Isolation of De-Colourizing Bacteria for Bioremediation of Waste-Water From Textile Industry

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Abstract: *Textile industries are well-characterized for consuming large quantities of water, energy, and discharging high volumes of waste into public sewage treatment plants. The present study was carried out with the aim to isolate and use bacteria for decolourization of textile effluents. Bacteria were isolated from wastewater sample collected in July, 2023 from one of the textile industries in Kano, Nigeria. Twenty (20) different colonies of bacteria grown on nutrient agar were tested for their ability to grow on wastewater agar prepared using textile wastewater as the only carbon source. However, only four (4) isolates with identification codes; 3, 14, 18 and 20 were found to have the ability to grow on the wastewater agar and thus; were used for the treatment in the research. Consortium of all the isolates was also included in the treatment in order to determine their synergistic performance and the highest percentage color removal was 85.83% achieved using consortium. Physicochemical parameters such as pH, chemical oxygen demand (COD), total suspended solids (TSS), and ammonical-nitrogen concentration of the textile wastewater sample were determined both before and after the treatments. The results revealed a significant decolourization in the treatment samples incubated anaerobically without shaking. Therefore, the bacteria strains isolated have potential applications for bioremediation of textile effluent and could be used in bioreactors to treat waste water streams.*

Keywords: *Wastewater effluent; Bacterial isolate; Consortium; Decolourization; Physicochemical parameters.*

INTRODUCTION

Wastewater or effluents from the textile and other dye-stuff industries contain significant amounts of synthetic dyes that require treatment to prevent groundwater contamination [1]. The textile industry is a diverse sector in terms of production of raw materials, operating processes, product development, and equipment. Textile industries are well-characterized for consuming large quantities of water, energy, and discharging high volumes of waste in to public sewage treatment plants [2]. Reactive dyes, including many structurally different dyes, are extensively used in the textile industry because of their wide variety of color shades, high wet fastness profiles, ease of application, brilliant colors, and minimal energy consumption. The three most common groups are azo, anthraquinone and phthalocyanine dyes [3], most of which are toxic and carcinogenic [4]. The delivery of colour in the form of dyes onto textile fibres is not an efficient process, as a result, most of the wastewater produced by the textile industry is coloured [2]. Discharge of these dyes into the environment causes serious damage, since they may significantly affect the photosynthetic activity of hydrophytes by reducing light penetration and also they may be toxic to some aquatic organisms due to their breakdown products [5].

Major characteristics of environmental concern that are associated with textile wastewater include residual dyes especially **azo dyes**, toxic organic compounds, pH, colour, large amount of suspended solids, high temperature and biochemical/chemical oxygen demand (BOD_5/COD) [6]**.** Many dyes contain organic compounds with functional groups, such as carboxylic (–COOH), amine (–NH2), and azo (–N=N–) groups, so treatment methods must be tailored to the chemistry of the dyes. The need for a cost-effective process to remove the colour from wastewater produced by the textile industry has been recognised. Alternative approaches to colour removal, utilising microbial biocatalysts to reduce the dyes that are present in the effluent offer potential advantages over physio-chemical processes. Such systems are now the focus of recent research. The use of microbes and Membrane filtration processes such as nanofiltration reverse osmosis are promising technologies for an eco-friendly approach to treating textile effluent for **reuse** since it consumes less water and energy. Researchers have revealed that organic materials in wastewater can be remove biologically by the action of microorganisms especially bacteria [6]**.**

Many azo dyes and their degradation intermediates are mutagenic and carcinogenic [7, 8] and contribute immensely to the mutagenic activity of ground and surface waters that are polluted by textile effluents [9, 10]. For the degradation of dyes in coloured wastewater, the use of whole cells rather than isolated enzymes is advantageous, because costs associated with enzyme purification are negated and the cell can also offer protection from the harsh process environment to the enzymes [11]. Also, degradation is often carried out by a number of enzymes working sequentially [2].

Textile wastewater being generated by textile industries contains highly toxic compounds that pose serious environmental problem [12]. For the degradation of dyes

in coloured wastewater, the use of whole cells rather than isolated enzymes is advantageous, because costs associated with enzyme purification are negated and the cell can also offer protection from the harsh process environment to the enzymes. Also, degradation is often carried out by a number of enzymes working sequentially [2]. There are so many physicochemical tecniques for color removal from wastewater effluents containing dyes [13, 14].

Approaches in Biotechnology for bioremediation of polluted wastewaters are also receiving attention, but are not yet utilized by the industries because more concern has been given to aromatic amines production generated under anaerobic conditions [15]. Nevertheless, a lot of investigations have shown that anaerobic– aerobic treatment processes are very effective for complete colour removal of wastewater effluent and that should ultimately prove to be environment-friendly and cost-effective alternative to chemical decomposition processes [16]. Moreover, decolourization and degradation can also detoxify the effluents. Recent fundamental work has revealed the existence of a wide variety of microorganisms capable of decolourizing a wide range of dyes [16].
Wastewater effluent is mainly used as an inoculum to initiate biodegradation, and it shows that various microbes can decolorize azo dyes [7, 17, 18]. Accumulation of dyestuff and dye wastewater creates not only environmental pollution, but also medical and aesthetic problems. Most of the conventional methods used in the treatment are either ineffective or very expensive. For this reason, there is an urgent need for alternative approaches that are technically feasible and cost-effective. Microbial and enzymatic decolorization and degradation of dyes have significant potential to address this problem due to their eco-friendly and inexpensive nature [16]. Further development of biotreatment processes will be enhanced by identification of the most effective microbes and ways to minimize the time required to process polluted wastewater effluents for decolourization during treatment. In research aimed at biotechnology for treatment of textile waste-water, the current study was conducted to isolate dye-decolorizing bacteria from textile wastewater and to utilize them in the treatment of real textile effluent.

2.0 MATERIALS AND METHODS

2.1 Materials

Some of the materials used include: micropipette, pipette tip, test tubes, centrifuge tubes, cuvette, glass spreader, membrane filter, wire loop, Bunsen burner, ethanol sprayer, beaker, conical flask, Scott bottles, HACH tube, BOD glass bottles (300-Ml), petri dishes, universal bottles, measuring cylinder, nutrient agar, nutrient broth, wastewater sample, distilled water. Reagents for COD, BOD, and ammonical-nitrogen were also provided for use.

2.2 Isolation of Bacteria from the Textile Wastewater

The sample of wastewater was collected in July, 2023 from one of the textile industries in Kano, Nigeria and transported to the Environmental Laboratory and kept until it assumed room temperature. Serial dilutions of sample were prepared by transferring 1ml of the sample into 9ml of sterile distilled water in a test tube to make 10^{-1} dilution, it was then serially diluted until 10^{-7}. Nutrient agar plates were prepared and separately inoculated with 100µL aliquot from 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} respectively using spread plate technique. The procedure was duplicated to allow for incubation at two different temperatures. One set of the inoculated plates (1 from each of the four chosen dilutions) were incubated for 24 hours at 30°C and the other set at 37°C. After 24 hours of incubation, the colonies grown were observed and their respective morphological appearance was recorded. Twenty separate colonies (five from each of the four positive N.A plates) were then assigned numbers and sub-cultured on both nutrient agar and wastewater agar (50% wastewater + 50% Dh_2O + agar) using streaking method. Both plates were then incubated at 30°C and 37C for 2 days.

2.2.1 Selection of decolouring bacteria

After 2 days of incubation, the colonies that were able to grow on both nutrient agar and wastewater agar (positive results) were selected and sub-cultured into nutrient broth for multiplication of the bacteria to be used for the treatment. The selected colonies were also inoculated onto agar slant to keep the isolates for subsequent used/identification. Both nutrient broth and slant agar were incubated for 24 hours at 30°C.

2.3 Wastewater Treatment for Decolourization

2.3.1 Preparation of broth culture for the Treatment

The four bacteria isolates selected based on their ability to grow on both Nutrients agar and Wastewater agar were separately inoculated into four different test tubes each containing about 30ml of sterile nutrient broth. The tubes were then incubated in the oven at 37^0C for 24hour in order to get actively growing cells for the treatment.

2.3.2 Treatment procedure

The treatment of the textile wastewater was carried out under two different conditions (Aerobically with shaking and anaerobically without shaking). Six test tubes were used for each treatment condition. Initially, the wastewater was sterilised by autoclaving at 121^0C for 15minutes and allowed to cool to room temperature after which about 35ml was transferred to each of the test tubes labelled according to the isolate number (3, 14, 18 and 20) with the

fifth and sixth test tubes labelled as consortium and negative control respectively for set A. Each of the first four test tubes (3, 14, 18 and 20) was then inoculated with 1000µL of the respective broth culture of the isolates (3, 14, 18 and 20), the fifth test tube (consortium) was inoculated 250 µL from all the four different broth culture, while the sixth test tube (negative control) was left uninoculated. Another set (B) was prepared using the same procedure. Finally, set A tubes were incubated aerobically at 37^0C with shaking at 150 rpm for 24hours to check for decolourization. Set B were incubated at 30^0C without shaking for 24hours. After incubation, the tubes were observed for decolourization and samples were taken for colour determination and other physicochemical analysis.

2.4 Characterization of the Textile Wastewater effluent

Physicochemical parameters of the wastewater sample such as pH, colour, Total Suspended Solid (TSS), COD and Ammonical-nitrogen were determined both before and after the treatments.

2.4.1 pH Measurement

The pH was measured using digital pH meter (Delta 320) according to standard method. Initially, the pH meter was calibrated using standard pH solutions. Supernatant of the sample was used for the measurement after centrifugation at 4000rpm for 10 minutes. Electrode of the pH meter was rinsed with distilled water after every usage. Measurement was repeated after treatment procedure.

2.4.2 Determination of Colour Intensity

Colour intensity determination was done according HATCH reactor digestion method. The sample was initially centrifuged at 4000rpm for 10 minutes. 1:10 dilution of the supernatant was prepared using phosphate buffer (pH 7.6). 100µl of the diluted supernatant was transferred into 1.5ml cuvette after which the optical density (OD) was measured using Spectrophotometer (HACH DR5000) at 620nm wave length. The final value of colour intensity was determined by multiplying ADMI value measured by the spectrophotometer and the dilution factor.

2.4.3 Determination of Total Suspended Solid

To obtain an estimated total suspended solid (TSS), a well-mixed sample was filtered through an inorganic filter. Nylon filter paper was initially dried in oven at 105^0C for 1 hour and allowed to cool in desiccator. The initial weight of the filter paper was measured and recorded as W1. The filter paper was then mounted unto the filtration apparatus after which 10ml of the textile wastewater was filtered using vacuum pump. After filtration, the filter paper was transferred into the muffle furnace and dried at 105^0C for 24hours followed by cooling in desiccator for 20-30 minutes and then measurement of the final weight W2.

Calculation;

$$\text{Total suspended solids (mg /Ml)} = \frac{(W2\text{-}W1)}{\text{Sample volume, Ml}}$$

Where:

W2 = weight of filter + dried residue in mg, and
W1 = weight of filter in mg.

2.4.4 Determination of Chemical Oxygen Demand (COD)

The supernatant of the sample was used for COD measurement after centrifuged at 4000rpm for 10 minutes. 1ml of each sample was transferred into COD tube; 1.75ml of silver sulphate solution was then added followed by 0.75ml of dichromate reagent. The COD tubes were heated at 150˚C for 2 hours using COD reactor (HACH DRB 200). After 2 hours, the tubes were allowed to cool down and then the COD level of each tube was measured using Spectrophotometer (HACH DR5000).

2.4.5 Determination of Ammonical-Nitrogen

Determination of ammonical-nitrogen concentration was achieved using HACH program 2400. 1:50 dilution was initially prepared by mixing 0.5 Ml of wastewater sample with 24.5Ml deionized water in a 50 Ml centrifuge tube. After that, 1ml of Nessler reagent, 3 drops of mineral stabilizer and 3 drops dispersing agent (alcohol) were added and the miture shaken vigorously for 1 minute. The mixture was allowed to settle for 10 minutes to allow reaction to take place. After 5 minutes, the sample was poured into a sample cell, and then transferred into the cell holder with a lid. Blank was prepared by adding all three reagents to 25Ml deionized water into another sample cell. Concentration of ammonical-nitrogen was measured by using Hach DR 4000 Spectrophotometer. A yellow colour would appear if ammonical-nitrogen was present in the sample.

3.0 RESULTS

3.1 Bacterial isolation

For bacteria isolation, after 24 hours incubation, only plates inoculated with 10^{-4} and 10^{-5} dilutions showed positive growth (Table 1). The morphological features of the colonies on nutrient agar differ only slightly from white to milky coloured and they are mostly circular with smooth edges.

Table 1: *Shows results of spread plate technique*

Dilution factor	Incubation Temp. (°C)	Growth of bacteria on Nutrient Agar
10^{-4}	30	+
	37	+
10^{-5}	30	+
	37	+
10^{-6}	30	-
	37	-
10^{-7}	30	-
	37	-

3.2 Selection of bacteria for the treatment

Out of the twenty colonies (numbered 1-20) tested on the wastewater agar, only four (i.e. 3, 14, 18 and 20) were able to grow after 24hour of incubation. These isolates were considered as potential isolates for the treatment. Table 2 shows the results of selection.

Table 2: *Showing the growth response of the selected colonies on wastewater agar*

Test	Isolate number																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
GR	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	+

KEY: GR= Growth on wastewater agar

3.3 Treatment for decolourization (or Bioremediation)

Biological treatment using the selected bacteria isolated from the textile wastewater was carried out. Set B which was subjected to facultative anaerobic conditions (incubated at 30°C without shaking), revealed positive result for treatment process. The color of the sterile wastewater sample apparently disappeared in all the test tube including consortium except for the negative control as shown in figure 1. For set A which was provided by aerobic condition (kept at 37°C with shaking) no significant colour change was observed.

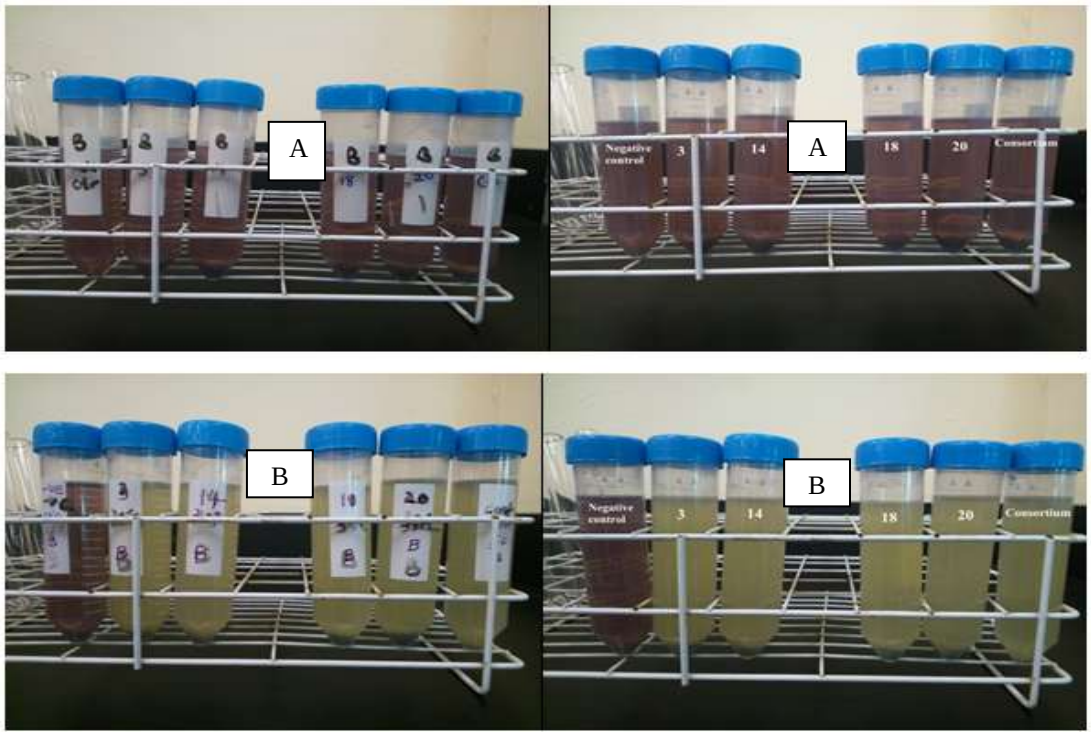

Figure 1: Shows set A (top) and set B (bottom) test tubes used for the treatments

Table 3: *Treatment plan based on the number of selected bacteria*

Tube	Isolates
1	-ve control
2	3
3	14
4	18
5	20
6	Consortium (3+14+18+20)

3.4 Characterization of the Wastewater effluent

The colour intensity of the sample before the treatment was found to be 1270 ADMI. After the treatment, the measurements of colour intensity were significantly reduced in which the highest percentage colour removal was 85.83% achieved using consortium of the isolates (table 4).

Table 4: *Shows colour intensity and percentage (%) reduction of the sample*

Isolate code	Color intensity (ADMI)			
	Set A		Set B	
	After treatment	% reduction	After treatment	% reduction
-ve control	480	62.20	620	51.18
3	520	59.06	460	63.78
14	570	55.12	370	70.87
18	780	38.58	630	50.39
20	740	41.73	440	65.35
Consortium	740	41.73	180	85.83

Table 5: *Shows concentrations of pH, Ammonical-nitrogen, Total suspended solid, and COD after treatments*

Isolate code	pH				Ammonical-nitrogen (mg/L)		Total suspended solid (mg/L)		COD (mg/L)			
	Set A		**Set B**		**Set A**	**Set B**	**Set A**	**Set B**	**Set A**		**Set B**	
	After treatment	% reduction	After treatment	% reduction	After treatment	After treatment	After treatment	After treatment	After treatment	% reduction	After treatment	% reduction
-ve control	8.76	7.88	7.79	18.09	5.5	11.5	0.15	0.19	1740	71.66	1550	74.76
3	7.85	17.46	6.88	**27.66**	17	145	0.13	0.28	230	96.25	1590	74.10
14	7.84	17.56	7.01	26.29	16.5	131	0.21	0.16	2150	64.98	1340	78.18
18	8.03	15.56	7.00	26.39	15	142	0.18	0.51	1940	68.40	2950	51.95
20	7.92	16.72	7.23	23.97	16	114	0.13	0.10	1620	73.62	1190	80.62
Consortium	7.92	16.72	7.08	25.55	19	**111**	0.11	0.39	1760	71.34	3820	37.79

The Initial pH of the sample before the treatment was 9.51. After the treatment, a significant reduction in pH was observed in all the test samples. The highest % reduction in pH was observed in a treatment tube inoculated with isolate code number 3 as shown in table 5 (set-B). For ammonical nitrogen concentration, the initial value has revealed 7mg/L of the effluent sample. After treatment, the highest reduction value was obtained with c**onsortium** which is 111mg/L as shown in table 5 (set-B). The TSS and COD of the sample before the treatments were found to be 0.63mg/L and 6140mg/L. After the treatments, their measurements were also reduced significantly as compared with the initial values (table 5). The highest reduction value for TSS was obtained with isolate code 20 (set-B) which is 0.10mg/L, and the highest % reduction in COD obtained was obtained was 80.62%.

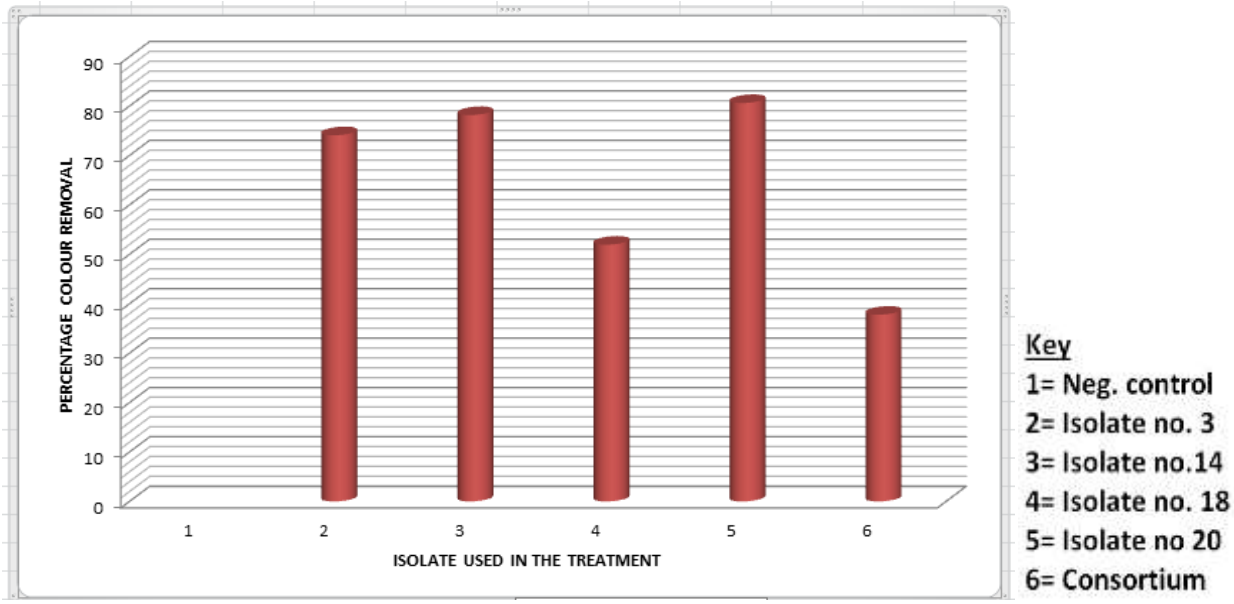

Figure 2: *A Bar chart showing comparison of color removal performance of the isolates*

4.0 DISCUSSION

Isolation of decolourizing bacteria and their utilization in the treatment of textile wastewater was the focus of this project, the results obtained have revealed the presence of four bacteria isolates designated (3,14,18 and 20) that are capable of decolourizing textile waste effluent. Although, these isolates were not the only bacteria presence in the textile wastewater sample, the result of selection indicated that they have high potentiality to decolourize textile wastewater due to their ability to grow on the wastewater agar. This result is in agreement with previous researches in which few isolates from a large number showed higher performance in decolourization of textile waste. For instance, Mahmood *et al.,* [19] isolated up to 200 bacteria out of which only five were found to have the decolourizing ability.

Relative performance of the isolated bacteria for the decolorization of textile wastewater clearly implies that these bacteria can be effectively used for the removal of dye from contaminated industrial wastewater. Consortium of the bacteria showed a better performance in the treatment and significant decrease in colour up to 85.35%. This could probably be due to synergistic action of the different isolates involved. Colour in wastewater is due to the presence of azo dyes, most literature on the decolourization of azo dyes reveals that it occur under facultative anaerobic condition [20]. Azoreductase is reported to be the key enzyme expressed in azo-dye-degrading bacteria and catalyses the reductive cleavage of the azo

bond [21]. Azoreductase activity had been identified in several species of bacteria recently, such as *Staphylococcus aureus*, *Shewanella putrefaciens*, *Shewanella* strain J18 143 and *Pseudomonas* spp. [21, 22, 23].

Reduction of azo dye is non-specific, it can be enzymatic with the vital enzyme identified as azoreductase or by low molecular weight redox mediators. Only few aerobic bacteria are able to utilize azo dyes as growth substrate [18]. Under facultative anaerobic condition, the dyes are reduced producing colourless solution containing aromatic amines. In the presence of oxygen, decolourization may not be achieved due to the competition between azo dye and oxygen for reduced electron carriers, oxygen may dominate the utilisation of NADH there by hindering the electron transfer to the azo dyes [20].

In this project Set B which was subjected to facultative anaerobic conditions (incubated at 30°C without shaking), achieved higher positive results for treatment process. Comparing with the negative control (original wastewater), colour of wastewater disappeared in all other test tubes as Shown in figure 1. While set A in which the treatment was done under aerobic condition (kept at 37°C with shaking) did not show significant colour change. Thus, major decolourization activity could be attributed to facultative anaerobic bacteria.

Changes observed in the physicochemical parameters such as pH, TSS, COD and ammonical nitrogen could be attributed to the metabolic activity of the bacteria used in the treatment. In this study, the bacteria slowly reduce colour during the aerobic phase of growth, however, higher colour removal was achieved during the facultative anaerobic phase of treatment during which % of colour removal was achieved; the increase in colour removal may be due to the absence of oxygen which may have slow down the decolourisation process.

5.0 CONCLUSION

It can be concluded that the sample of textile waste-water used in this research harbours indigenous bacteria that could be isolated and used as agents for bioremediation of textile waste-water. Also the four bacteria isolates used in the treatment process have the ability of decolorizing textile effluent. Furthermore, certain analytical methods such as spectroscopic measurement of colour intensity were used to measure the decolourization in the treatment process. Finally, our experimental results suggest that these bacteria strains isolated, have potential applications for bioremediation of textile effluent or dye-polluted waters and could be used in bioreactors to treat waste water streams.

FUTURE WORK

Identification of the bacterial isolates using molecular approach, and recovery of biodegradation products of textile effluent generated by the isolated bacterial species including verification of the enzymes responsible for rapid decolorization of the dyes could be carried out as the future work.

ACKNOWLEDGEMENTS

More grease to the elbow of Tertiary Education Trust Fund (TETfund) Nigeria for supporting this research because, this study would not have been possible without the financial and moral support of TETfund, Nigeria. We also immensely thanked Kano State Polytechnic and the Department of Science Laboratory Technology (SLT) for the opportunity given to us to pursue this important task.

AUTHOR CONTRIBUTIONS

All authors contributed toward data analysis, drafting and critical revision of the paper and agreed to be accountable for all aspects of the research study.

CONFLICTS OF INTEREST

The research was conducted with no financial conflict or other factors which is considered to be declared as conflict.

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