but the critical environmental problem facing humankind globally its low quantity beside the pollution. Nowadays, there are urgently to find ways to manage water, particularly industrial drainage, as it constitutes a threat to the surrounding environment and its use in the field of agricultural irrigation (Hussona & Abdullah, 2014; MWRI, 2014; Nour El-Din M.M, 2013; Waseem A. Gad, 2017).

One of the foremost essential industries currently is that the leather industry, but the most problem is the disposal of water resulting from this industry because it contains many complex pollutants (Lofrano et al., 2013). Such as chlorine, heavy metals, toxic chemicals, and lime with highly soluble and suspended salts and other pollutants (Elmagd & Mahmoud, 2014). During the tanning process, Chromium salt had been used. As a side component of the process, generation of two sorts of chrome; hexavalent and trivalent chromiums. Hexavalent chromium is very toxic to living organisms (Lee et al., 1995). Soluble trivalent chromium causes toxicity in anaerobic digestion (Alkan et al., 1996).

Many components within the effluent contain nitrogen as a part of their chemical structure, which might lead to the development of anaerobic conditions harmful to the aquatic life (Midha & Dey, 2008; Srivastava et al., 2007). In Egypt, the tannery wastewater is disposed of on to the domestic sewage pipeline system without a significant treatment, which adds difficulties to the facility and the wastewater treatment plants (Abdulla et al., 2010). Treatment of tannery wastewater is administrated by biological or chemical or physical or combination of these methods (Lefebvre et al., 2005; Lofrano et al., 2010, 2011; Schrank et al., 2009).

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Bacterial isolation in microbiology, the term isolation refers to the separation of a strain from a natural, mixed population of living microbes, as present within the environment. It is accustomed to enhance the biological treatment process by culturing the active bacteria to degrade the specified matter.

Global biological methods like up-flow Anaerobic Sludge Blanket Reactor (UASB) and activated sludge process (ASP). Biological treatment methods are often divided into aerobic and anaerobic methods, supported the availability of dissolved oxygen (Irfan, 2009). Biological methods, like activated sludge processes, are repeatedly utilized for the secondary treatment of the many business wastewaters. A widely known of the microbial kinetics and determination of the kinetic parameters for a selected wastewater type be obliged to the rational design of treatment facilities (Contreras et al., 2001; Haydar et al., 2016).

Biological processes are usually prescribed for treating industrial effluents to cut back organic content as they need economic advantages over chemical oxidation (Dogruel et al., 2006). However, a high concentration of tannins and other poorly biodegradable compounds also as metals can inhibit biological treatment (Ganesh et al., 2006; Murat et al., 2006). The activated sludge process is the most universal and multifaceted process used worldwide for the secondary treatment of domestic, municipal, and industrial wastewater. By the time, several modifications of the ASP are made to extend the degree of treatment following firm effluent standards (Association et al., 2005) **.**

The main goal of this study is to treat the tannery wastewater by using ASP with or without additives to use the treated effluent in the irrigation process.

2. MATERIAL AND METHODS

2.1. Sample Collection

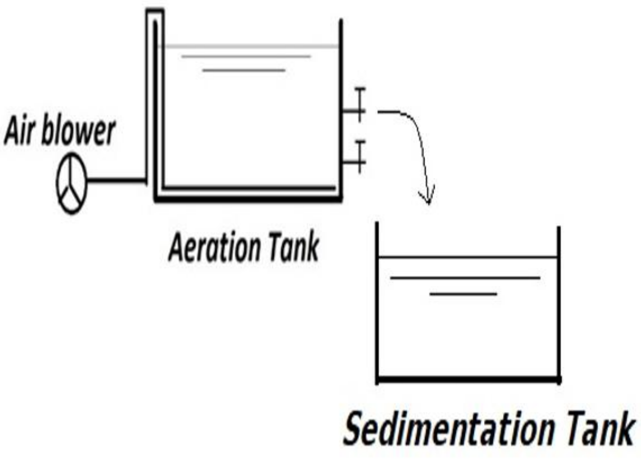
Industrial wastewater was collected from a leather tanning factory at Elfostat city, Egypt. The containers filled through the plastic vessel; the containers are prepared to be transferred to the workplace to process the industrial wastewater. Five plastic containers were collected with 20 liters for each one. Samples were collected and transferred to the National Research Center for the experiments.

2.2. Site Description

The factory is located at Elfostat city, Egypt. It occupies an area of 450 square meters. Its daylily process around 9 tons of leather. The effluent discharged through an open channel to the sewerage system.

2.3 Pilot Description

A pilot was made by an aeration tank with dimensions of 30*30*60 cm with a total volume of 54 litters and an actual working volume of 45 litters considering 5 cm freeboard and followed by a settling tank with dimensions of 30*30*60 cm with a total volume of 54 litters and a working volume of 45 litters considering 5 cm freeboard. Figure 1 shows the schematic diagram of the pilot and the actual pilot components.



a) Shows components of the system

b) The activated sludge system including the aerators

Fig 1: Components of the activated sludge model.

2.4 Experimental Run Description

Aeration tank was filled with wastewater leather tanning. The aeration tank duration was tested by changing the aeration time throughout five trials, as listed below.

- The first trial was selecting the aeration duration to be 6 hours without any additives.
- The second trial was increasing the aeration duration to 8 hours without any additives.
- The third trial was increasing the aeration duration to 12 hours without any additives.
- The fourth trial was selecting the aeration duration of 12 hours by applying the first isolated type of bacteria (Bacillus type).
- The fifth trial was selecting the aeration duration of 12 hours by applying the second isolated type of bacteria (Staphylococcus type).

All these trials were followed by 2 hours of settling stage. Samples were taken before each trial, after applying aeration duration and after the settling stage

2.5 Bacterial Isolation Concept

A sample of the wastewater was taken and apply the isolation concept in the National Research Center Microbiology laboratory. The sample produces two major types of bacteria, Bacillus, and Staphylococcus. Each type of bacteria was tested on the best aeration cycle resulting best physiochemical parameters for the treated tannery wastewater. Most of the currently known species of bacteria are identified using traditional microbiological techniques, just like the Gram method reaction, morphology, and metabolic reactions. Bacteria rarely live alone but in communities with other bacteria. This will be true both within the environment and in and on our bodies. The first requirement for physically isolating a bacterium is that it will be cultured within the laboratory. This needs knowledge of optimal temperature for growth, optimal oxygen requirements, and optimal nutritional needs

There are two main ways to isolate organisms;(1) Streaking for isolation on an agar plate, (2)the pour plate method. Streaking for isolation on an agar plate involves the successive dilution of organisms until the cells at an occasional enough density that single cells are physically isolated spatially to convey rise to recognizable individual colonies. Within the pour plate method, dilute the sample sufficiently before adding it to molten cooled agar then pour this mixture in an exceeding dish. The isolated cells create individual colonies growing in agar. This method is slightly tricky. If the melted agar is solely too hot, the bacteria will die while in the melted agar is solely too cold, land up with a large lump in r Petri dish. The streaking method yields individual colonies on the surface of the agar. The isolation process done by taking 1 mL of industrial wastewater sample was inoculated in 100 ml sterile distilled water in 250 mL Erlenmeyer flask. Inoculated flasks were incubated in a shaker incubator at 120 rpm and 35°C for 24h. The obtained suspensions were diluted ten times with sterile saline solution, and 0.1 mL of each dilution was streaked on four nutrient agar plates, each plate containing 300 ppm of single studied dye and incubated at 35°C for 24h and decolorization zones were measured after the incubation period. Colony showing the largest decolorization zone in each plate, was picked up and preserved on nutrient agar slants for further studies. The picked-up bacterial isolates were identified using the Biolog Gen III (Biolog® Inc., USA) identification system.

2.6 Chemical and Physical Analysis

Characterization of raw and treated leather tanning wastewater was carried out through measuring some physicochemical and bacteriological parameters. These parameters were biological oxygen demand (BOD_5), chemical oxygen demand (COD), total suspended solids (TSS), pH, NH3-N, TN and TP. All these parameters were measured according to the standard methods for the examination of water and wastewater APHA2010 at the national research center(Association et al., 2005)

3. RESULTS AND DISCUSSION

3.1 Characterization of Raw Leather Tanning Sample

Five samples had been taken from the industrial wastewater, which is raw water (untreated), and tested at the National Research Center to know the rate of pollutants and pathogens in it. The characteristics of raw industrial wastewater are given in Table (1).

Table 1: Raw industrial wastewater samples

Parameters	Raw sample 1	Raw water 2	Raw water 3	Raw water 4	Raw water 5
COD mg L^{-1}	3600	3500	4000	3700	3600
BOD mg L^{-1}	1300	1350	1400	1450	1400
pH	4.3	4.2	4.4	4.2	4.3
Modified PH	6.7	6.7	6.8	6.7	6.9
TSS mg L^{-1}	510	490	520	520	500
NH3-N mg L^{-1}	800	750	770	780	750
TN mg L^{-1}	700	600	700	640	600
TP mg L^{-1}	4.2	3.8	4.2	4.3	4

Table (1) shows the values for measured physicochemical parameters of raw industrial wastewater to evaluate and determine the efficiency of the industrial wastewater treatment process. The raw industrial wastewater was highly polluted due to the high values of physicochemical parameters. In this study, the pH of raw industrial wastewater ranged acidic side (4.2–4.4). The values of pH are not suitable for bacteria to be active. So the pH value must be modified by adding a sodium hydroxide solution and testing the obtained pH until reaching the required pH range of (6-7).

High concentrations of suspended matters were measured by TSS. TSS average concentrations were higher than those reported by some researchers 357.8-492 mg L^{-1}. (Sugasini & Rajagopal, 2015). Also, raw industrial wastewater showed high concentrations of organic matter fraction expressed as COD and BOD5, which derives primarily from leather treatment. The high value of COD concentrations higher than reports by “Arasappan Sugasini and Kalyanaraman Rajagopal” (2749.8-3420.3) mg L^{-1} (Sugasini & Rajagopal, 2015). High concentrations of BOD5 are attributed to the presence of organic matters and microorganisms.

3.2 Effect of Different Aeration Cycle and the Bacteria Addition

Table (2) shows the final results of all trials of treatment of tannery wastewater. All the results were compared with the Egyptian standards for reusing the treated industrial wastewater for irrigation purposes. As shown in Table (2), the final results of the fourth trial used in industrial wastewater treatment were less than the Egyptian standards for reusing the treated industrial wastewater for irrigation purposes.

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Table 2: Final results of all trials of industrial wastewater treatment

Parameters	Activated sludge Without additive											
	Trial 1				Trial 2				Trial 3			
	R 1	A 6h	Sed 2h	Ov. Eff.%	R 2	An 8h	Sed 2h	Ov. Eff.%	R 3	A 12h	Sed 2h	Ov. Eff.%
COD mg L^{-1}	3600	3200	2800	22.22	3500	3100	2500	28.57	4000	2900	2700	32.50
BOD mg L^{-1}	1300	1250	1180	9.23	1350	1200	1050	22.22	1400	1200	1008	28.00
pH	4.3	7.7	7.2	-----	4.2	7.8	7.3	-----	4.4	7.5	7.2	-----
TSS mg L^{-1}	510	400	250	50.98	490	380	220	55.10	520	390	270	48.08
NH3-N mg L^{-1}	800	300	250	68.75	750	250	270	64.00	770	330	220	71.43
TN mg L^{-1}	700	330	280	60.00	600	310	280	53.33	700	350	260	62.86
TP mg L^{-1}	4.2	2	1.7	59.52	3.8	1.6	1.6	57.89	4.2	1.9	1.5	64.29

Parameters	Activated sludge With Bacterial Isolation Additive (Bacillus (B1)) and (Staphylococcus (B2)) types								Law 48 for 1982
	Bacillus (B1) bacterias				Staphylococcus (B2) bacterias				
	Trial 4				Trial 5				
	R 4	A 12h + B1	Sed 2h	Ov. Eff.%	R 5	A 12h + B2	Sed 2h	Ov. Eff.%	
COD mg L^{-1}	3700	120	90	97.57	3600	220	180	95.00	100
BOD mg L^{-1}	1450	90	58	96.00	1400	100	80	94.29	60
pH	4.2	8	8	-----	4.3	8.1	8.1	-----	9-6
TSS mg L^{-1}	520	75	45	91.35	500	80	60	88.00	60
NH3-N mg L^{-1}	780	50	30	96.15	750	80	60	92.00	40
TN mg L^{-1}	640	32	9	98.59	600	45	18	97.00	10
TP mg L^{-1}	4.3	1.7	1.6	62.79	4	3	3.4	15.00	10

Table key:

- R 1 : Raw sample 1
- R 2 : Raw sample 2
- R 3 : Raw sample 3
- R 4 : Raw sample 4
- R 5 : Raw sample 5
- A 6h : Aeration for 6 hours
- Ov Eff.% : Overall Efficiency percentage
- A 8h : Aeration for 8 hours
- A 12h : Aeration for 12 hours
- A 12h + B1 : Aeration for 12 hours with adding Bacillus bacteria
- A 12h + B2 : Aeration for 12 hours with adding Staphylococcus bacteria
- Sed 2h : sedimentation for 2 hours

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Generally, raw industrial wastewater showed higher average values of physicochemical and bacterial parameters than the permissible limits of treated wastewater reuse in irrigation purposes according to the Egyptian guidelines.

3.2.1 Chemical oxygen demand (COD)

The experiment result with an activated sludge system indicates that the removal efficiency of COD increased significantly from the first trial to the second trial to the third trial. Raw industrial wastewater showed high concentrations of organic matter fraction expressed as COD and BOD, which derives primarily from tanning leather processes. The obtained results in Table (4.6) showed that industrial wastewater had a relatively high average concentration of COD (3500-4000 mg L^{-1}). During the total operational period, high COD removal was achieved in the system by applying the trial of adding the Bacillus bacteria as isolated bacteria to the industrial wastewater and using a 12 hour aeration period, and the removal efficiency approaches to 97.57 %. At other studies, a less removal efficiency for COD by applying only activated sludge treatment method (Haydar et al., 2016). The variation between results is due to the highly polluted raw tannery wastewater.

3.2.2 Biochemical oxygen demand

One of the commonly measured parameters of wastewater is the biochemical oxygen demand (BOD). Wastewater contains inorganic and organic matters. Organic matter is the molecules that are supported by carbon and include stool. These organic molecules are easily digested by bacteria within the biological treatment system. However, oxygen is required for this process of breaking large molecules into smaller ones and at last into water and carbon dioxide. The number of oxygen required for this process is referred to because of the biological oxygen demand. BOD removal efficiency approach to 96% while applying the trial of adding the Bacillus bacteria as isolated bacteria to the economic wastewater and using a 12 hour aeration period. Treatment of tannery effluent through the use of activated sludge process has been reported by many research workers (Ahmad, 2002; Eckenfelder, 2002; Emerson & NEMEROW, 1969; Haydar et al., 2016; Jawahar et al., 1998; Murugesan & Elangoan, 1994; Tare et al., 2003). All these studies indicate a BOD5 removal of 90 to 97% for the tannery effluent, concluding the activated sludge process as highly useful for the treatment purpose.

3.2.3 Total suspended solids (T.S.S)

Total suspended solids include all particles suspended in water, which can't be filtered. Suspended solids are present in many sorts of business wastewater. As levels of total suspended solid concentration increase, a water body loses its capability to a miscellany of life. Suspended solids absorb heat from sunlight, which maximizes water temperature and subsequently minimize levels of dissolved oxygen. The TSS removal rate was varied during the deferent treatment trials. The upper removal percentage achieved when applying the trial of adding the Bacillus bacteria as isolated bacteria to the commercial wastewater and using a 12 hour aeration period, which reaches 91.35%. High total suspended solids value in an exceeding water body can often mean higher concentrations of bacteria, nutrients, pesticides, and metals within the water. The foremost issue when using recycled water is that the potential risk to human health, the standards are usually supported by microbial content. It had been clear that raw industrial wastewater contains high concentrations of suspended matters measured by either TSS or turbidity. TSS ranged from (490-520 mg L-1). At other studies, a removal efficiency achieves 99.9% by applying activated sludge treatment methods(Elmagd & Mahmoud, 2014)

3.2.4 Ammonia-nitrogen (NH3-N)

The data was collected by taking the effluent samples and tested for the removal performance. It had been found that the transformation of ammonia to nitrite and nitrate happened successfully within the method of activated sludge treatment. Ammonia concentration was decreased from 780 to 30 mg L^{-1} with a removal efficiency of 96.15%. The whole process of nitrification requires oxygen. Aerobic autotrophic bacteria are chargeable for nitrification, where nitrification is that the biological oxidation of ammonia or ammonium to nitrite followed by the oxidation of the nitrite to nitrate. The transformation of ammonia to nitrite is typically the rate-limiting step of nitrification. Nitrification is a crucial step within the organic process in soil. In other studies, a removal efficiency achieves 98.8% by applying the activated sludge treatment method(Elmagd & Mahmoud, 2014).

3.2.5 Total nitrogen (TN)

The data was obtained by analyzing the effluent samples at laboratories of the national research center and also tested for the removal performance. It was found that the transformation of ammonia to nitrite and nitrate happened successfully within the method of activated sludge treatment, which lead to decrees the value of TN. Total nitrogen was varied 600-700 mg L^{-1} in raw samples. After applying the treatment cycles, the value of TN decreased from 640 to 9 mg $L^{-1,}$ achieving a removal efficiency of 98.59%. This achievement happens when adding the Bacillus bacteria as isolated bacteria to the industrial wastewater and using a 12 hour aeration period followed by 2 hours of settling period.

3.2.6 Total phosphorus (TP)

Phosphorus is a component of the various living cells and also the metabolism of animals and plants. Phosphorus found in sewage in its most highly oxidized forms. Therefore it is not an oxygen-consuming matter. Nevertheless, phosphorus is also an enormous problem because, in an aquatic environment, it is generally a potent factor for the evolution of organisms—the whole phosphorus removal average within 62.79 %. Where the worth of TP varied from 4.3-1.6 mg L-1after applying a 12-hour aeration cycle with adding the bacillus bacteria to the treatment trial followed by a 2-hour settling cycle, however, it is noticed the worth of the raw industrial wastewater TP is varied from 4.3-3.8 mg L-1, which is not up to the law limitation of reusing the treated water for irrigation purposes. In other studies, a removal efficiency achieves 98.6% by applying the activated sludge treatment method (Elmagd & Mahmoud, 2014).

4. CONCLUSION

The Activated Sludge Process (ASP) is a possible wastewater treatment technology mainly if the space is inadequate for the use of other biological methods. According to the results obtained from the experimental program:

• The raw industrial sample is highly polluted due to the manufacturing process for tanning leather.

• Activated sludge biological treatment without additive and 6 hours of air blower reduce water properties by an overall 45.12 percent.

• Activated sludge biological treatment without additive and air blower treatment for 8 hours reduces water properties by 46.85% overall.

• Using Activated Sludge biological therapy with no chemicals and an air blower for 8 hours reduces water properties for over 6 hours.

• Activated sludge treatment without the use of an additive and an air blower for 12 hours lowers the total water properties by 51.19 percent.

• The use of biological treatment without additive by the active sludge and the use of a blowing air for 12 hours minimize the water characteristics by more than eight hours.

• The use of biological treatment of activated sludge with the addition of bacteria with air blowers for 12 hours significantly reduces water properties.

• A water sample for industrial wastewater contained bacterial insulation and two types of bacteria, Bacillus (B1) and Staphylococcus (B2), were found. A single separate test was used for industrial wastewater treatment with a 12 hour aeration period and 2-hour settlement stage.

• The use of biological therapy with the introduction of Bacillus bacteria (B1) with the use of air blowers for 12 hours greatly decreases water properties by an average percentage of 90.41%.

• Biological treatment by using the Staphylococcus bacteria activated sludge (B2) addition with an air blower for 12 hours reduces the water properties by 80.21 percent overall.

• A comparison of the outcomes of various treatment experiments used in industrial wastewater treatment was made with the Egyptian irrigation or drainage method.

• The final results of industrial wastewater treatment with Bacillus bacteria, 12-hour aeration followed by 2 hours settlement, were less than the Egyptian irrigation water standard.

• The results obtained indicate that the incorporation of Bacillus bacteria into industrial wastewater is appropriate as the final properties of treated industrial wastewater were consistent with Egyptian irrigation water specifications.

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