



Anions Bioremediation Potential of Immobilized Bacteria in Tannery Industrial Effluents from Kano State, Nigeria

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ABSTRACT

The present study aims to assess the potentials of immobilized bacteria in the remediation of anions within the effluents by determining the levels of the anions before and after the remediation. Industrial Effluents Samples from Gashash Tanneries (TAN1), Larabee Tannery Industry (TAN2) and Z Tannery Industries (TAN3) in Kano State, Nigeria were collected monthly for six months (August 2017 to January 2018). Bacteria were isolated from the effluents and immobilized on agar-agar. The effluent samples were analyzed for the anions before and after the treatment using DR/2010 HACH portable data logging spectrophotometer. Different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria were used in the treatment of 250 ml of the effluents for ten days in a shaker incubator (Gallenkamp-OC-4364-L) at the temperature 30 °C and speed of 60 rpm. Pre-treatment analysis of the effluents for nitrate (NO₃⁻), Sulphate (SO₄²⁻), Phosphate (PO₄³⁻) and Chloride (Cl⁻) give the following results; NO₃⁻ ranged (25.35±17.16-28.12±10.72 mg/l); SO₄²⁻ (67.00±5.93-114.83±53.20 mg/l); PO₄³⁻ (26.16±22.93-34.17±16.73 mg/l) and Cl⁻ (22.57±9.64-26.00±4.86 mg/l). No statistical difference (p ≤ 0.05) was observed for all the anions among the different industries. The bacterial isolates were identified as *Neisseria spp*, *Bacillus cereus*, and *Staphylococcus aureus*, in TAN1, TAN2, and TAN3, respectively. After treatment of the effluents with the different masses of the isolated bacteria, the mean level of NO₃⁻ were found to range as (6.17±6.67-20.78±7.76); SO₄²⁻ (25.73±17.84-46.00±46.81); PO₄³⁻ (11.45±12.02-28.19±9.09) and Cl⁻ (0.38±1.83-15.36±13.58). The results of Post-treatment analysis showed that there is an overall decrease in the levels of the anions determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilized bacteria in the treatment of the respective effluents was in the order TAN3 (57%)>TAN1 (49%)>TAN2 (47%). Therefore, the immobilized bacteria are having higher potentials for the treatment of the anions in the effluents.

1. Introduction

Tanning is the chemical process that converts animal hides and skin into leather [1]. The release of effluents directly from tanneries into bodies of water has become a growing environmental challenge as it is one of the major sources of pollution [2]. Most of these effluents are complex mixtures containing inorganic compounds [3] including anions such as SO₄²⁻, NO₃⁻, PO₄³⁻ [2]. The characteristics of tannery effluent vary considerably from tannery to tannery depending upon the size of the

tannery, chemicals used for a specific process, amount of water used, and type of final product produced by a tannery effluent from a typical tannery [4]. There are more than 6000 tanneries in Nigeria with an annual processing capacity of 700,000 tons of hides and skins [5, 6]. It was reported that the total amount of waste produced per animal slaughtered is approximately 35% of its weight [7]. 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) [8].

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The excessive concentration of nutrients in wastewater, particularly nitrogen and phosphorus leads to the eutrophication of water bodies (such as lakes and rivers) and subterranean water contamination [9-11]. Additionally, high concentrations of ammonium ion in water bodies can be toxic to aquatic organisms and ultimately to humans when converted to nitrate [12]. Contamination with high concentrations of chloride ions in water can damage metal pipes and concrete structures [13]. The presence of chloride in tannery effluents is the result of the addition of NaCl in a large number of common salts for the preservation and pickling processes of skins and hides. The presence of chloride ions increases the salinity of the receiving water body. The high level of chlorides in effluents pollutes the drinking water is responsible for the growth of microbes. Hence, resulting in water-borne diseases

Many conventional processes such as oxidation, chemical, and biological processes were carried out to treat tanneries wastewater [14]. Numerous physiochemical methods utilized for wastewater treatment is also applicable to tannery wastewater, but these processes are expensive. Biological treatment of wastewater is more favorable and cost-effective as compared to other physiochemical methods [14]. Various microorganisms are capable of reducing the content of pollutants significantly by utilizing them as energy and nutrient sources in the presence or absence of oxygen [15]. Biological processes have received more attention because of their cost-effectiveness, lower sludge production, and environmental friendliness [16]. Naturally occurring micro-organisms degrade the hazardous organic wastes including xenobiotic compounds, such as pesticides, polycyclic aromatic hydrocarbons (PAHs), and polychlorinated biphenyls (PCBs) in due course of time [17]. Bioremediation is based on the idea that all organisms remove substances from the environment to carry outgrowth and metabolism [18]. Bacteria, protists, and fungi are found to be very good at degrading complex molecules and incorporating the breakdown products into their metabolism [18]. The resultant metabolic wastes that they produce are generally safe and somehow recycled into other organisms [17]. An acclimatized indigenous population of microorganisms capable of degradation of the compounds of interest must exist at the site for a successful bioremediation mode [17].

Microbial biochemical processes play useful roles in the safe disposal of tannery waste. These processes are influenced by pH, temperature, nutrient availability, temperature, biomass behavior, and concentration [19]. Organisms in the tannery effluents have been reported to adopt measures that enable them to tolerate and utilize the hazardous constituent of tannery effluents. Such measures include the acquisition

of plasma [20]. Indigenous organisms thus are well adapted for the treatment of tannery effluent [21]. In a bid to use indigenous organisms that have been well adapted to the constituents of tannery effluent, it is imperative that optimum conditions that will enhance their ability to detoxify the effluent be investigated [21]. The knowledge of these conditions which will enhance their optimal growth will provide information on the conditions that will make them function optimally for the elimination of the toxic components of tannery effluent [21].

Indigenous or native bacteria are bacteria that are naturally present in the sample due to their adaptation. The growth and activity of these bacteria are normally stimulated by adding nutrient (minimum basal medium) to the effluent. Immobilized bacteria are bacteria that have been confined to a limited region by attachment to a solid structure. Immobilized bacteria can withstand various temperatures, pH, and substrate concentrations; consequently, increasing the efficiency and the lifespan of the bacteria [22]. Immobilized bacteria are widely applied in the treatment of wastewater and can be separated and recovered after the treatment with the same efficiency [22].

2. Experimental

2.1. Study Area

This study was carried out in Bompai, Sharada, and Challawa industrial estates in Kano, Figure 1. Kano lies on Latitude 11°30'N and 8°30'E and Longitude 11°5'N and 8°5'E in Northern Nigeria. It is one of the developed industrial cities in Nigeria. Tannery activities are the dominating industries and this could be one of the reasons for her high population density [23]. Many researchers have studied the bioremediation of tannery effluent using microorganisms. However, limited literature is available on the remediation of anions in tannery effluents in Kano industrial estates using immobilized bacterial cells. This research work focuses on the potential of the use of the indigenous immobilized bacterial isolates in the treatment of anions in tannery effluents in the industrial estates. The aim of the present study is to assess the potentials of immobilized bacteria in the remediation of anions within the effluents by determining the levels of the anions before (Pre-treatment) and after (Post-treatment) the remediation

2.2. Sample Collection

Effluents were collected from the Tannery Industries in Bompai, Challawa, and Sharada Industrial Estates, Kano, Nigeria. The effluents were collected monthly for six months (August 2017 to January 2018). Samples collected in a sterile 4-liter plastic container with a unique identification number were preserved using an ice-box that was transported to the

Microbiology Laboratory, Department of Microbiology, Bayero University of Kano for analysis

2.3. Sample Pre-treatment Analysis

Immediately after the collection of the effluent, Nitrate, Sulphate, Phosphate, and Chloride levels were determined before treatment (Pre-treatment determination) using DR/2010 HACH portable data logging spectrophotometer [24].

Nitrates in the wastewater samples were determined by using cadmium Reduction method 8036 (Powder Pillows). Phosphates were determined by using Ascorbic Acid method 8048 (Powder Pillows). Sulphates were determined by using by using Sulfa Ver method 8051 (Powder Pillows) while Chlorides were

determined by using Mercuric Thiocyanate method 8113 [24].

2.4. Identification and Biochemical Characterization of the Bacterial Isolates

The bacteria were isolated from the effluents using Serial Dilution according to the method described by APHA [25]. The biochemical tests such as oxidase, catalase, coagulase, indole (from 1% tryptone broth), citrate (Simmons citrate agar), methyl red/Voges-Proskauer (MR/VP), nitrate reduction, 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.* [26].

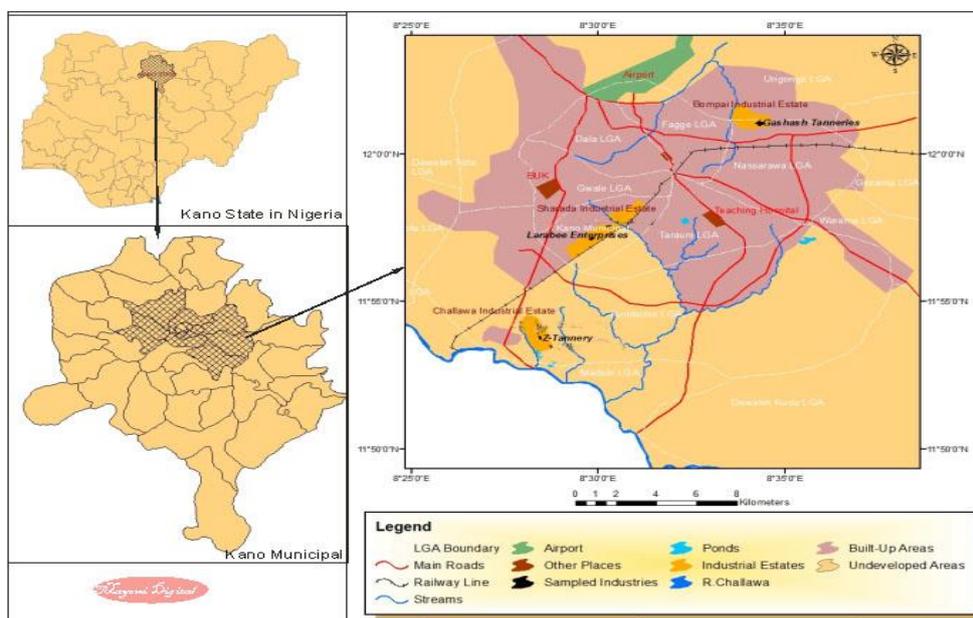


Fig. 1 Map showing the study areas Source: Mayomi Digital Productions, GIS Laboratory, Department of Geography, UNIMAID (2017)

2.5. 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 (Giffirin cool) for 10 days. Controls were 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.

2.6. 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 [26]. 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. These were kept in the refrigerator for bioremediation [22].

2.7. 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 [27]. The phosphate buffer was decanted after 1 hour and the cubes were washed with sterile distilled water 3-4 times before it was used [27].

Two liters (2 L) of the effluent was supplemented with the minimum basal medium in g/L: NaCl (0.8), MgSO₄·7H₂O (0.001), KH₂PO₄ (2), NaNO₃ (2), CaCl₂·2H₂O (0.5) and NaHPO₄·12H₂O (2) and sterilized in an autoclave at 121°C for 15 minutes [28]. 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. The same procedure was 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 a temperature 30 °C and speed of 60 rpm.

2.8. Sample Post-treatment Analysis

The treated effluent samples were taken on the tenth day and analyzed for the anions (Post-treatment determination) for the different grams of bacteria to evaluate and compare the bioremediation potential [22, 24].

2.9. 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.

3. Results and Discussion

3.1. Results of the anions in the Industrial Effluents before the bioremediation.

Results of the anions in the industrial effluents before the Biodegradation is shown in table 1.

3.1.1. Nitrate (NO₃⁻)

The mean level of Nitrate (mg/l) observed in TAN1, TAN2, and TAN3 samples were 28.12±10.72; 26.27±4.27 and 25.35±17.16 respectively. The order of the mean nitrate in the samples from the three industries can be arranged as TAN1>TAN2>TAN3. The nitrate observed in TAN1, TAN2 and TAN3 samples fall below the recommended limits of WHO (50 mg/l) and NESREA (40 mg/l). 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 the proteinaceous skins from unhairing activities. The presence of nitrate in receiving water can lead to eutrophication and consequently unfavorable algal growth.

Statistical analysis shows that there was no significant difference ($p < 0.05$) between the mean values

of nitrate among the industries. This might be due to the related tannery processes involved in the tannery industries at the time of sampling. The average values of nitrates observed in this present study are less than that observed by Baba et al. [22] but higher than that of Mohammed et al. [29].

3.1.2. Sulphate (SO₄⁻)

The mean Sulphate (mg/l) observed in TAN1, TAN2, and TAN3 samples were 114.83±53.20; 67.00±5.93 and 105.72±43.08 respectively. The order of the mean sulphate in the samples from the three industries can be arranged as TAN1>TAN3>TAN2. The mean level of sulphate observed in TAN1, TAN2 and TAN3 samples fall below the recommended limits of NESREA (500 mg/l). Also, the mean sulphate in TAN1, TAN2, and TAN3 samples fall below the recommended limits of WHO (100 mg/l) except TAN1 and TAN3. The high concentrations of the sulphate in the effluents can lead to eutrophication and consequently unfavorable algal growth. The high level of sulphate in the tannery effluents might be due to the use of sulphuric acid. The high level of sulphate was observed in the effluent because of the use of sulphuric acid or product with high sodium sulphate content during the tanning process [30].

Statistical analysis shows that there was no significant difference ($p < 0.05$) between the mean values of sulphate among the industries. This might be due to the related tannery processes involved in the tannery industries at the time of sampling. The average values of sulphates observed in this present study are less than those observed by Mohammed et al. [29] and Baba et al. [22].

3.1.3. Phosphate (PO₄³⁻)

The mean levels of phosphate (mg/l) observed in TAN1, TAN2 and TAN3 samples were 26.16±22.93; 34.17±16.73 and 31.6±7.73 respectively. The order of the mean phosphate in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The mean levels of Phosphate observed in TAN1, TAN2 and TAN3 samples were far above the recommended standard limits of regulating bodies WHO (0.1 mg/l) and NESREA (3.5 mg/l). The high phosphate concentration detected in the effluents can lead to eutrophication and consequently unfavorable algal growth.

Statistical analysis shows that there was no significant difference ($p < 0.05$) between the mean values of phosphate among the industries. This might be due to the related tannery processes involved in the tannery industries at the time of sampling. The average values of phosphates observed in this present study are higher than those observed by Mohammed et al. [29] and Baba et al. [22].

3.1.4. Chloride (Cl)

The mean levels of chlorides (mg/l) observed in TAN1, TAN2 and TAN3 samples were 22.57±9.64;

26.00±4.86 and 24.93±4.85 respectively. The order of the mean chloride in the samples from the three industries can be arranged as TAN2>TAN3>TAN1. The mean level of chloride observed in TAN1, TAN2 and TAN3, samples fall below the recommended limits of WHO (250 mg/l) and NESREA (350 mg/l). High levels of chlorides in the tannery effluent could be attributed to the soaking and pickling processes [31]. The chloride content in the water sample gives an idea of the salinity of the water sample [31]. In the current study, the presence of chloride in the tannery effluents might be due to the use of sodium chloride in hide and skin preservation. A high level of chlorides prevent the growth of plants and fish in waters.

Statistical analysis shows that there was no significant difference ($p<0.05$) between the mean values of chloride among the industries. This might be due to the related tannery processes involved in the tannery industries at the time of sampling. The average values of chlorides observed in this present study are less than those observed by Mohammed et al. [29] and Baba et al. [22].

3.2. Identification, Biochemical Characterization and growth rates of the Bacterial Isolates

Results of identification and biochemical characterization of the bacterial isolates were shown in table 2. After 24 hours of incubation, the nutrient agar media plates were checked for bacterial growth.

The results showed the presence of different strains in the samples. The TAN1 bacteria isolate was found to be gram-negative cocci while TAN3 was gram-positive cocci. TAN2 bacteria isolate was found to be gram-positive, rod-shaped. TAN1, TAN2, and TAN3 bacteria isolates recorded positive results for spore former. The results of the biochemical tests indicated that all the bacteria were positive for catalase, oxidase, citrate, maltose, glucose, lactose (negative in TAN1), mannitol (negative in TAN2), starch hydrolysis and coagulase (negative in TAN2) tests. The bacteria showed negative

results for nitrate reduction, MR (positive in TAN2), VP (positive in TAN1), Indole (positive in TAN2) tests. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacteria isolates were identified to be *Nesseria spp.*, *Bacillus cereus*, and *Staphylococcus aureus* respectively.

The growth rate of the TAN1, TAN2, and TAN3 Isolates were shown in figure 2. Generally, the optical density increase with the increase in time (day) and decreases as time goes on. The highest optical density was shown by *bacillus cereus* in TAN2 while the lowest was shown by *Staphylococcus aureus* in TAN3.

The initial growth phase of TAN1 Isolate bacteria occurred within 2-day of incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

A similar trend of growth was also observed for TAN2 Isolate as the initial growth phase also occurred within 2-day of incubation but maximum growth rate observed on the 4th day of incubation. The stationary stage occurred between the 4th day and the 8th day. Beyond the 8th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluents).

The initial growth phase of TAN3 bacterial Isolate occurred within the 3-day incubation as the growth rate increases up to the 6th-day incubation when the maximum growth was observed. Beyond the 6th day, the growth of the bacteria declined (which might be due to a shortage of nutrients in the effluents) until it reached its death phase (which might be due to the unavailability of nutrients in the effluent).

Table 1. Mean Values ± S.D of anions in effluents from the Tannery Industries before Treatment.

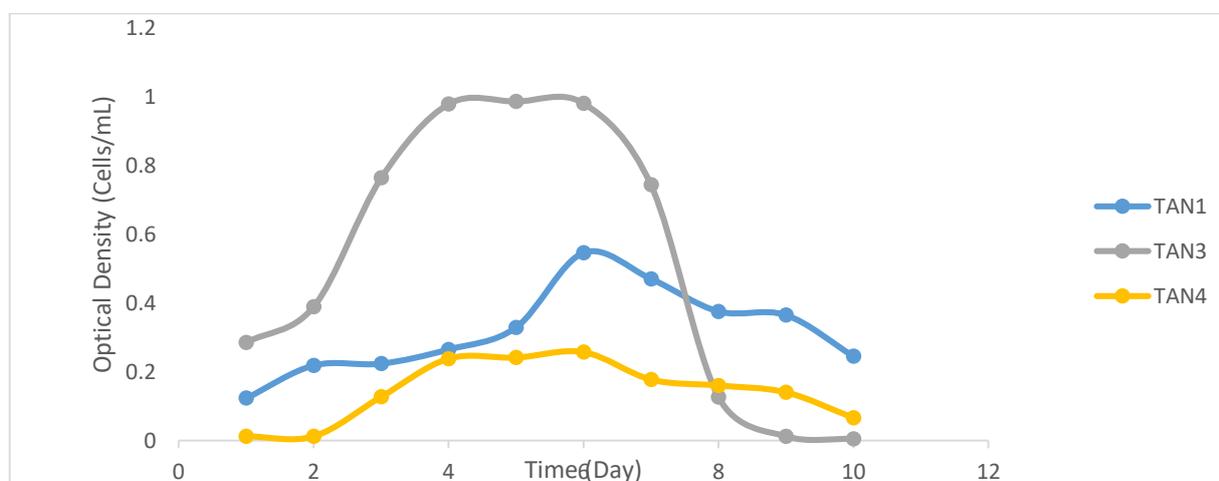
Anions (mg/l)	Tannery 1	Tannery 2	Tannery 3	Mohammed et al.	Baba et al.	WHO, 2006	NESSRA, 2009
Nitrate	28.12a±10.72	26.27a±4.27	25.35a±17.16	12	97.20±146.80	50	40
Chloride	22.57a±9.64	26.00a±4.86	24.93a±4.85	1011.2	22.59±19.64	250	350
Sulphate	114.83a±53.20	67.00a±5.93	105.72a±43.08	1522	268.34±411.01	100	500
Phosphate	26.16a±22.93	34.17a±16.73	31.6a±7.73	2.2	18.82±34.71	0.1	3.5

The values given in the table above are means of six replicate values, n=6 (1 sample was taken monthly for 6 months). Within the rows, means with different alphabets are statistically different ($p<0.05$). WHO: World Health Organization [32]. NESREA: National Environmental Standard and Regulatory Enforcement Agency [33]

Table 2. Morphological and Biochemical characteristics of bacterial isolates

Bacterial Isolates		TAN1	TAN2	TAN3
<i>Morphological characteristics</i>				
	Shape	Cocci	Rod	Cocci
	Spore former	+	+	+
	Gram reaction	-	+	+
<i>Biochemical characteristics</i>				
	Citrate	+	+	+
	Catalase	+	+	+
	Coagulase	+	-	+
	Starch	+	+	+
	Glucose	+	+	+
	Oxidase	+	+	+
	Indo	-	+	-
	Lactose	-	+	+
	Mannitol	+	-	+
	Maltose	+	+	+
	MR	-	+	-
	VP	+	-	-
	Nitrate Reduction	-	-	-
	Bacterial name	Neisseria spp	Bacillus Cereus	Staphylococcus aureus

+ = Positive; - = Negative; MR=Methyl Red; VP= Voges-Proskauer

**Fig. 2** Growth rates (Cells/mL/day) of the isolates in the effluents from the Tannery Industries

3.3 Results of the anions in the Industrial Effluents after the bioremediation.

Table 3 shows the mean results of the Anions before and after bioremediation using the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the respective immobilized bacteria. Also, Table IV shows the mean results of the correlation coefficient (r) between different masses of bacteria and anions.

The mean values (mg/l) of the nitrate after the bioremediation varies between 6.17 ± 6.67 and 20 ± 78 . The mean concentration (mg/l) of nitrate remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of

the bacteria varies. There is a reduction in the levels of all the nitrate of the samples after the bioremediation process when compared with the nitrate of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating nitrate in TAN1, TAN2, and TAN3 samples were in the order $10g > 20g > 25g > 5g > 15g$, $25g > 10g > 20g > 15g > 5g$ and $15g > 25g > 20g > 10g > 5g$ respectively. Statistical analysis shows that there is no significant difference ($p < 0.05$) between the mean values of nitrate among the masses in the respective industries. The decrease in the concentration of the anions after the bioremediation was

not only due to the increase in the mass of immobilized bacteria but might be also due to the increase in surface area of the different mass of immobilized bacteria [22]. Negative and insignificant correlations exist between the masses of bacteria and nitrate. The negative correlations showed that there is a general decrease in the levels of the nitrates as the masses of the immobilized bacteria increases. TAN1 (1%) showed a very low correlation with the masses of the bacteria while TAN2 (63%) and TAN3 (60%) showed a high correlation. According to Baba *et al.* (2020), the decrease in the concentration of the anions after the bioremediation was not only due to the increase in the mass of immobilized bacteria but might be also due to the increase in surface areas of the different masses of immobilized bacteria.

The mean values (mg/l) of the sulphate after the bioremediation varies between 25.73 ± 17.84 and 48.35 ± 34.85 . The mean concentration (mg/l) of sulphate remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the sulphate levels of all the samples after the bioremediation process when compared with the sulphate of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating sulphate in TAN1, TAN2, and TAN3 samples were in the order $25g > 15g > 20g > 10g > 5g$,

$20g > 10g > 25g > 5g > 15g$ and $10g > 25g > 20g > 15g > 5g$ respectively. Statistical analysis shows that there is no significant difference ($p < 0.05$) between the mean values of sulphate among the masses in the respective industries. Negative and insignificant correlations exist between the masses of bacteria and sulphate. The negative correlations showed that there is a general decrease in the levels of the sulphates as the masses of the immobilized bacteria increases. TAN1 (75%) showed a high correlation with the masses of the bacteria while TAN2 (23%) and TAN3 (28%) showed low correlation. According to Baba *et al.* (2020), the decrease in the concentration of the anions after the bioremediation was not only due to the increase in the mass of immobilized bacteria but might be also due to the increase in surface areas of the different masses of immobilized bacteria.

The mean values (mg/l) of the phosphate after the bioremediation varies between 25.73 ± 17.84 and 48.35 ± 34.85 . The mean concentration (mg/l) of phosphate remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in all the phosphate levels of the samples after the bioremediation process when compared with the phosphate of the raw samples before the bioremediation. The relative potential or efficiency of

the different masses of the bacteria in remediating phosphate in TAN1, TAN2, and TAN3 samples were in the order $5g > 25g > 20g > 15g > 10g$, $20g > 25g > 10g > 15g > 5g$ and $5g > 15g > 25g > 20g > 10g$ respectively. Statistical analysis shows that there are significant differences ($p < 0.05$) between the mean values of phosphate among the masses in the respective industries with the exception in TAN2. Positive (with the exception in TAN2) and insignificant correlations exist between the masses of bacteria and phosphate. The positive correlations showed that there is a general increase in the levels of the phosphate as the masses of the immobilized bacteria increases. This might be due to the addition of the nutrients (basal medium) containing the phosphate for the growth and activities of the immobilized bacteria. TAN2 (66%) showed a high correlation with the masses of the bacteria while TAN1 (4%) and TAN3 (5%) showed a very low correlation. According to Baba *et al.* (2020), the decrease in the concentration of the anions after the bioremediation was not only due to the increase in the mass of immobilized bacteria but might be also due to the increase in surface areas of the different masses of immobilized bacteria

The mean values (mg/l) of the chloride after the bioremediation varies between $0.38a \pm 1.83$ and $15.36a \pm 13.58$. The mean concentration (mg/l) of chloride remediated by the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria varies. There is a reduction in the chloride levels of all the samples after the bioremediation process when compared with the chloride of the raw samples before the bioremediation. The relative potential or efficiency of the different masses of the bacteria in remediating chloride in TAN1, TAN2, and TAN3 samples were in the order $5g > 10g > 15g > 25g > 20g$, $25g > 20g > 5g > 10g > 15g$ and $10g > 20g > 25g > 15g > 5g$ respectively. Statistical analysis shows that there is no significant difference ($p < 0.05$) between the mean values of chloride among the masses in the respective industries with the exception in TAN3. Negative (with the exception in TAN1) and insignificant (with the exception in TAN1) correlations exist between the masses of bacteria and chloride. The negative correlations showed that there is a general decrease in the levels of the chloride as the masses of the immobilized bacteria increases. TAN1 (71%) showed a high correlation with the masses of the bacteria while TAN2 (33%) and TAN3 (25%) showed low correlation. According to Baba *et al.* (2020), the decrease in the concentration of the anions after the bioremediation was not only due to the increase in the mass of immobilized bacteria but might be also due to the increase in surface areas of the different masses of immobilized bacteria

Table 3. Mean Values (mg/l) \pm S.D of anions in effluents from the Tannery Industries before and after Treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the bacteria.

Anions	Industries	Before	After				
			5 g	10 g	15 g	20 g	25 g
Nitrate	TAN1	28.12±10.72	15.40a±25.76	7.73a±2.49	20.55a±17.47	10.82a±8.76	12.32a±8.33
	TAN2	26.27±4.27	20.78a±7.76	12.05a±6.72	14.7a±6.44	13.28a±10.24	9.88a±9.06
	TAN3	25.35±17.16	19.21a±16.64	15.49a±17.18	6.17a±6.67	9.77a±10.47	9.15a±10.48
Sulphate	TAN1	114.83±53.20	48.35a±34.85	46.00a±46.81	34.75a±29.75	38.75a±37.14	34.48a±39.10
	TAN2	67.00±5.93	38.67a±24.95	33.83a±20.26	39.83a±23.85	27.17a±12.53	34.50a±23.71
	TAN3	105.72±43.08	36.17a±21.75	25.73a±17.84	34.25a±14.46	29.92a±7.90	26.25a±14.59
Phosphate	TAN1	26.16±22.93	11.45a±12.02	21.02b±27.90	20.54b±27.87	16.70b±21.24	16.08b±19.67
	TAN2	34.17±16.73	28.19a±9.09	18.92b±4.33	20.60b±5.68	16.38ab±5.00	17.46ab±7.12
	TAN3	31.6±7.73	13.67a±4.73	18.95a±5.66	14.51a±5.01	17.63a±6.04	15.83a±6.22
Chloride	TAN1	22.57±9.64	10.34a±7.46	10.88a±8.00	11.63a±8.70	15.36a±13.58	13.69a±5.74
	TAN2	26.00±4.86	12.5a±6.79	14.02a±11.15	14.72a±9.58	10.87a±6.67	10.83a±8.83
	TAN3	14.82±7.98	0.38a±1.83	0.44b±1.47	0.64a±1.27	0.46b±1.40	0.39a±1.68

Replicate= 6 (months).

Within the rows, for the same anions, means with different alphabets are statistically different ($p < 0.05$).

Table 4. The correlation coefficient (r) between different masses of the bacteria and the anions.

Industries	Anions	Correlation coefficient (r)	Percent dependence ($r \times 100$) (%)
TAN1	Nitrate	-0.10	1
	Sulphate	-0.86	75
	Phosphate	0.20	4
	Chloride	0.84*	71
TAN2	Nitrate	-0.79	63
	Sulphate	-0.48	23
	Phosphate	-0.81	66
	Chloride	-0.58	33
TAN3	Nitrate	-0.77	60
	Sulphate	-0.53	28
	Phosphate	0.22	5
	Chloride	-0.50	25

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

Generally, there was an overall decrease in the concentration of these anions after the bioremediation using the different masses of the bacterial isolates. The negative correlations showed that there is a general decrease in the levels of the chloride as the masses of the immobilized bacteria increases. This is in line with the work of Jimoh et al. [34] and Baba et al. [22]. According to Baba et al. (2020), the decrease in the concentration of the anions after the bioremediation was not only due to the increase in the mass of immobilize bacteria but might be also due to the increase in surface areas of the different masses of immobilized bacteria.

3.4. Percentage reduction of the Anions

Table V shows the percentage reduction of the anions in industrial samples before and after the treatment of the effluents (250 ml) with the different

masses (5 g, 10 g, 15 g, 20 g, and 25 g) of the Immobilized Bacteria.

In TAN1 samples, the percentage reduction (%) of Nitrate ranged (21-73); Sulphate (32-54); Phosphate (58-70), and Chloride (20-56). In TAN2 samples, the percentage decrease (%) of Nitrate ranged (21-62); Sulphate (43-58); Phosphate (41-59), and Chloride (18-52). In TAN3 samples, the percentage decrease (%) of Nitrate ranged (24-76); Sulphate (47-61); Phosphate (66-76), and Chloride (40-57). The average percentage reduction of anions in the present study was found below the percentage reduction of anions obtained by Baba et al. (2020).

Sreemoyee and Priti [35] assessed and reduced several Physico-chemical parameters of dairy

wastewater using *Neisseria sp.* and concluded that the species are well known to degrade organic compounds.

The optimum pH Biosorption of Chromium by *Bacillus* spp and *Staphylococcus* spp. from tannery effluent was investigated by Mythili and Karthikeyan, [36]. The maximum adsorption of Chromium (86.4 mg/L) was showed by *Bacillus* spp and *Staphylococcus* spp showed 70.6 mg/L at an initial concentration of 100 mg/L. In the present study, immobilized *Bacillus* spp and *Staphylococcus* spp. from the tannery industrial effluents reduced the levels of the pollutants with high potential as indicated by the percentage reduction of the anions.

Uma *et al.* [37] studied the effect of free and immobilized cells of effluent tolerant bacteria (*Bacillus cereus*) isolated from the tannery effluents of Vaniyambadi to treat effluent water. The percentage reduction of Nitrate was ranged from 60 to 79% in the

15thday of the treatment. It was concluded that the *B. cereus* investigated in the study is highly recommended for beneficial bioremediation applications for in-situ and off-site removal of pollutants.

Enzymes often can work in multiple environments especially if they are immobilized. This makes the microorganisms' enzymes even more resistant to harsh environments and enables the enzymes to be recovered and recycled after they are no longer needed [38]. Ramesh and Singh [39] reported that the immobilized bacteria having more efficiency to remove the suspended particles than free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which makes it more economical. Similar work was done by Sikander *et al.* [40]. Results indicated that immobilized *B. cereus* could be efficiently used for the reduction of Cr (VI).

Table 5. Percentage reduction of the anions from the tannery industrial samples before and after treatment of the effluents (250 ml) with the different masses (5 g, 10 g, 15 g 20 g and 25 g) of the Immobilized Bacteria.

Industries	Anions (mg/l)	Percentage Reduction (%)				
		5 g	10 g	15 g	20 g	25 g
TAN1	Nitrate	45	73	27	62	56
	Sulphate	54	52	48	32	39
	Phosphate	58	60	70	66	70
	Chloride	56	20	21	36	39
TAN2	Nitrate	21	54	44	49	62
	Sulphate	52	46	43	58	58
	Phosphate	42	50	41	59	49
	Chloride	18	45	40	52	49
TAN3	Nitrate	24	39	76	61	64
	Sulphate	47	61	51	58	57
	Phosphate	66	76	68	72	75
	Chloride	57	40	54	44	50

Percentage Reduction= (B-A)/B ×100%

A= Concentration of the anions after treatment, B= Concentration of the anions before treatment

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 [41, 42-44]. Immobilized cells have been used and studied extensively for the production of useful chemicals [45], the treatment of wastewaters [46]. There is limited literature on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

4. CONCLUSION

The samples contained variable levels of the anions. The results of the Analysis of variance revealed that no statistical difference ($p < 0.05$) was observed for all the anions among the three tannery industries before the treatment. The levels of phosphates in the samples and

sulphates (in some samples) were found above the recommended limits of WHO and NESREA, which called for the treatment of the effluents before discharge into the environment. Base on the morphological and biochemical test results, TAN1, TAN2, and TAN3 bacterial isolates were identified to be *Neisseria spp*, *Bacillus cereus*, and *Staphylococcus aureus* respectively. The results of Post-treatment analysis showed that there is an overall decrease in the levels of the anions determined when compared with that of the pre-treatment. The overall percentage reduction of the immobilized bacteria in the treatment of the respective effluents was in the order TAN3 (57%)>TAN1 (49%)>TAN2 (47%). Therefore, the immobilized bacteria are having higher potentials for the treatment of the anions in the effluents.

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 [41, 42-44]. Immobilized cells have been used and studied extensively for the production of useful chemicals [45], the treatment of wastewaters [46]. There is limited literature on the bioremediation of tannery effluent using immobilized bacterial cells in the Kano Industrial Estates.

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Compliance with ethical standards

Conflicts of interest No potential conflict of interest was reported by the authors.

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