Journal of the Turkish Chemical Society Section A: Chemistry
Turkish Chemical Society

Bioremediation Potential of Immobilized *Corynebacterium kutsceri* in the Treatment of Tannery Industry Effluent from Challawa Industrial Estate, Kano State, Nigeria

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Abstract: In the present study, bioremediation potentials of indigenous bacteria (*Corynebacterium kutsceri*) in the treatment of tannery effluent was investigated. Industrial tannery effluent samples from Mamuda Tannery Industries in Challawa Industrial estate, Kano State, Nigeria were collected for a period of six months (August 2017 to January 2018) for the experiment. Bacteria were isolated from the effluents using serial dilution, immobilized on agar-agar and biochemical tests were carried out to identify the bacteria. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the identified bacteria were used in the treatment of 250 mL of the effluents. Temperature, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Suspended Solid (SS), Total Dissolved Solids (TDS), sulfate, phosphate, nitrate, chloride, and some heavy metals (Cr, Fe, Mn, Ni, Pb, Zn, Cd, and Cu) were determined before and after treatment of the effluents with the bacteria. The pre-treatment analysis showed that the values, 29.50±4.68, and 5.35±1.57, were observed for temperature (^{o}C) and the pH respectively. The levels (mg/L), 26.17±9.49; 3106±2753; 562±482; 444±507; 97.20±146.80; 268.34±411.01; 8.82±34.71 and 22.59±19.64 were observed for BOD, COD, SS, TDS, nitrate, sulfates, phosphate, and chlorides, respectively. The concentration (mg/L) of the heavy metals, before treatment (with the bacteria) were as; Cr (7.528±4.530); Fe (1.263±0.502); Ni (0.023 ± 0.021); Mn (0.277 ± 0.03); Pb (0.304 ± 0.20); Zn (0.058± 0.05); Cd (0.068±0.02) and Cu (0.012 ±0.02). The bacteria were identified to be *Corynebacterium kutsceri*. The post-treatment analysis showed that the COD (1376±248−2681±867 mg/L) has the highest value while Cu (ND −0.007±0.00 mg/L) has the lowest value. The post-treatment analysis using the different masses of the *Corynebacterium kutsceri* indicates that there is a great decrease in the levels of the physicochemical parameters and the heavy metals as well when compared with the levels observed at the pre-treatment analysis. The decrease could be attributed to, not only due to the increase in the exact mass of the bacteria but also to the multiplicity in the mass of the *Corynebacterium kutsceri* which subsequently increases the surface area for the remediation. The average high percentage reduction (70% to 100%) of these parameters and heavy metals implies that the *Corynebacterium kutsceri* has a higher potential for the treatment of effluents from the textile industries.

Keywords: Bioremediation, *Corynebacterium kutsceri*, effluent, immobilization, tannery.

Submitted: November 07, 2019. **Accepted:** February 27, 2020.

Cite this: Baba A, Garba S, Bello H. Bioremediation Potential of Immobilized Corynebacterium kutsceri in the Treatment of Tannery Industry Effluent from Challawa Industrial Estate, Kano State, Nigeria. JOTCSA. 2020;7(2):335–50.

DOI: https://doi.org/10.18596/jotcsa.643771.

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INTRODUCTION

With rapid industrialization, water pollution has become a major problem. The characteristics of industrial effluent depend upon the type of industrial raw material and the output of the product (1). Industrial effluents account for several point sources of water pollution, while developed nations adopt stringent water quality requirement to control river pollution from point and non-point sources, the situation is different in the most developing countries like Nigeria. Wastewater treatment in Nigeria is not given the necessary priority it deserves and therefore, industrial wastes are discharged into receiving water bodies without treatment. The consequences of this include among others, river pollution, loss of aquatic life, uptake of polluted water by plants, disease burden, and shorter life expectancy in developing countries (2).

Tannery industrial wastewater is a serious consequence of the pollution point of view for streams, freshwater, and land used for agriculture. The lack of awareness in the modern industrial practice has resulted in the discharge of tannery effluents which exhibit the very high value of Cr, Sulfide, and chloride, Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water stream or land (3). Tannery effluent refers to as wastewater from the process of converting skin and hides into leather. The process of tanning requires a large volume of water, which is used to either cleanse the hides and skins or as an interaction medium between the hides and skin. During the tanning process, a large volume of effluents is discharged into the surrounding soil as well as a water source. These effluents may contain a variety of chemicals such as sodium sulfate, chromium sulfate, and non- ionic wetting agents that are used in the tanning process and may accumulate in the immediate environment of the tannery(4). There are two types of tanning. The first is the vegetable tanning in which plant extracts are employed to produce heavy leather such as belts and soles of footwear. The second is the chrome tanning which involves the treatment of skins/hides with chromium salts obtained by reduction of sodium dichromate reducing agent produces lighter leathers such as gloves and upper part of shoes. During the tanning process, at least about 300 kg chemicals are added per tonne of hides (5). A tannery is one of the important industries causing water pollution (6). There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins (7)(8). It was reported that the total amount of waste produced per slaughtered animal is approximately 35% of its weight (9). Also, for every 1000 kg of carcass weight, a slaughtered beef produces 5.5 kg of manure (excluding rumen contents or stockyard manure) and 100 kg of paunch manure (undigested food)(10). wastewater in the range of 30-35 L kg^{-1} skin/hide processed with variable pH, BOD, COD, high concentrations of suspended solids, and tannins including chromium (11). A single tannery can cause the pollution of groundwater around the radius of 7-8 kilometers (7).

Tannery effluent, when discharged into water bodies, alters the physical, chemical, and biological characteristics of water and depletes the dissolved oxygen, increases alkalinity, suspended solids, and sulfides which are deleterious to fish and other aquatic lives (12). Many conventional processes such as oxidation, chemical, and biological processes were carried out to treat wastewater from tanneries (6). Biological processes have received more attention because of their cost-effectiveness, lower sludge production and environmental friendliness in contrast to chemical/physical methods which are invariably cost-intensive and cannot be conveniently employed in all industries, especially in developing and underdeveloped countries (12). Microbes in the environment play an important role in cycling and destroying pollutants through bio-degradation (11). In the course of the last two decades, a wide variety of technologies had been developed for clean-up operations of contaminated sites. Bioremediation has evolved as the most promising one because of its economical safety and environmental features since organic contaminants become transferred and some of them are fully mineralized. Bioremediation of tannery effluents is an attractive environment-friendly, safe and cost-effective alternative technology to conventional methods. Microbes in the environment play an important role in cycling and destroying them through bio-degradation (3).

Bioremediation is an option that offers the possibility to destroy or render harmless various contaminants using natural biological activity. It is based on the idea that all organisms remove substances from the environment to carry out growth and metabolism. The principles of bioremediation are that the microbe feeds on contaminants, digest it, and this waste is transformed into water and harmless gases which are later expelled out of the microbes. The resultant metabolic wastes that they produce are generally safe and somehow recycled into other products. Although the microorganisms are present in contaminated water, they cannot necessarily be there in the numbers required for bioremediation of the site. Their growth and activity must be stimulated. Naturally occurring degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in due course of time. However, metallic residues cannot be degraded in composting but may be converted into organic combinations that have less bioavailability than mineral combinations of the heavy metals. Microbial transformation of metals serves various function generally, it occurs either by redox conversions of inorganic forms or conversions from inorganic to organic forms and vice versa. Bioremediation is amenable to a variety of organic and inorganic compounds and may be applied either *in situ* or *ex-situ*. In addition to this, it is easy to implement and maintain, does not require the use of expensive equipment or highly specialized personnel and is environmentally friendly and aesthetically pleasing to the public (13).

Immobilized bacteria can withstand various temperatures, pH and substrate concentrations; consequently increasing the efficiency and the lifespan of the bacteria. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency.

This study was carried out in Kano. Kano lies on Latitude 11°30′N and 8°30′N and Longitude 11°5′N and 8°5′E and 8°5′E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery and textile-related activities are the dominating industries and this could be one of the reasons for her high population density(14). The climate is characterized by well-defined wet and dry seasons. The wet season spreads from May to October, August usually being the wettest whereas the dry season lasts from November to April. River Challawa originates from the Challawa Gorge dam in Challawa village and stretches to River Kano that empties into Lake Chad. The river receives waste from tanneries and textile industries, urban water storm, and agricultural runoffs from farming communities along its course. River Challawa serves as fishing, farming, and water supplies for the communities in the area. The domestic water supply for Challawa, Sharada, and Bompai industrial areas and their surroundings originates from the Challawa River (14). Several workers have studied bioremediation of tannery effluent using microorganisms. However, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the Challawa industrial estate. This research work focused on the potential of the immobilized bacteria in the treatment of industry tannery effluent in the Challawa industrial estate.

MATERIAL AND METHODS

Sample Collection

Effluents were collected from the Mamuda Tannery Industry in Challawa Industrial Estates, Kano, Nigeria. The effluents were collected for a period of six months (August 2017 to January 2018). It was collected from the effluent reservoir in the industries in sterile 4-liter plastic containers with a unique identification number and was preserved using an ice-box that was transported to Microbiology Laboratory, Department of Microbiology, the Bayero University of Kano for analysis.

Sample Preparation and Analysis

Immediately after the collection of the effluent, pH, SS, TDS, COD, BOD, nitrate, sulfates, phosphates, chlorides, and heavy metals (Cr, Fe, Mn, Ni, Pb, Zn, Cd, and Cu) concentrations were determined. pH was determined using Ecotests pH meter and TDS was determined using AQUALYTIC TDS-Salinometer. BOD was determined as described by the standard method (7). COD, SS, nitrate, sulfates, phosphates, and chlorides were determined using DR/2010 HACH portable data logging spectrophotometer (8). The heavy metals were determined using Atomic Absorption Spectrophotometer (BUCK Scientific ACCUSYS 211) according to the manufacturer's instructions.

Isolation and Identification of the Bacteria from the Tannery Effluents

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA (15). The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red (5 drops of MR), nitrate reduction, Voges-Proskauer (VP), Starch Hydrolysis, Glucose, Maltose, and Lactose tests were carried out on the bacterial isolates to identify the bacteria through the bacteria biochemical characteristics according to Ajao *et. al.* (16).

Determination of Growth Rate of the Bacteria in Effluent Sample

The bacteria growth rates were determined by transferring 2 mL of the bacterial isolates from the tannery effluent in broth medium into 100 mL sterile effluents in conical flasks and kept in an incubator (Giffrin cool) for 10 days. Control was also set up by incubating another 100 mL each of the sterile effluents without the bacteria. The optical density of the content was determined at the wavelength of 600 nm on a daily interval and recorded.

Immobilization of Bacteria

Agar solution and inoculi were prepared separately. Fifty milliliters (50 mL) of nutrient broth each of the inoculi was prepared in a McCartney bottle and incubated for 24 hours. A solution of agar-agar was prepared by dissolving 10 g of the powder in distilled water and made up to 500 mL mark in an Erlenmeyer flask and was sterilized in an autoclave (280A) for 15 minutes and allowed to cool to 40-45 ˚C (16). Four milliliters (4 mL) of the bacterial isolates in the nutrient broth was mixed with 36 ml of the prepared agar-agar media in petri-dish plates and then allowed to solidify. This was kept in the refrigerator for bioremediation.

Bioremediation (Treatment) of the Effluents

The solidified agar block (immobilized bacteria) was cut into cubes using a sterile knife; 0.1 mL phosphate buffer (pH 7.0) was added and kept in the refrigerator for 1 hour for curing. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used. Five grams (5 g), 10 g, 15 g, 20 g and 25 g of the immobilized bacteria were then weighed (17).

Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), $MgSO_4.7H_2O$ (0.001), KH_2PO_4 (2), $NaNO_3$ (2), $CaCl_2.2H_2O$ (0.5) and $NaHPO_4.12H_2O$ (2) and sterilized in an autoclave at 121 °C for 15 minutes. Two hundred and fifty milliliters (250 mL) of the effluents were transferred into different 250 ml conical flasks. The content was covered with a cotton-wool ramped with foil paper to avoid contamination. Five grams (5 g) of the immobilized bacteria were quickly transferred into each of the effluents in the conical flasks in an inoculating chamber (18). The same procedures were carried out for the 10 g, 15 g, 20 g and 25 g of the immobilized bacteria in separate 250 mL effluents in conical flasks and agitated for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and velocity of 60 rpm.

The treated effluent samples were taken on the tenth day and analyzed for the parameters pH, SS, TDS, COD, BOD, nitrate, sulfates, phosphates, chlorides, and heavy metal concentrations (Post-treatment determination) at the different grams of bacteria to evaluate and compare the bioremediation efficiency.

Statistical Analysis

The data were represented as Mean ± Standard deviation and analyzed statistically using one-way Analysis of Variance (ANOVA) and Tukey's HSD as Post Hoc Tests with the aid of SPSS 16.0. The correlation coefficient was also used to measure the strength of the relationship between the different masses of the bacteria and the parameters. All $p \leq 0.05$ were considered as statistically significant.

RESULTS AND DISCUSSION

Results of the Physico-Chemical Parameters and Heavy Metals in Industrial Effluents before the Bioremediation

Results of the physicochemical parameters in the industrial effluents before the bioremediation were shown in Table 1. The mean level of temperature (29.50±4.68 °C) was found below the World Health Organization (WHO) (35 °C) and NESREA (National Environmental Standards and Regulatory Enforcement Agency) (40 °C) recommended standard limits; the average value of temperature observed in this present study is less than that observed (56.23 to 74.54 °C) by another researcher. High temperature brings down the solubility of gases in water that ultimately expresses as high BOD and COD (19). pH (5.35±1.57) was below the WHO (7.0-8.5) and NESREA (6-9). Maheshwari et al. (2017) reported that the level of pH in the effluents from the tannery industry in Vaniyambadi, India was 6.5 which was higher than that observed in the present study (20).

BOD (26.17±9.49 mg/L) was below the NESREA (200 mg/L) but above the WHO (10 mg/L). Maheshwari et al. (2017) reported that the level of BOD in the effluents from the tannery industry in Vaniyambadi, India was 348 mg/L which was higher than that observed in the present study. BOD is a measure of the content of organic substances in the wastewater which are biologically degradable with consumption of oxygen usually indicated as 5-day BOD. This is the amount of oxygen in milligrams per liter (mg/L) that consumed by microorganisms in 5 days at 20 °C for the oxidation of the biologically degradable substances contained in the water. The high values of BOD recorded are indicative of the presence of total solids in the effluents which are known to be organic with high oxygen demands for their oxidation under required conditions of temperature and oxidants and time, as a result, will naturally lead to the depletion of dissolved oxygen in the aqueous body. The biochemical oxygen demand of the untreated textile wastewater can cause rapid depletion of dissolved oxygen if it is directly discharged into the surface water sources another researcher (21).

The Chemical Oxygen demand (COD) is the amount of oxygen, in mg/L, required for the degradation of the compound of wastewater to occur. COD (3106±2753 mg/L) above the WHO (40 mg/L) and NESREA (40 mg/L). The level of COD in the effluents from the tannery industry in Vaniyambadi, India was 860 mg/L (20) which was lower than that observed in the present study. The higher the COD value of wastewater, the more oxygen demand to discharge water bodies another researcher(22). Akan et al. (2009) reported that a high COD value shows that the effluents have high oxygen demanding wastes which cause the depletion of Dissolved Oxygen (DO) which is a fundamental requirement for aquatic life.

SS (562±482 mg/L) is above the WHO (30 mg/L) and NESREA (10 mg/L). These high TSS values observed in all the industries understudied might be possible due to the presence of fine leather particles, residues from various chemical discharges and reagents from different waste liquors from tanneries and textile industries (23). The composition of solids present in tannery effluent mainly depends upon the nature and quality of hides and skins processed in the tannery (24). The average value of SS observed in this present study is less than that observed (3422±122 to 3700±122 mg/L) by Maheshwari et al. (2017). It is observed that a suspended solid absorb heat from sunlight, causing an increase in water temperature and subsequently decreases the level of dissolved oxygen (25).

TDS (444±507 mg/L) was above the WHO (250 mg/ L) but below the NESREA (500 mg/L). The composition of solids present in tannery effluent mainly depends upon the nature and quality of hides and skins processed in the tannery (24).

Nitrate (97.20±146.80 mg/L) was found above the WHO (50 mg/L) and NESREA (40 mg/L). Maheshwari et al. (2017) reported that the level of nitrate in the effluents from the tannery industry in Vaniyambadi, India was 95 mg/L which was lower than that observed in the present study. The high level of nitrate in the tannery effluents in the present study might be due to the release of ammonia from the deliming process and the presence of nitrogen in proteinaceous materials from unhairing operations. Momodu et al. (2017) have reported the presence of nitrate in receiving water which can lead to extensive undesirable algal growth associated with eutrophication.

Sulfates (268.34±411.01 mg/L) were below the NESREA (500 mg/L) but above the WHO (100 mg/L). The high level of sulfate in the tannery effluents in the present study might be due to the use of sulfuric acid. The sulfate and high phosphate concentration detected in the effluents are also known to cause eutrophication and algal boom (26).

Phosphate (18.82±34.717 mg/L) was found far above WHO (0.1 mg/L) and NESREA (3.5 mg/L). The sulfate and high phosphate concentration detected in the effluents are also known to cause eutrophication and algal boom (26).

Chlorides (22.59±19.64 mg/L) were below the recommended limits of WHO (250 mg/L) and NESREA (350 mg/L). The presence of chloride in the tannery effluents in the present study might be due to the use of sodium chloride in hide and skin preservation. Chlorides inhibit the growth of plants, bacteria, and fish in surface waters growing. The presence of these physicochemical parameters in the tannery effluent of the present study might be due to the nature of the raw materials and processes used in the tannery industries at the time of sampling.

The results of the heavy metals in the industrial effluents before the bioremediation were shown in Table 2. The mean level of chromium (7.528±4.530 mg/L) was found above the WHO (0.05 mg/L) and the NESREA (0.5 mg/L) recommended standard limits. Maheshwari (20) reported that the level of chromium in the effluents from the tannery industry in Vaniyambadi, India was 435 mg/L which was higher than that observed in the present study. The high concentration of chromium might be due to the use of chromium salts for tanning processes. Chromium can cause allergic reactions in the skin, damage the lungs, and asthma attacks maximum concentration of 0.1 mg/L was set up (27).

Iron (1.263±0.502 mg/L) was found above WHO (0.5 mg/L) and the NESREA (1.0 mg/L). Higher iron content may produce undesirable effects such as astringent taste, coloration, turbidity, deposits, and growth of iron bacteria in pipes affecting the acceptability of water for domestic use. Iron is an essential element in Maheshwari nutrition, and the health effect of iron in drinking water may include warding off fatigue and anemia (28).

Manganese (0.277 ± 0.03 mg/L) was above the NESREA (0.01 mg/L) and the WHO (0.02 mg/L). In another study, 1.02 mg/L of manganese was reported Yusuff and Sonobare, 2004 (29). Depending upon the exposure route, manganese may be among the least toxic of the trace elements if ingested low IQ of children is attributed to high manganese intake and hence at high concentration lead to neurotoxins and harms the brain (30).

Nickel (0.023 ± 0.021 mg/L) was above the NESREA (0.01 mg/L) and the WHO (0.02 mg/L). The concentration of nickel in the effluents from textile industries in Kano ranged (3.11 to 3.4 mg/L) (31) which is the same order of magnitude reported in the study by Ali et al (32). The most obvious anthropogenic source of nickel is scrap metal waste, notably alloyed metals including stainless steel. Nickel is considered as an essential trace element at very low concentrations. It does bioaccumulate in aquatic systems, and as such elevations above normal concentrations can result in deleterious aquatic effects (25).

Lead (0.304 ± 0.20 mg/L) was above the WHO (0.05 mg/L) and NESREA (0.1 mg/L). Galadima et al. (2012) (30) reported that the concentration of lead in the effluents from textile industries in Kano ranged (2.45 to 2.46 mg/L) which is the same order of magnitude reported in the study by Ali et al (32). Excess quantities of lead may impact Maheshwari health, especially affecting small children (33). The value 0.646 mg/L and 0.289 mg/L were reported for lead in wastewater samples from the textile industry in Kaduna (33).

Zinc (0.058± 0.05 mg/L) was below the WHO (5 mg/L) and the NESREA (0.2 mg/L). The concentration of zinc in the effluents from textile industries in kano ranged from 2.52 to 2.45 mg/L (31), which is the same order of magnitude reported by Ali et al. (2009). Zinc is a trace element that is essential for Maheshwari health; the danger of which can be to an unborn child when mothers absorbed large concentration of zinc and other health problem such as stomach cramps, skin irritation, vomiting and anemia (34).

Cadmium (0.068±0.02 mg/L) was above the NESREA (0.01 mg/L) but below the WHO (0.5 mg/ L). Galadima et al. (2012) reported that the concentration of cadmium in the effluents from textile industries in Kano ranged from 1.23 to 2.06 mg/L, which is the same order of magnitude reported by Ali et al. (2009). Cadmium is a nonessential trace element that enters the environment via anthropogenic activities such as industrial effluent, sewage-sludge, fertilizers, and pesticides. Cadmium adsorbs strongly to sediments and organic matter (35). It has a range of negative physiological effects on the organism, such as decreased growth rates and negative effects on embryonic development and children are likely to be exposed to cadmium when is highly toxic and absorbed in the skin and can cause lung damage and irritation with shortness of breath dry throat, headache, vomiting, extreme restlessness or irritability, etc (36). Other potential long-term effects are pulmonary damage and fragile bones (37).

Copper (0.012 ±0.02 mg/L) was found below WHO (1.00 mg/L) but above NESREA (0.01 mg/L) the recommended limits. Galadima et al. (2012) reported that the concentration of copper in the effluents from textile industries in Kano ranged from 1.06 to 1.01 mg/L. Copper is a reddish metal in a color that occurs naturally in rocks, soil, water, industrial activities, and sediment and has some practical uses in our society and are found in pipes, electrical wiring, and coins. The level of copper reported from the textile industry in Lagos ranged from 4.0 mg/L to 5.14 mg/L (29). Copper is generally remobilized with acid-base ion exchange or oxidation mechanism (33).

The presence of these heavy metals may be due to the addition of some raw materials containing these ions during the production process. It has been reported that the major problem associated with industrial effluents is the presence of heavy metal ions, which arise from the material used in the production process or a considerably high amount, from metal-containing raw materials (37). Heavy metals present as impurities in dye effluents or chelated as part of dye molecules. In metal complex dyes, the metal is coordinated or forms a chemical bond with the organic dye molecules. Thus, it is an indispensable constituent of dye and governs the fastness in absorbing colors. The highest value of heavy metal ions in the effluents severely affects soil fertility and depletes the soil quality and its nutrients. Besides, the variations of heavy metals concentration in the wastewater sample were due to the different types of dyestuff used in a different production of the threads when the samples were taken. The concentration of heavy metals could be a serious environmental nuisance if a large volume of such effluents is released into the environment regularly without proper treatment (31). In line with the findings by Yusuff and Sonibare (2004), Dan Azumi and Bichi (14) analyzed heavy metal in Kano from Challawa industrial estate and found high concentrations of metal ions. The presence of these heavy metals in the tannery effluent of the present study might be due to the nature of the raw materials and processes used in the textile industries at the time of sampling.

Table 1: Mean values (mg/L) ± S.D of physicochemical parameters in effluents from the industries before and after treatment of the effluents with the different masses of the bacteria.

Parameter	**Before**	**After**				
		5 g	**10 g**	**15 g**	**20 g**	**25 g**
Temperature(ºC)	29.50±4.68					
pH	5.35±1.57					
COD	3106±2753	1376ab±248	1588a±489	2279b±924	2681b±867	1881ab±626
BOD	26.17±9.49	0.81c±0.67	0.80b±0.61	0.80b±0.61	0.58bc±0.45	0.73b±0.38
SS	562±482	118ab±136	230ab±252	78ad±89	196bd±302	273ab±375
TDS	444±507	27be±16	25bc±15	25bc±16	27bc±18	25bd±20
Nitrate	97.20±146.80	18.97a±15.93	14.43a±8.88	18.67a±16.94	26.52a±13.06	20.28a±7.85
Chloride	22.59±19.64	12.90a±18.39	3.95bc±4.78	8.87a±10.95	5.54ab±4.66	8.25a±10.68
Sulfate	268.34±411.01	18.12a±19.26	15.99a±17.37	5.95b±8.91	13.31b±18.55	8.59b±12.82
Phosphate	18.82±34.71	2.03a±3.11	1.90a±3.15	2.10a±3.00	1.76b±2.74	1.76b±2.64

Replicate= 6 (months), Within the columns, means with different letters are statistically different ($p<0.05$).

Table 2: Mean values (mg/L) ± S.D of heavy metals in effluents from the industries before and after the treatment of the effluents with the different masses of the bacteria.

Parameter	**Before**	**After**				
		5 g	**10 g**	**15 g**	**20 g**	**25 g**
Cr	0.171a±0.12	0.049a±0.05	0.046a±0.07	0.013a±0.02	ND	0.170a±0.00
Fe	0.822±0.63	0.532c±0.11	0.488b±0.18	0.485b±0.36	0.202a±0.21	0.287ab±0.29
Mn	0.217a±0.48	0.002a±0.00	ND	ND	ND	ND
Ni	0.285±0.06	0.180a±0.07	0.180a±0.09	0.100a±0.10	0.070a±0.11	0.055a±0.11
Pb	0.273±0.20	0.118a±0.05	0.076a±0.04	0.073a±0.04	0.051a±0.05	0.043a±0.05
Zn	0.035±0.03	0.028a±0.02	0.028ac±0.03	0.027a±0.02	0.020ab±0.02	0.013a±0.02
Cd	0.068±0.02	0.058a±0.02	0.058a±0.03	0.050a±0.02	0.058a±0.03	0.063a±0.02
Cu	0.172±0.16	0.125b±0.12	0.068b±0.08	0.122b±0.13	0.100b±0.11	0.078b±0.08

Replicate= 6 (months), Within the columns, means with different alphabets are statistically different ($p<0.05$).

Table 3. Preliminary identification of the bacterial isolates.

Gram staining	Shape	Spore formation
+	Rod	-

+ = Positive - = Negative

Table 4. Biochemical characteristics of the bacterial isolates.

Citrate	Catalase	Coagulase	Starch	Glucose	Oxidase	Lactose	Mannitol	Maltose	MR	Nitrate Reduction
-	+	+	+	+	-	-	-	+	-	-

MR=Methyl Red

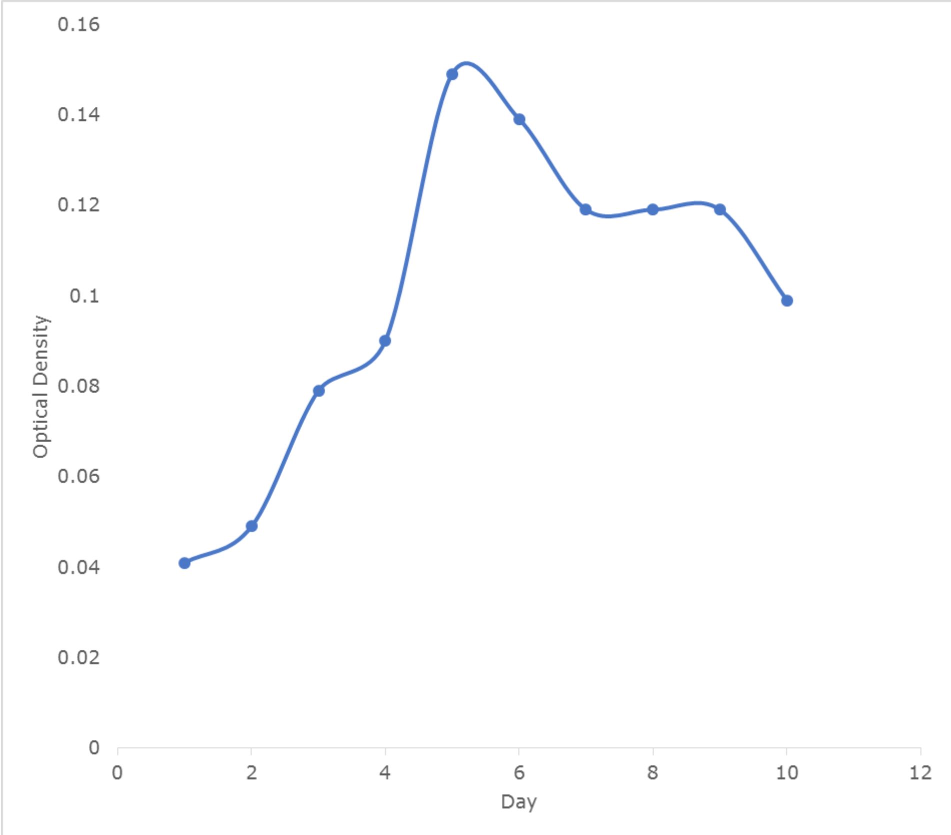

Figure 1: Growth rates (Cells/mL/day) of the *Corynebacterium kutsceri* in the effluent sample from the tannery industry.

Table 5: The correlation coefficient (r) between different masses of bacteria and physicochemical parameters.

Parameter	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
SS	0.54*	30
TDS	-0.35	12
BOD	-0.62	39
COD	0.63*	40
NITRATE	0.53*	28
SULFATE	-0.68	46
PHOSPHATE	-0.69	48
CHLORIDE	-0.37	14

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

SS=Suspended Solid, TDS=Total Dissolved Solid, BOD=Biochemical Oxygen Demand and COD=Chemical Oxygen Demand

Table 6: The correlation coefficient (r) between different masses of bacteria and heavy metals.

Heavy metals	Correlation coefficient (r)	Percent dependence (rxrx100) (%)
Cr	-0.29	9
Fe	-0.85	72
Mn	-0.76	58
Ni	-0.99	97
Pb	-0.89	79
Zn	-1.00	99
Cd	0.33	11
Cu	-0.39	15

The correlation coefficient (r) with * is statistically significant ($p<0.05$).

Table 7: Percentage reduction of the physicochemical parameters after-treatment of the effluents (250 mL) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the *Corynebacterium kutsceri.*

Parameter (mg/L)	Percentage reduction (%)				
	5 g	10 g	15 g	20 g	25 g
COD	56	49	27	14	39
BOD	97	97	97	98	97
SS	79	59	86	65	51
TDS	94	94	94	94	94
Nitrate	80	85	81	73	79
Chloride	43	83	61	75	63
Sulfate	93	94	98	95	97
Phosphate_	89	90	89	91	91

Table 8: Percentage reduction of the heavy after-treatment of the effluents (250 mL) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Corynebacterium kutsceri.

Parameter (mg/L)	Percentage reduction (%)				
	5 g	10 g	15 g	20 g	25 g
Cr	33	60	28	46	52
Fe	42	55	62	54	75
Mn	36	57	71	50	86
Ni	38	43	50	64	72
Pb	48	58	51	67	73
Zn	17	26	34	40	51
Cd	15	15	27	15	7
Cu	86	43	100	71	86

Results of the Preliminary Identification and Biochemical Characteristics of the Bacterial Isolates

Results of preliminary identification and biochemical characteristics of bacterial isolates were shown in Tables 3 and 4 respectively. After 48 hours of incubation, the nutrient agar media plates were checked for bacteria growth. The results showed that the bacterial isolate was found to be gram-positive, rod-shaped and recorded negative results for spore formation. The results of the biochemical test indicated that the bacteria were positive for catalase, maltose, glucose, starch hydrolysis, and coagulase tests. The bacteria showed negative results for citrate, oxidase, nitrate reduction, MR, lactose and mannitol tests. Base on the Preliminary Identification and biochemical test results, the bacteria isolated were identified to be *Corynebacterium kutsceri (C. kutsceri)*.

Mohammed *et al*., (2012) (3) investigated the enriched inoculums of *Corynebacterium sp* and *Pseudomonas putida,* separately inoculated into anthracene-contaminated water at the temperature of 28 °C under an optimum pH of 7.2 for 96 hours. Maheshwari et al., (2017) (20) isolated and immobilized *Pseudomonas putida* and *Bacillus cereus* isolated from the tannery effluents of Vaniyambadi, India.

Figure 1 shows the growth rates of *C. kutsceri* in the effluent sample from the tannery industry. The initial growth phase of the *C. kutsceri* occurred on the 3rd day of incubation as the growth rate increases up to the 5th-day incubation when the maximum growth was observed. Beyond the 5th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its phase of death (which might be due to the unavailability of nutrients in the effluents). This implies that the isolate can bioremediate most in the effluent on 5th day.

Results of the Physicochemical Parameters and Heavy Metals in the Industrial Effluents after the Bioremediation

Table 1 shows the mean results of the physicochemical parameters in the industrial effluents before and after the bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the *C. kutsceri*. Also, Table 5 shows the results of the correlation coefficient (r) between the different mean masses of bacteria and the physicochemical parameters.

The mean value (mg/L) of the SS after the bioremediation varies between 78±89 and 273±375. The relative potential or efficiency of the different masses of the *C. kutsceri* in remediating the SS was in the order 15 g>5 g>20 g>10 g>25 g. A positive and significant correlation (0.54*) exists between the masses of the *C. kutsceri* and the SS.

There was a low correlation (30 %) between the masses of the *C. kutsceri* and the SS.

The mean value (mg/L) of the TDS after the bioremediation was between 25±15 and 27±16. A negative and no significant correlation (-0.35) exist between the masses of the *C. kutsceri* and the TDS. There was a low correlation (12%) between the masses of the *C. kutsceri* and the TDS.

The mean value (mg/l) of the BOD after the bioremediation varies between 0.58b±0.45 and 0.81±0.67. In the present study, the values of the BOD agree with the report Maheshwari *et al*. (2017) (20) in which the concentration of nitrate decreased in biologically (immobilized *Pseudomonas putida* and *Bacillus cereus*) treated tannery wastewater. The relative potential or efficiency of the different masses of the *C. kutsceri* in remediating the BOD was in the order 20 g>25 g>15 g=10 g>5 g. A negative and no significant correlation (-0.62) exists between the masses of the *C. kutsceri* and the BOD. There was a low correlation (39%) between the masses of the *C. kutsceri* and the BOD.

The mean value (mg/L) of the COD after the bioremediation varies between 1376±248 and 2681±867. In the present study, the values of the COD agree with the report Maheshwari *et al*. (2017) (20) in which the concentration of nitrate decreased in biological (immobilized *Pseudomonas putida* and *Bacillus cereus*) treated tannery wastewater. The relative potential or efficiency of the different masses of the *C. kutsceri* in remediating the COD was in the order 5 g>10 g>25 g>15 g>20 g. A positive and significant correlation (0.63*) exists between the masses of the *C. kutsceri* and the COD. There was a low correlation (40 %) between the masses of the *C. kutsceri* and the COD.

The mean value (mg/L) of the nitrate after the bioremediation varies between 14.43±8.88 and 26.52±13.06. In the present study, the values of the nitrate agree with the report Maheshwari *et al*. (2017) (20) in which the concentration of nitrate decreased in biological (immobilized *Pseudomonas putida* and *Bacillus cereus*) treated tannery wastewater. The relative potential or efficiency of the different masses of the *C. kutsceri* in remediating the nitrate was in the order 10 g>5 g>15 g>25 g>20 g. A positive and significant correlation (0.53*) exists between the masses of the *C. kutsceri* and nitrate. There was a low correlation (28%) between the masses of the *C. kutsceri* and the nitrate.

The mean value (mg/L) of the sulfate after the bioremediation varies between 5.95±8.91 and 18.12±19.26. The relative potential or efficiency of the different masses of the *C. kutsceri* in remediating the sulfate was in the order 15 g>25 g>20 g>10 g>5 g. A negative and no significant correlation (-0.68) exist between the masses of the *C. kutsceri* and the sulfate. There was an average correlation (46 %) between the masses of *C. kutsceri* and the sulfate.

The mean value (mg/L) of the phosphate after the bioremediation varies between 1.76±2.64 and 2.10±3.00. The relative potential or efficiency of the different masses of the *C. kutsceri* in remediating the phosphate was in the order 25 g>20 g>10 g>5 g>15 g. A negative and no significant correlation (-0.69) exist between the masses of *C. kutsceri* and the phosphate. There was an average correlation (48%) between the masses of *C. kutsceri* and the phosphate.

The mean value (mg/L) of the chloride after the bioremediation varies between 5.54±4.66 and 12.90±18.39. The relative potential or efficiency of the different masses of the *C. kutsceri* in remediating the chloride was in the order 10 g>20 g>25 g>15 g>5 g. A negative and no significant correlation (-0.37) exist between the masses of *C. kutsceri* and the chloride. There was a very low correlation (14%) between the masses of *C. kutsceri* and the chloride. The decrease in the concentration of the physicochemical parameters after the bioremediation was not only due to the increase in the mass of *C. kutsceri* but might be also due to the increase in surface area of the different mass of *Corynebacterium Kutsceri.*

Table 2 shows the mean results of the heavy metals in the industrial effluents before and after the bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the *C. kutsceri*. Also, Table 6 shows the results of the correlation coefficient (r) between the different mean masses of bacteria and heavy metals.

The mean value (mg/L) of the chromium after the bioremediation varies between 3.048±2.70 and 5.416±4.07. This is in agreement with the work of Maheshwari *et al*. (2017) (22) in which the concentration of chromium decreased in biologically (immobilized *Pseudomonas putida* and *Bacillus cereus*) treated tannery wastewater. The relative potential or efficiency of the different masses of the *C. kutsceri* in remediating the chromium was in the order 10 g>25 g>20 g>5 g>15 g. A negative and no significant correlation (-0.29) exists between the masses of the *C. kutsceri* and the chromium. There was a very low correlation (9%) between the masses of the *C. kutsceri* and the chromium.

The mean value (mg/L) of the iron after the bioremediation varies between 0.310±0.20 and 0.732±0.11. The relative potential or efficiency of the different masses of the *C. kutsceri* in remediating the iron was in the order 25 g>15 g>10 g>20 g>5 g. A negative and no significant correlation (-0.85) exist between the masses of the

C. kutsceri and the iron. There was a high correlation (72%) between the masses of the *C. kutsceri* and the iron.

The mean value (mg/L) of the manganese after the bioremediation varies between 0.003±0.01 and 0.015±0.01. The relative potential or efficiency of the different masses of the *C. kutsceri* in remediating the manganese was in the order 25 g>15 g>10 g>20 g>5 g. A negative and no significant correlation (-0.76) exists between the masses of the *C. kutsceri* and the manganese. There was an average correlation (58 %) between the masses of the *C. kutsceri* and the manganese.

The mean value (mg/L) of the nickel after the bioremediation varies between 0.077±0.06 and 0.172±0.05. The relative potential or efficiency of the different masses of the *C. kutsceri* in remediating the nickel was in the order 20 g>25 g>15 g>10 g>5 g. A negative and no significant correlation (-0.99) exists between the masses of *C. kutsceri* and the nickel. There was a very high correlation (97%) between the masses of the *C. kutsceri* and the nickel.

The mean value (mg/L) of the lead after the bioremediation varies between 0.082±0.11 and 0.158±0.20. The relative potential or efficiency of the different masses of the *C. kutsceri* in remediating the lead was in the order 25 g>20 g>10 g>15 g>5 g. A negative and no significant correlation (-0.89) exists between the masses of the *C. kutsceri* and the lead. There was a high correlation (79%) between the masses of the *C. kutsceri* and the lead.

The mean value (mg/L) of the zinc after the bioremediation varies between 0.028±0.03 and 0.048±0.04. The relative potential or efficiency of the different masses of the *C. kutsceri* in remediating the zinc was in the order 20 g>25 g>15 g>10 g>5 g. A negative and no significant correlation (-1.00) exist between the masses of the *C. kutsceri* and the zinc. There was a very high correlation (99%) between the masses of the *C. kutsceri* and the zinc.

The mean value (mg/L) of the cadmium after the bioremediation varies between 0.050±0.02 and 0.063±0.02. The relative potential or efficiency of the different masses of the *C. kutsceri* in remediating the cadmium was in the order 15 g>5 g>20 g=1 g>25 g. A positive and no significant correlation (0.33) exists between the masses of the *C. kutsceri* and the cadmium. There was a very low correlation (11%) between the masses of the *C. kutsceri* and the cadmium.

The mean value (mg/L) of the copper after the bioremediation varies between not detected and 0.007±0.00. The relative potential or efficiency of the different masses of the *C. kutsceri* in remediating the copper was in the order 15 g>25 g>20 g>5 g>10 g. A negative and not significant correlation (-0.39) exists between the masses of the *C. kutsceri* and the copper. There was a low correlation (15%) between the masses of the *C. kutsceri* and the copper. There was a reduction in the concentration of all the heavy metals after the bioremediation process when compared with the concentration of the raw samples before the bioremediation. This is in agreement with the work of Maheshwari *et al*. (2017) in which the concentration of chromium decreased in biologically (immobilized *Pseudomonas putida* and *Bacillus cereus*) treated tannery wastewater. This is also in line with the report made by Ajao et al. (2011) and Galadima et al. (2012). Also, Table 6 shows the results of the correlation coefficient (r) between the different mean masses of bacteria and heavy metals. In the present study, the decrease in the concentration of the heavy metals after the bioremediation was not only due to the increase in the mass of the bacteria but might be also due to the increase in surface area of the different mass of the bacteria.

Percentage reduction of the physicochemical parameters and the heavy metals by the *Corynebacterium Kutsceri*

Table 7 shows the percentage reduction of the physicochemical parameters after the treatment of the effluents (250 mL) by the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the *C. kutsceri.* The percentage reduction (%) of SS ranged (51-86); TDS (94); BOD (97-99); COD (14-56); nitrate (73-85); chloride (43-83); sulfate (93-98); phosphate (89-92).

Table 8 shows the percentage reduction of the heavy metals after-treatment of the effluents (250 mL) with the different masses (5 g, 10 g, 15 g 20 g, and 25 g) of the *C. kutsceri.* The percentage reduction (%) of Cr ranged (28-60); Fe (42-75); Mn (36-86); Ni (38-72); Pb (48-73); Zn (17-51); Cd (7-27) and Cu (43-100). Maheshwari *et al*. (2017) studied the bioremediation by free and immobilized bacteria isolated from tannery effluent. The percentage reduction of nitrate and nitrite was ranged from 45 to 75% when treated with *P. putida*, whereas they were ranged from 60 to 79% on the 15^{th} day of the treatment. The level of BOD was reduced drastically after the 15^{th} day of exposure by the free cells of both bacteria. The maximum percentage of reduction (83%) was shown by immobilized *B. cereus.* The percent reduction of COD ranged from 16% to 65% in both bacterial strains. The percentage of removal of Cr(VI) was 86% and 91% for free and immobilized cells, respectively. In *B. cereus* treated sample, it reached 84% and 89% on the 15^{th} day respectively. It was concluded that both *P. putida* and *B. cereus* investigated in this study are highly recommended for beneficial bioremediation applications for *in-situ* and off-site removal of pollutants.

Bioremediation of textile industrial effluent using the mixed culture of *Pseudomonas aeruginosa* and *Bacillus subtilis* immobilized on agar-agar in a bioreactor was carried out by Ajao et al. (2011). The result indicates overall % reduction in COD, BOD, nitrate, sulfate, phosphates as 83%, 97%, 61.3%, 62.8%, 61.2% respectively. Heavy metals were also bio-sorbed. It was concluded that immobilized cells represent a promising application in the bioremediation of textile industrial effluent and possible reusability of the cells for its commercial application can be achieved.

In general, immobilization makes the enzyme more resistant to temperature, pH, and substrate concentration swings giving it a longer lifetime and higher productivity per active unit (35). Although several workers described microbial degradation of tannery effluent, limited literature is available on the bioremediation of tannery effluent using immobilized bacterial cells in the present study area.

CONCLUSION

The tannery industrial effluent samples contained variable levels of the physicochemical parameter and heavy metals, in which some of them were higher than the recommended standard limits. The presence of these physicochemical parameters and heavy metals might be due to the nature of the raw materials and processes used in the tannery industries at the time of sampling. Based on the preliminary identification and biochemical test results, the bacterial isolate was identified to be *C. kutsceri*. There was an overall decrease in the concentration of the physicochemical parameters and the heavy metals after the bioremediation using the different masses of the immobilized *C. kutsceri*. However, the decrease was not only due to the increase in the mass of the *C. kutsceri* but might be also due to the increase in surface area of the different mass of the immobilized *C. kutsceri*. The average high percentage reduction (70% to 100%) of these parameters and heavy metals implies that the immobilized *C. kutsceri* is having a higher potential for the treatment of the tannery industrial effluents.

ACKNOWLEDGMENTS

The authors are grateful to the Kano State Ministry of Environment for their support in obtaining the effluent samples.

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