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Microbial Biotechnology for the Decolourization and Mineralization of Organic Components of Textile Wastewater by Single and Mixed Microbial Consortium Isolated From Effluent Treatment Plant of African Textiles Industry Kano, Nigeria

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Abstract: In this study, four (4) resident bacterial strains were selected from 127 different microbial strains isolated from textile effluent treatment plant of African Textiles Industry Kano and 16S rDNA gene sequence analysis revealed closely related organisms to species of Acinetobacter, Pseudomonas, Micrococcus and Alcaligenes. The bacterial strains inoculum was cultured in mineral salt medium and mutated using UV-irradiation inducement method. Both the wild and mutant types of the bacterial strains were separately cultured and their decolorization activities on organic dyes effluent were monitored and compared. It was observed that, these organisms are compatible with each other and capable of utilizing and degrading organic components of the textile waste effluent. Decolorization efficiency of the bacterial strains was evaluated as a function of the operational parameters (i.e. aeration, temperature, pH, dye concentration, incubation condition, salt and nutrient supplement) at optimal operational conditions of pH 7 and 37 °C temperature. The bacterial isolates showed increase in cell number as the concentration, absorbance and pH decreases. The adsorption capacity for organic dyes effluent was determined spectrophotometrically by monitoring absorbance of the different organic dyes effluent at a constant wavelength (λ max). The mixed cultures were observed to be better textile effluent degraders than the single cultures.

Keywords: Bacterial strains, decolorization, effluent, mineralization, organic dyes

I. Introduction

Rapid industrialization has resulted in disproportionate environmental pollution, with one of the major sources being textile industry effluents [1]. Synthetic organic dyes are used in textile, cosmetic, and food industries [2; 3]. Dyes are deeply colored synthetic compounds with an array of structural diversity, which made it have widespread application. More than 10,000 different dyes with an annual production of more than 7×10^5 metric tons worldwide are commercially available and are intensively used in paper printing, pharmaceutical, food, textile industries for dyeing nylon, polyacylonitrile modified nylon, wool, silk, cotton etc. [4; 5]. Amongst various applications of synthetic dyes, about 30,000 tons of different dye stuffs are used per year for textile dyeing operations, thus dye houses are the major consumers of synthetic dyes and consequently are the main cause of water pollution and impose severe damage to the quality of the soil [6; 7].

The largest group of all synthetic dyes represents 70 % of all organic dyes used by the textile industry [8]. Azo dyes constitute a major class of environmental pollutants and some of the azo dyes and their breakdown products have toxic and mutagenic effects on living organisms [9]. Textile industries generate wastewater with varied characteristics, depending on the process employed [10]. The concentration of dye contained in the effluent varies depending on the dyeing process, but the content is generally in the range of 10 - 200 mg/l [11]. The high concentration of dyes in water causes many water borne diseases and increases BOD of the receiving water because of the complex structure and large molecular size of dyes. Dyes when present in water and on contact can cause ulceration of skin and mucous membrane, dermatitis, perforation of nasal septum and severe irritation of respiratory tract. Its injection into the body may cause vomiting, haemorrhage and severe diarrhoea [12]. The presence of carcinogens has also been reported in combined wastewater of dyeing and printing [13].

As the dyes present in wastewater decompose very slowly under normal conditions, a treatment method to remove such dyes is very essential [10]. The solution to the environmental problems caused by the textile dye effluent is being sought by physical, chemical and biological treatment processes. The physicochemical methods

include adsorption, chemical precipitation, flocculation, electrofloatation, oxidation via chlorine, peroxide, electrolysis and ozone treatment, reduction, electrochemical destruction and ion-pair extraction [14]. But due to their limitation and disadvantages as narrow range of application, high cost, produced large secondary hazardous sludge, intensive energy requirements; biological treatment has been increasing interest [15; 16]. Biological methods of removal involve the use of microorganisms such as bacteria and fungi to turn these pollutants into non-toxic harmless substances. Biological processes convert organic compounds completely into water and carbon dioxide and the process is economical and the by-products are easy to use [17]. Due to catabolic diversity, wide spectrum of microorganisms has been widely utilized in biodegradation of dyes containing wastewater [18]. Various bacterial species have been reported to decolorize and degrade a wide range of organic dyes [15; 19; 20; 21; 22]. The microbial degradation and decolorization of dyes have received considerable attention in the recent past from the viewpoint of treating industrial wastewater containing textile dyes. As wastewater treatment facilities are often unable to completely remove commercial dyestuffs, thus contributing to the pollution of aqueous habitats. In this regard, this investigation was aimed to exploit the biodegradation abilities of single and mixed microbial cultures of resident microbial flora for remediation purposes, when used on the synthetic organic dyes effluents which are extensively used in textile industry and to study the effect of optimizing the operational parameters.

2.1 Sample collection

II. Materials And Methods

The textile dye effluent samples were collected from common effluent treatment plant of Africa Textiles Company, in a screw capped sterilized bottles - 'Klas'. The samples were brought to the laboratory and stored at 4 °C for 48 hrs, before use.

2.2 Pre-treatment of the sample

A filter paper was folded and inserted on the mouth of the soxhlet extractor unto which some quantity of XAD-2 resin was carefully dispensed. Measured 2.5 litres of textile wastewater was carefully dispensed in the XAD-2 resin. The organic components of textile waste effluent were retained within the organic resin as residues and the filtered were collected and discarded. The resulting residues being the desired component needed for the analyses were allowed to dry for 2 hrs in the soxhlet extractor. Then, 50 cm³ of ether was passed through the dried residues of XAD-2 resin contained in the soxhlet extractor, and all the organic components in the XAD-2 were miscible with ether as organic solvent which are collected as a filtered and warmed gently in a hot air oven at 180 °C to allow the ether to evaporate in 48 min. The solid deposit was allowed to cool to 35 °C and weighed to 30 g, which were diluted to 1000 cm³ with sterile de-ionized water and properly labelled as stock solution (1000 mg/dm³) [23].

2.3 Isolation and identification of dyes decolorizing bacterial strains from textile effluents

Total bacteria were enumerated by spread plate method using 0.1 ml of the dilution 10^{-1} to 10^{-4} onto nutrient agar. All cultures were incubated for 24 hr to 48 hr at 37 °C. The bacterial colonies, which developed on the plate were randomly picked and purified by sub culturing unto fresh agar plates using the streak-plate technique. Isolated colonies, which appeared on the plates, were then transferred unto nutrient agar slants properly labelled and stored as stock cultures. All the strains were identified based on their morphology, gram staining, and other standard conventional biochemical tests according Bergey's Manual of Systematic Bacteriology [24]. For identification of bacterial strains the genomic DNA and RNA was extracted from culture following the methods described by [25]. The universal primers were used and PCR amplification was performed in total reaction volume 20 µl. The PCR programming used were 94 °C for 5 min, 30 cycles at 94 °C for 1 min and 72 °C for 3 min and final extension was at 72 °C for 10 min. The amplified PCR product was detected on 0.8 % agarose gel in TBE buffer. Nucleotide sequences were determined by DNA sequencing method. The sequences of 16S rDNA and rRNA gene of the bacterial strains were subjected to BLAST search tool in gene database of NCBI.

2.4 Compatibility analysis for the selected microbes

Microbes that are involved in a consortium should not exhibit any antagonistic effect over the other microbes that are in the consortium, only when the organisms are compatible with each other the remediation capacity of the consortia would be efficient. The compatibility analysis was done with the selected efficient microbial strains. Mueller Hinton Agar plates were swabbed with one of the selected microbe. Four wells were cut and 10 μ l of the culture supernatant (after 72 hrs of incubation) of the other organisms selected were added to the well. The test was repeated by changing the swabbed organism with the three selected cultures used in the study and the culture supernatants of three other cultures which were not swabbed. The organism was said to be incompatible with the other if a zone of clearance was observed around the well [26].

2.5 Growth conditions, media and irradiation

After 70-80 % confluent cells were cultured in mineral salt medium overnight, UV-irradiation was performed on the cells according to the method described by [27]. UV-irradiation was carried out in a chamber safe for UV-B exposure. The UV-dose was quantified in joules per square meter with the use of a microvolt ammeter. The strains were grown in mineral salt medium on Luria-Bertani (LB plates). In all experiments, the strains were grown from a single colony in mineral salt medium at 30 °C until they reached an optical density at 600 nm. For UV-experiments, serial dilutions of bacterial cultures were plated on LB plates and irradiated with a dose of UV (280 nm) light of 10 j/m². The dose rate was 0.25 j/m²/s. Colonies of survivors were scored after 24 to 48 hrs of incubation at 30 °C and later sub-cultured on nutrient agar, now prepared for further studies [28].

2.6 Mineral salt broth for wild and mutant types of microbial strains

Measured volume of 99.9 cm³ of mineral salt medium was dispensed into 250 ml Erlenmeyer flask and distinct wild and mutant type of bacterial strains from nutrient agar slants were picked gently using a sterile wire loop and inoculated into the mineral salt medium. The mixture was shaken and incubated at room temperature of 30 °C for 24 hrs. The mineral salt broths for bacterial strains were kept near a freezing point in order to have a control of microbial load (inoculums size) [23].

2.7 Growth of bacterial strains in organic effluent

Mineral salt broth of bacterial strains were dispensed 99.0 cm³ into 250 ml Erlenmeyer flasks arranged sub sequentially in order of 48 hrs interval at pH 5. To each reaction flask was added 1 cm³ of organic effluent sample, cultures were established in the incubator shaken at 200 rpm and allowed to grow for twenty four days (24 days). At the end of the days organic component of textile dyes effluents were dissolved in methanol at 1.0 mg/l concentration. Controls were run with the same reaction conditions but excluding bacterial strains. These experiments were replicated three times. The absorbance (optical density) of the supernatant was measured at different wavelengths using spectrophotometer at regular intervals of 48 hrs, during the decolorization process. The following optimal and operational parameters i.e. total viable count (TVC), concentration; pH and optical density at different wavelengths, until the high peak was attained using UV-Visible spectrophotometer were also determined [28].

2.8 Dye decolorization assay

The decolorization assay of organic dyes effluent was carried out in 100 ml MSM medium at 30 mg/l dyes concentration with inoculum concentration 10 % (v/v) at 37 °C and 200 rpm. Two types of control were used, uninoculated sterile medium supplemented with dye (abiotic control), while the other inoculated medium has no dye (biotic control). After incubation under shaking condition for 48 hrs, aliquot (2 ml) culture media was withdrawn aseptically, centrifuged at 10,000 rpm for 10 min to separate the bacterial cell mass and clear supernatant. Decolorization of dyes was determined by monitoring the decrease in absorbance at the maximum absorption wavelength (λ max) for the organic components of dyes effluents in an UV-Visible scanning spectrophotometer. Decolorization activity was calculated by applying the formula used previously by [29].

Decolorization rate (%) = $(I-F) \times 100$ I

(1)

Where I = initial absorbance of the coloured sample and F = final absorbance of the decolorized sample.

2.9 Establishment of optimum operational parameters on dyes decolorization

2.9.1 Effect of pH on decolorization

For determination of pH dependence of bacterial bio-decolorization, medium was supplemented with organic dyes effluents at low concentration of dye 200 mg/l. The medium pH was adjusted between 5.0 to 9.0 with hydrochloric acid and sodium hydroxide and inoculated with 10 % (v/v) bacterial culture and incubated at 25 °C. Samples were collected at regular time intervals up to 24 hrs. Decolorization % was calculated based on the OD values at 518 nm [30].

2.9.2 Effect of oxygen requirement in dyes decolorization

Stab culture method was adapted to determine the requirements of oxygen for dye decolorizing bacterial strains. A loop full of selected bacterial strains was inoculated by stab inoculation into test tubes containing the screening soft medium (i.e. Luria-Bertani agar) with 100 mg/l concentration of organic dyes effluent. The inoculated test tubes were incubated at ambient temperature $(30 \pm 1 \text{ °C})$ for 48 hrs. At the end of

incubation period colour changes were qualitatively observed and it was compared with un-inoculated test tubes as the control [31].

2.9.3 Effect of different concentrations of dye on decolorization

To check the efficiency of dye decolorization by the bacterial isolates, decolorization assay was carried out at different concentration of organic dyes effluents, 50 mg/l, 100 mg/l, 200 mg/l, 300 mg/l, 400 mg/l and 500 mg/l. Samples were withdrawn at the interval of 3 hrs and subjected to centrifugation at 10,000 rpm and supernatant was used to determine the decolorization of dye by UV-Vis spectrophotometer at 518 nm and the percentage decolourization was calculated [29].

2.9.4 Effect of yeast extract as a co-substrate

In this experiment dye concentration was gradually increased from 100 mg/l to 500 mg/l, while yeast extract concentration was maintained at 400 mg/l. To the flask containing 500 mg/l dyes, no yeast extract was added. Two controls were used; one is abiotic without inoculation, while other with 500 mg/l yeast with inoculation in which organic dyes has been omitted. The inoculated flasks were incubated at 25 °C in stationary condition. Concentration of organic dyes was determined by taking absorbance at 517 nm at regular intervals of 48 hr with Spectrophometer and decolorization was determined [32; 33].

2.9.5 Effect of incubation conditions on dye decolorization

A conical flask containing 100 ml LB broth, 200 mg/l dyes and inoculum (10 % v/v) were added. One set of flasks were incubated at 25 °C under static conditions and the other at same temperature on an orbital shaker at 200 rpm. The same controls were used as mentioned previously, and samples were withdrawn at regular interval of 3 hrs, centrifuged at 10,000 rpm for 10 min and the absorbance of supernatant was read at 518 nm in a spectrophotometer and the percentage decolorization was calculated [29].

2.9.6 Effect of salt on the growth of bacterial strains

Bacterial strains vary in their ability to tolerate salt (NaCl). The growth at different salt concentration was therefore tested (i.e. at 10, 20 and 30 g/l) amended with nutrient agar medium. Nutrient agar plates with different concentrations of sodium chloride and the bacterial isolates were inoculated by making a single line loop inoculation. Plates were incubated at 30 °C in for 48 hrs. At the end of incubation the growth was observed as salt tolerant or not [29].

2.10 Microcosm experiment

Textile polluted water was collected from the river where the industry discharges its wastewater. The water had a pH of 8 - 9. Then clean water was collected at a nearby well with a pH 7 - 8.5. Measured 10 ml of the polluted water as well as that of the clean water were incubated with 100 mg/l concentration of organic dyes put into the test tubes. The screened bacterial strains were grown in nutrient agar for 24 hrs and were then transferred into test tubes containing both waters. The experimental test tubes were incubated statically at 30 ± 1 °C, till disappearance of color was observed [34].

III. Results and discussion

Microorganisms play an important role in the decolorization and degradation of environmental pollutants. The science of bioremediation is greatly enhanced by the presence of a diversity of microbes which strive even in extreme conditions and concentrations of pollutants. However, microbial growth and metabolism in impacted areas can be mitigated by a number of factors such as pH, temperature, concentration of pollutant, moisture content, conductivity, oxygen content, nutrient availability and bioavailability and the property of the medium. [21].

The organic dyes decolorizing bacterial strains was identified by 16S rDNA gene sequence analysis were designated as *Acinetobacter*, *Pseudomonas*, *Micrococcus* and *Alcaligenes* species and compatibility analysis showed that the organisms were found to be compatible with each other as there was no zone of inhibition around the wells in all the plates tested. This may be as a result evolution and/or co-existence of the isolated organisms in a common environment for a longer period.

However, in the present studies, the result obtained by single and mixed cultures of wild type of *Acinetobacter, Pseudomonas, Micrococcus* and *Alcaligenes* species, demonstrate their potentials to substantially decolorized organic dyes effluent by 52.3 %, 65.7 %, 72.4 %, 42.2 % and 100 %, while mineralized by 47.6 %, 63.4 %, 67.5 %, 51.0 % and 99.7 % respectively (Fig 1A). Also the visible portion of the spectrum of organic dyes effluent showed a major peak at 532 nm, 510 nm, 512 nm, 515 nm and 525 nm after 24 days period of treatment, this peak shifted to 316 nm, 315 nm, 332 nm, 328 nm and 308 nm respectively for bacterial strains. In the case of mutant type, they showed a remarkable potentials to decolorize organic dyes effluent by 49.2 %, 87.6

%, 1.37 %, 92.6 % and 38.67 % respectively, and mineralizes organic dyes by 48.6 %, 82.7 %, 0.08 %, 83.9 % and 25.7 % respectively with the shift in major peak from 535 nm, 495 nm, 508 nm, 547 nm and 498 nm respectively, after 24 days period of treatment, to 322 nm, 310 nm, 327 nm, 319 nm and 337 nm respectively (Fig. 1B). Generally, the results of this studies indicates that a pro-biotic microorganism has demonstrated a high potentiality for decolorization and degradation of organic components of textile effluent and there is increased in cell number as the concentration, absorbance and pH decreases. Mixed culture of microbial consortium demonstrate an excellent result, which indicates that mixed cultures for wild type were observed to be better textile effluents degraders than the single cultures or mixed culture of the mutant types, but the mutant type of *Micrococcus* specie, on the twelfth (12^{th}) day suddenly shows a drastic dropped in cell number and pH, with an increased in concentration and optical density, which indicates that the strain generates degradation residual products which are more persistent or toxic than the intermediate compounds on the tenth (10^{th}) day period of treatment.

The reduction of major peak in the visible region indicates substantial decolorization. This was indicated by the decrease in absorption at the maximum wavelength and the decrease in the area under the absorption curve in the visible region. No new peak appeared in the UV-Vis region after decolorization. The absorptivity are intrinsic properties of a colorant and measuring the spectral absorption curves of dyes during biodegradation provided a means of determining the reaction rate, which provide evidence of structural change of dyes during biodegradation. Previous experience showed that fading of the original color of the dyes is sometimes accompanied by a colour change (wavelength shift) in the solution. Because of this, both the ultraviolet and visible regions were monitored by a UV-Vis spectrophotometer to provide more information about the biodegradation. The decrease of the maximum absorbance of the dyes occurred primarily in the first several days of treatment, with no substantial additional decrease in absorbance as the treatment time increased. The pH of the culture medium is another important factor that plays a critical role for the optimal performance of microbial cells and has marked effect on cell growth. For decolorization process an optimal pH was indicated

of microbial cells and has marked effect on cell growth. For decolorization process an optimal pH was indicated that the bacterial culture generally exhibited maximum decolorization rate at pH values near 7.0 (Fig. 2). An increase in pH from 5.0 to 9.0 did not show any marked changes in the values of percentage decolorization indicating that the organism could be used in treatment of effluents which are either acidic or alkaline. The optimum pH for decolorization of organic components of textile effluents however remained 7.0.

Typical microbial oxygen requirement mechanism was studied by stab culture method. Screening result using the stab culture method shows that all the four strains showed the ability to decolorize organic dyes effluent after 24 hrs of incubation at room temperature. The place of the decolorization was only present in the inside of tubes indicating the oxygen requirements for the four strains are anoxic or microaerophilic state of affairs only.

Dyes being toxic compounds have an inhibitory effect on the decolorization process at higher concentration. So the influence of different organic dyes concentration was evaluated ranges from 50, 100, 200, 300, 400, 500 mg/l was used for the determination of the effect of dyes concentration on the decolorization. After 48 hrs it was found that with increase in dye concentration, the dyes decolorizing efficiency of the bacterial strains decreases. The maximum decolorization was achieved at 50 mg/l concentration and minimum decolorization was found with 500 mg/l concentration (Fig. 3).

Different concentrations of yeast extract (100 - 500 mg/l) were used to determination of effect of yeast extract as co-substrate for decolorization efficiency of bacterial strains (Fig 4). With increase concentration of organic dyes effluent (0 - 500 mg/l) and decrease concentration of yeast extract (500 - 0 mg/l) the time taken for decolorization was found to be 72 hrs. At 500 mg/l dyes concentration and in absence of yeast extract the decolorization after 70 hrs was found to be 81.94 %, suggesting that the organism could utilize organic dyes as a sole carbon source (Fig. 4).

The effect of incubation conditions (shaking and static) on decolorization of organic dyes effluent by bacterial strains revealed that static condition was more suitable for decolorization, where the activity was found to be 95 % and at shaking condition it was 84.83 %. This showed that static condition is more appropriate for the decolorization of the dyes by the bacterium (Fig. 5).

Textile effluents generally contains chloride of sodium and potassium, which are frequently employed for salting out of dyes and are therefore discharged into the effluent. Hence, screening of dyes decolorizing bacteria for salt tolerance results showed that all the four strains showed growth at 10 to 30 g/l sodium chloride concentration. The growth rate of dyes decolorizing bacteria increases with the increase in the concentration of sodium chloride up to 30 g/l and then decreased with further increase in the salt concentration. Effect of salt tolerance on the bacteria for dyes decolorization suggests a steady increase in the rate of decolorization but excess salt in the medium inhibit the microorganism ability to decolorize the organic dyes. This result was supported by [35], who reported that the salt tolerance of bacterial consortium for dye decolorization was tested up to 5.0 % (w/v).

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The adaptation of the microorganisms to their natural environment was studied using microcosm experiment and also their dves decolorizing efficiency in relation to their natural ecosystem. Dves contaminated effluent and clean water (unpolluted water) were inoculated using the four monocultures and mixed culture containing organic dyes effluent and dye concentration (100 mg/l) incubated statically for 30 ± 1 °C (ambient temperature). Both the monoculture and mixed culture were able to decolorize the polluted water in the presence of organic dyes. The mixed culture efficiently decolorizes the polluted water with the organic dyes effluents compare to monoculture in 24 - 48 hrs. Un-inoculated polluted water was not decolorized even with the presence of resident organism in the polluted water. Mixed culture efficiently decolorized organic dyes effluent due to the presence of resident microorganism and other organic matter in the polluted water may have helped in the degradation of the organic dyes effluent. In the present study natural organic matters, resident microorganisms drastically influence dyes decolorizing activity compared with control. This report was supported by [34], who explained that resident microorganism alone could not have decolorized the dyes themselves. The reason behind this was microorganism present in the polluted water do not produce the enzymes to reduce organic dyes effluent. Another explanation was the organism were not exposed to organic dyes prior to the experiment and not adapted to the presence of dyes. Bacteria need to be adaptable to the pollutant before they can degrade it [36]. The environmental conditions must be favorable for the degradation of certain compounds including pH, electron acceptor, organic materials, inorganic materials, nitrogen and phosphorus that are essential to carry out perspective of bioremediation.

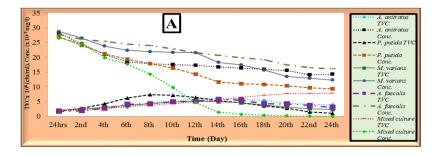


Fig 1A. Growth profile of wild types of *Acinetobacter anitratus*, *Pseudomonas putida*, *Micrococcus varians* and *Alcaligenes faecalis* and mixed cultures in mineral salt medium containing textile organic dyes effluent as a sole source of carbon and energy

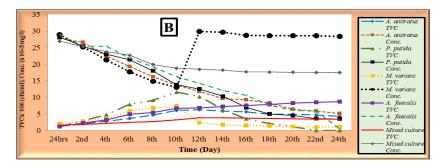


Fig1B. Growth profile of mutant types of *Acinetobacter anitratus*, *Pseudomonas putida*, *Micrococcus varians* and *Alcaligenes faecalis* and mixed cultures in mineral salt medium containing organic dyes effluent as a sole source of carbon and energy

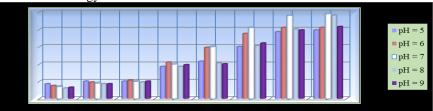


Fig 2. Effect of pH on the decolorization of organic dyes effluent

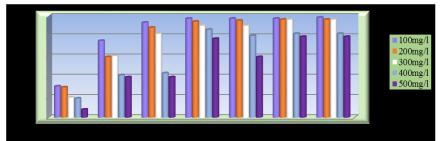


Fig 3. Effect of dye concentration on decolorization efficiency of bacterial strains

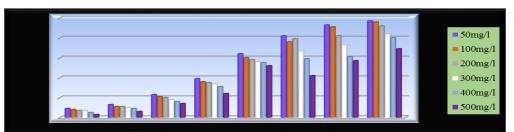


Fig 4. Shown is the utilization of organic dyes effluent as sole carbon source

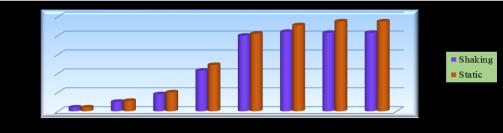


Fig 5. Effect of shaking and static conditions on organic dyes decolorization

IV. Conclusion

The viability studies indicated that a complete and partial decolourization and mineralization of a dye compounds can be successful, when required environmental conditions and nutrient are available, which encourages the organisms to grow maximally [37]. Complete pathways of degradation are more likely to occur through the combined effects of many organisms. Since there are active and adapted populations of microorganisms that showed potential to degrade organic textile effluent in the area, it may not be beneficial to consider augmenting the indigenous microbial populations by seeding with specialized microorganisms, except where the pollutant is found to be recalcitrant. Treating azo dye containing textile effluents using single pure culture of microbes is both time consuming and bio-recalcitrant under aerobic conditions. The use of a microbial consortium helps in reducing the azo dyes under aerobic conditions at a faster rate. Therefore, consortium of microbes with its wide spectral enzymatic activity, with or without substrate specificity helps in reducing a number of dyes containing aromatic rings present in the effluent [38]. The combination of chemical and physical processes with biological scheme is also promising [39]. The oxidase enzyme activity of the isolates is indicative of the indigenous microbial populations are capable of degrading textile waste effluents. Currently, the most reliable strategy which is generally accepted as an environmentally sound and economically feasible protocol for the treatment of hazardous waste and effluents is biodegradation using eco-friendly microbes. Hence microbes are the tribute for clean environment. This study clearly demonstrates that indigenous microbial community in textile waste effluents of African Textile Industry Kano, Nigeria has the ability to degrade and decolourize various types of dyes used in such industries. The potential of these bacterial strains can be exploited to remove residual dyes in textile wastes. Further research is needed to develop a comprehensive analytical method using GC/MS machine to monitor the residual products at designated period of time and also by optimizing the process parameters.

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