European Journal of Biotechnology and Bioscience

E J B B

Volume: 3, Issue: 9, 06-11
Sep 2015
www.biosciencejournals.com
ISSN: 2321-9122
Impact Factor: 3.742

M.S. Shinkafi
Department of Pure and Industrial Chemistry, Bayero University Kano, Nigeria

I.U. Mohammed
School of Science, Engineering & Technology, University of Abertay, Dundee, Scotland, United Kingdom

A.A. Audu
Department of Pure and Industrial Chemistry, Bayero University Kano, Nigeria

Correspondence
I.U. Mohammed
School of Science, Engineering & Technology, University of Abertay, Dundee, Scotland, United Kingdom.

Degradation and decolourization of textile dyes effluents

M.S. Shinkafi, I.U. Mohammed, A.A. Audu

Abstract
Microorganisms have potential ability to decolorize synthetic commercial dyes used for textile dyeing. Effluents from textile and dyeing industries cause serious pollution to air, soil and water environment. Therefore, this study was aimed at isolating potential dye degrading microorganisms from textile effluents and to evaluate their capability to decolorize commercially used textile dyes. Effluent sample were collected from the confluent of the textile wastewater discharge of African Textile Company in Kano, Nigeria. Two candidates' microorganisms (*Pseudomonas putida* and *Klebsiella ozaenae*) were used to decolorize and degrade dyes into non-toxic form. This same isolates were mutated using UV-irradiation inducement and resulted into the wild and mutant types of bacterial isolates. Cultures were made in thirteen (13) reaction flasks, each containing the same concentration of two different textile dyes; Congo red (Direct red 28) and Direct blue '80'. Degradation ability of dyestuffs by the isolates was observed by dye decolorization assay. The reactive capacity of dye Congo red and direct dye 80 were determined spectrophotometrically by monitoring the absorbance of different dyes at constant wavelength (λmax). Virtually all the isolates were able to decolorize the dyes considerably after specified periods of incubation.

Keywords: Effluents, textile dyes, wastewater, decolourization, degradation, Kano Nigeria

1. Introduction
Textile industries use large quantities of water and chemicals (Chakraborty *et al*., 2003; Mondol *et al*., 2015). Detergents and soda are used to remove dirt, grit, oils, and waxes (reviewed by Ranade *et al*., 2014). Bleach is used to improve whiteness and brightness of the fabric (Grancarić, *et al*., 2014; Chattopadhyay *et al*., 2015). Dyes, fixing agents, and many inorganic materials are used to provide the brilliant array of colours that the market demands (Naveed *et al*., 2006; Husaini *et al*., 2011). Sizing agents are added to improve weaving (Hasanbeigi & Price, 2015; Zhao *et al*., 2015). Oils are added to improve spinning and knitting (see reviews by Almeida, 2015; Dey & Islam, 2015), while latex and glues are used as binders (reviewed by Jyothi *et al*., 2012). A wide variety of special chemicals such as softeners, stain releasing agents, and wetting agents are used and many of these chemicals become part of the final product, whereas the rest are removed from the fabric and loaded into the effluent stream (see reviews by Chhabra *et al*., 2015; Dasgupta *et al*., 2015; Dey & Islam, 2015; Zeng *et al*., 2015).
Mixed bacterial cultures from different habitats have been shown to decolourise diazolinked chromophore of dye molecules in 15 days (Knapp & Newby, 1995). Nigam & Marchant (1995) and Nigam *et al*. (1996) demonstrated that a mixture of dyes were decolourised by anaerobic bacteria in 24 to 30 hrs, using free growing cells or in the form of biofilms on various support materials. Many bacteria have been used for degradation of azo dye in microbial systems, though this systems are associated with draw backs especially in the fermentation process and therefore unable to cope with large volumes of textile effluents (Ogawa & Yatome, 1990).
The ability of bacteria to metabolise azo dyes has been reported by different authors (see reviews by Jasińska *et al*., 2015; Karthikeyan, 2015; Singh & Singh, 2015). Under aerobic conditions azo dyes are not readily metabolised, although Robinson *et al*., (2001), reported the ability of *Pseudomonas strains* to aerobically degrade certain azo dyes. However, the intermediates formed by these degradation steps resulted in disruption of metabolic pathways and the dyes were not actually mineralised. Under anaerobic conditions, such as anoxic sediments, many bacteria gratuitously reduce azo dyes reportedly by the activity of unspecific, soluble, cytoplasmic reductases, known as azo reductases. These enzymes are reported to result in the production of colourless aromatic amines which may be toxic,

mutagenic, and possibly carcinogenic to animals. Increasingly literature evidence suggests that additional processes may also be involved in azo dye reduction. It has been reported that many bacteria reduce a variety of sulfonated and non-sulfonated azo dyes under anaerobic conditions without specificity of any significance (see reviews by Robinson *et al*., 2001; Stolz, 2001).

The prime objective of this research was to induce mutation in the organism(s) by irradiation/ photochemical mutagens and compare the activities of the mutant and wild type of bacteria (single and mixed microbial isolates) for their ability to decolorizes the most widely used reactive dyes used in textile industries and also monitor and quantify the degree of degradation of the organic effluent in different conditions for total viable count, pH, aeration, concentration and as well as the optical density. The results gained by these treatment methods will be used to develop of a comprehensive analytical method for investigating the degradation of dyes by microbial isolates.

2. Materials and Methods

2.1 Chemicals

Two dyes of relatively simple structures were chosen for this work, apart from the textile waste effluent collected directly from effluent treatment plant of textile industry. Congo red (60 percent purity) and Direct blue 80 (40 percent purity) were obtained from Aldrich Chemical Company (Milwaukee, Wis.), as was Congo red in a 90 percent pure form. The organic solvents employed in the experiments were at least of reagent grade and was used as purchased.

2.2 Microorganisms

The microorganisms used in this work were obtained from the Department of Pure and Industrial Chemistry Laboratory of BUK, Nigeria. The candidates include *Pseudomonas putida* and *klebsiella ozaenae*. The culture was maintained on malt agar plates at 30 °C, with subcultures routinely made every two weeks.

2.3 Isolation and enumeration of microbial cultures

Total bacteria were enumerated by spread plate method using 0.1ml of the dilution 10^{-1} to 10^{-4} onto nutrient agar. All cultures were incubated for 24hr to 48hr at 37 ^{o}C. The bacterial colonies, which developed on the plate were randomly picked and purified by sub culturing unto fresh agar plates by using the streak-plate technique. Isolated colonies, which appeared on the plates, were then transferred unto nutrient agar slants and stored as stock-cultures. The bacterial isolates were identified based on their morphology, gram reaction and as well as their biochemical reactions.

The fungi were isolated from the water samples using Czapek dox agar unto which sterile streptomycin (50 mg ml^{-1}) had been added to suppress bacterial growth. Pure cultures of the fungi isolates were made and transferred using Czapek dox agar slants as stock cultures. The microscopic and macroscopic features of the hyphal mass, morphology of cells and spores, and the nature of the fruiting bodies were used for identification.

2.4 Media, growth conditions and irradiation

The organisms were grown in mineral salt medium on Luria Bertani (LB plates). UV-irradiation was carried out in a chamber safe for UV-B exposure and was quantified in joules per square meter with the use of a microvolt ammeter. For UV-experiments, serial dilutions of cultures were plated on LB plates and irradiated with a dose of UV (280nm) light of $10j/m^2$ with a dose rate of $0.25j/m^2/s$. Colonies of survivors were scored after 24 to 48hrs of incubation at 30 ^{o}C and later sub-cultured on nutrient agar ready for broth formation (Cheesbrough *et al*., 2000).

2.5 Decolourization of textile waste effluent and commercial dyes

Mineral salt broth of fungal isolate were dispensed in $99.0cm^3$ quantities into six (commercial dyes) and eleven 250ml Erlenmeyer flasks which were arranged sub sequentially in order of 48hrs interval at pH 5.0. To each reaction flask was added $1cm^3$ of the effluent sample, cultures were established in the incubator shaken at 200 rpm and allowed to grow for twelve days (commercial dyes) and twenty four days (organic components of textile effluents sample). At the end of the days the two commercial azo dyes and 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 fungal strains. The optical density (absorbance) at different wavelengths, cell mass, concentration and pH of culture were determined (Cheesbrough *et al*., 2000).

2.6 Cells-mass measurement (dry weight)

Direct approached method was used to determine the microbial dry weight. Cells growing in liquid medium were collected by centrifugation, washed, dried in an oven and then weighed using Mettler Toledo as described earlier by Cheesbrough *et al*., (2005).

2.7 Degradation and decolourization assay

Aliquots of $2cm^3$ of a clear dye solution were taken from each of the reaction flasks at time intervals and measured immediately using a UV-Visible recording double beam spectrophotometer. Diluted cultures were used prior to the measurement to change the absorbance value to below 1.0 absorbance units per centimeter of path length. Because of the low water solubility of the organic dyes, an equal volume of methanol was mixed with the analytical solution to ensure complete solubilisation prior to measurement and decolourization was assessed in two ways; Firstly, by monitoring absorbance spectrophotometrically at maximum wavelength for each cultured solution and secondly by observing the reduction of the major peak area in the visible region for each cultured solution. Both approaches were used because colour change may occur during biodegradation with a concomitant wavelength shift. Therefore, the maximum wavelength of the cultured solution would not be sufficient to accurately reflect the full degree of decolourization. To obtain additional information regarding these changes, the area under the curve in the visible regions (400-800nm) was incorporated.

3. Results and Discussion

The absorptivity is intrinsic properties of a colorant. Measuring the spectral absorption curves of dyes during biodegradation provided a means of determining the reaction rates and provides evidence of the structural change of dyes during biodegradation. Previous experience showed that fading of the original colour 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 in the maximum absorbance of disperse dyes occurred primarily in the first several days of treatment, with no substantial additional decrease in absorbance as the treatment time increased.

The wild and mutant type of *Pseudomonas putida* when treated with Congo red dye after 12 days of treatment was found to decolorized by up to 90.67% and 91.62% and mineralized by up to 96.16% and 98.26% at a constant peak wavelength of 515nm and 510nm respectively. While for the wild and mutant types of Direct blue '80' were decolorized by 92.9% and 97.2% and were also mineralized by 96.9% and 98.9% respectively (shown in figure 1 and 2).

The result obtained when *Klebsiella ozaenae* was used for the treatment of Congo red dye in 12 days period, a remarkable decolorization and mineralization of up to 95.89% and 97.62% was achieved by mutant type at a constant wavelength of 519nm. While the wild type decolorized and mineralized Congo red dye by up to 89.2% and 95.1% respectively with a constant peak wavelength of 518nm. However, for direct blue '80' the decolorization and mineralization efficiency was in the same rate which is up to 91.6% and 97.9% with a constant peak wavelength of 538nm and 532nm for both the wild and mutant type respectively (shown in figure 3 and 4). This result shows that a mutant type of *Klebsiella ozaenae* was potentially effective in textile effluent degradation than wild type which shows that the mutant carries another form of genes (enzymes) responsible for textile degradation. Hence the result above solely agrees with the finding of Hai *et al*., (2007).

However, the result obtained by a mixed culture of wild and mutant type of *Pseudomonas putida* and *Klebsiella ozaenae* was able to shows a decolorization and mineralization efficiency in the same rate by up to 90.2% and 96.3%, while for mineralization by 95.3% and 98.3% in which the constant peak wavelength was 527nm and 521nm respectively as treated with Congo red dye in 12 days period. While for direct blue '80' was able to decolorized and mineralized in the same percentage efficiency by up to 98.7% and 99.3% with a constant peak wavelength of 537nm and 529nm for both the wild and mutant type respectively (shown in figure 5 & 6).

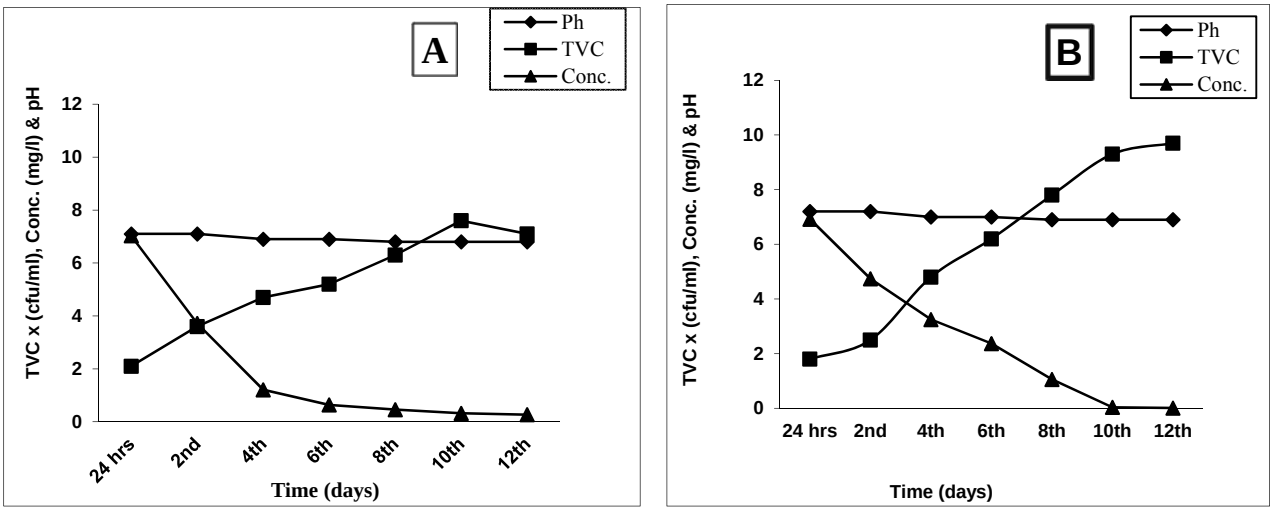

Fig 1: Growth profiles of wild (A) and mutant (B) types of *Pseudomonas putida* in mineral salt medium containing Congo red as sole source of carbon and energy (35 ^{o}C)

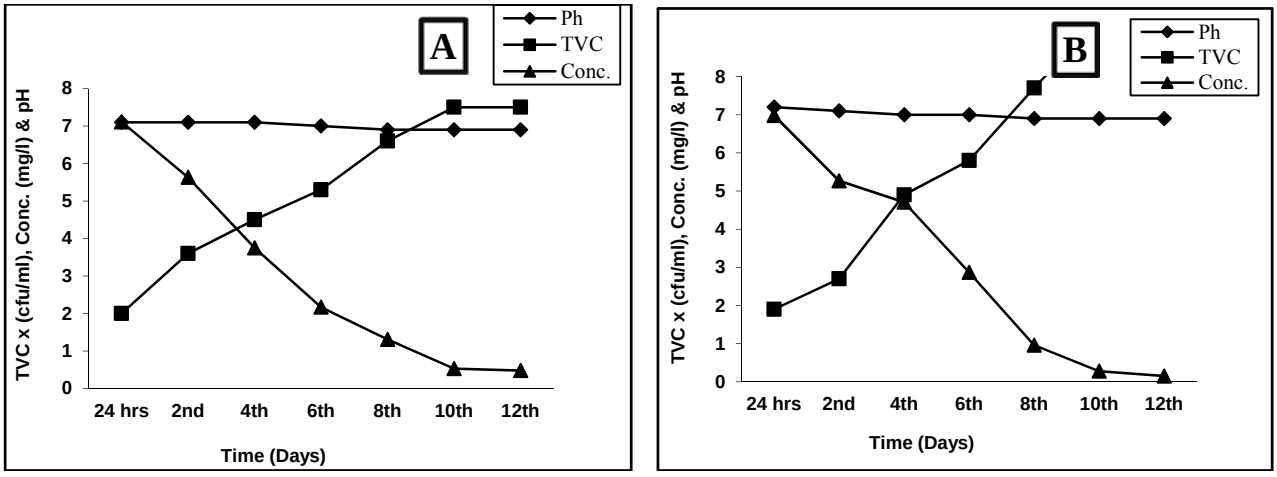

Fig 2: Growth profiles of wild (A) and mutant (B) types of *Pseudomonas putida* in mineral salt medium containing Direct blue '80' as sole source of carbon and energy (35 ^{o}C)

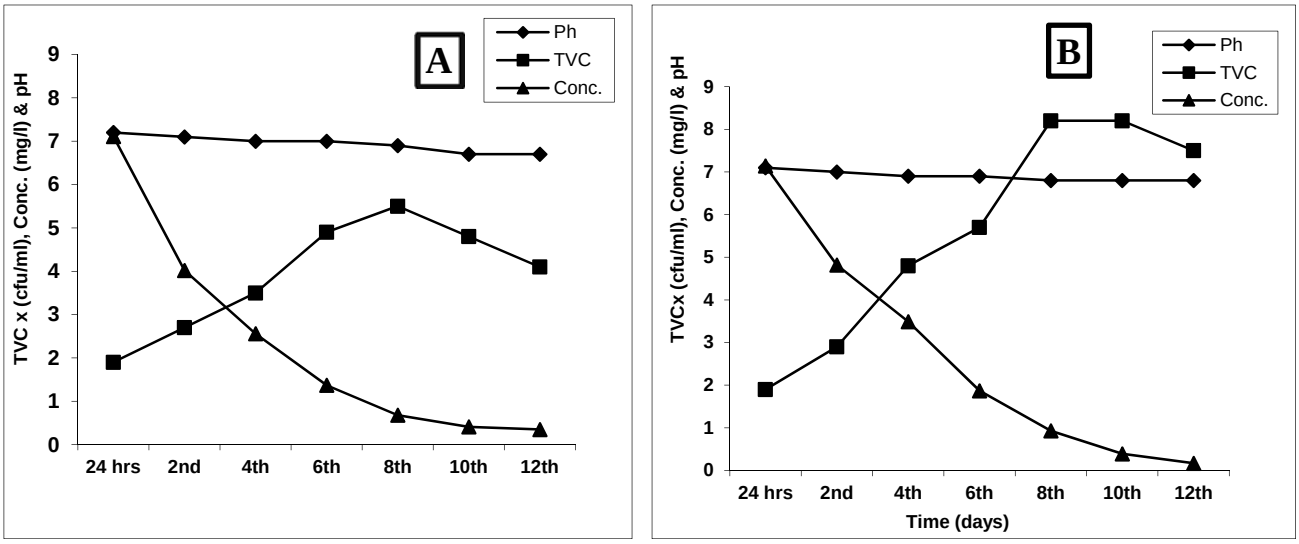

Fig 3: Growth profiles of wild (A) and mutant (B) types of *Klebsiella ozaenae* in mineral salt medium containing Congo red as sole source of carbon and energy (35 ºC).

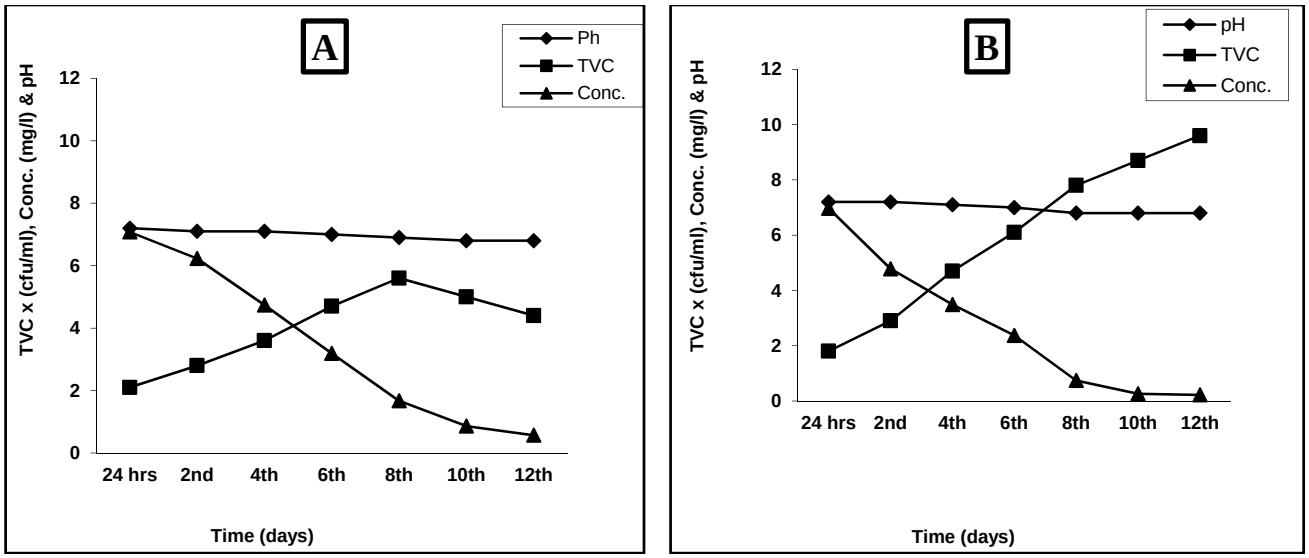

Fig 4: Growth profiles of wild (A) and mutant (B) types of *Klebsiella ozaenae* in mineral salt medium containing Direct blue '80' as a sole source of carbon and energy (35 ºC).

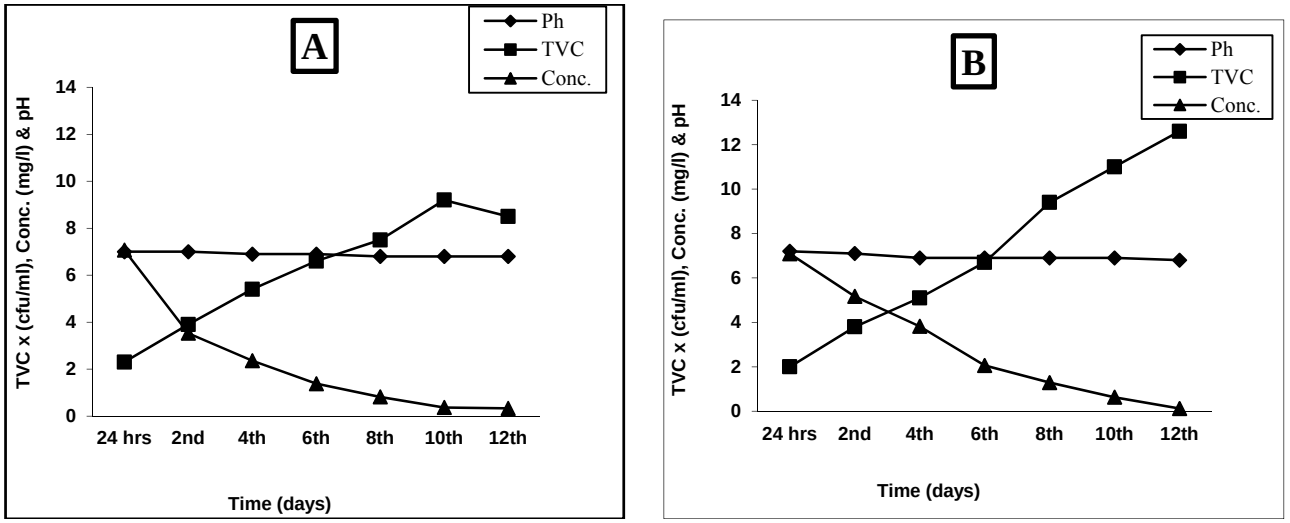

Fig 5: Growth profiles of wild (A) and mutant (B) types of *Pseudomonas putida* and *Klebsiella ozaenae* in mineral salt medium containing Congo red as a sole source of carbon and energy (35 ºC).

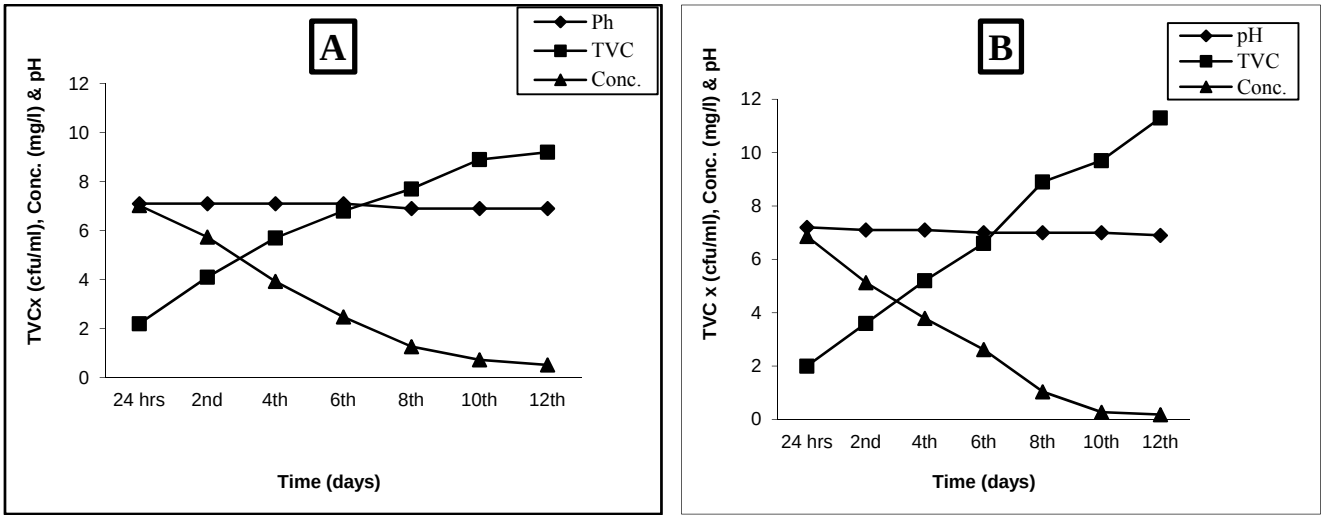

Fig 6: Growth profiles of wild (A) and mutant (B) types of *Pseudomonas putida* and *Klebsiella ozaenae* in mineral salt medium containing Congo red as sole source of carbon and energy (35 ^{o}C).

4. Conclusion

To achieve bioremediation, there needs to be a presence of textile dyes utilizing microorganisms and also optimum nutrient availability. The period of the research (duration) was pertinent in achieving higher rates of degradation. The present study clearly demonstrates that the indigenous bacterial community in textile dyes effluent of African textile industry Kano has the ability to degrade and decolorize various types of dyes emanating from such industries and therefore can be exploited for bioremediation of textile dyes containing wastes, due to their potentiality to degrade toxic reactive dyes into non-toxic form. Knowledge of bioremediation is important for the evaluation of indigenous microbial capabilities, the persistence of organic pollutants and the design of biodegradation facilities for large scale treatment applications. Further research is needed for optimization of the process, where the degradation of the fragmented products would be of great interest considering the aquatic toxicity of the overall effluent. The purification or reduction of contaminants in wastewater is a very crucial process before discharge into the environment as this will go a long way in preserving the quality of our environment, particularly our surface and ground water resources.

5. Acknowledgement

We appreciate the support and contributions of late Prof. J. T. Ayodele, Prof. J. Na'aliya, and Dr. J. Emmanuel, Head of Department of Microbiology, National Research Institute of Chemical Technology Zaria, Nigeria.

6. References

1. Almeida L. Ecolabels and Organic Certification for Textile Products. In Roadmap to Sustainable Textiles and Clothing, Springer Singapore, 2015, 175-196.
2. Asilian H, Moradian Fard SH, Rezae A, Mortazavi SB, khavanin A. The removal of color and COD from wastewater containing water base color by coagulation process. International Journal of Environmental Science & Technology. 2006; 3(2):153-157.
3. Chakraborty S, Purkait MK, DasGupta S, De S, Basu JK. Nanofiltration of textile plant effluent for color removal and reduction in COD. Separation and Purification Technology. 2003; 31(2):141-151.
4. Chattopadhyay SN, Pan NC, Roy AK, Khan A. Sustainable coloration of jute fabric using natural dyes with improved color yield and functional properties. AATCC Journal of Research. 2015; 2(2):28-36.
5. Cheesebrough M. District laboratory practice for tropical countries, part 2. Cambridge University Press, UK, 2005, 180-197.
6. Chhabra M, Mishra S, Sreekrishnan TR. Combination of chemical and enzymatic treatment for efficient decolorization/degradation of textile effluent: High operational stability of the continuous process. Biochemical Engineering Journal. 2015; 93:17-24.
7. Dasgupta J, Sikder J, Chakraborty S, Curcio S, Drioli E. Remediation of textile effluents by membrane based treatment techniques: A state of the art review. Journal of Environmental Management. 2015; 147:55-72.
8. Dey S, Islam AA. Review on textile wastewater characterization in Bangladesh. Resources and Environment. 2015; 5(1):15-44.
9. Dhanwada KR, Dickens M, Neades R, Davis R, Pelling JC. Differential effects of UV-B and UV-C components of solar radiation on MAP kinase signal transduction pathways in epidermal keratinocytes. *Oncogene.* 1995; 11(10):1947-1953.
10. Grancarić AM, Tarbuk A, Botteri L. Light Conversion and scattering in UV protective textiles. Autex Research Journal. 2014; 14(4):247-258.
11. Gupta GS, Prasad G, Singh VH. Removal of chrome dye from aqueous solutions by mixed adsorbents: Fly ash and coal. Water Research. 1990; 24(1):45-50.
12. Hai FI, Yamamoto K, Fukushi K. Hybrid treatment systems for dye wastewater. Critical Reviews in Environmental Science and Technology. 2007; 37(4):315-377.
13. Hasanbeigi A, Price LA. Technical review of emerging technologies for energy and water efficiency and pollution reduction in the textile industry. Journal of Cleaner Production. 2015; 95:30-44.
14. Husaini SN, Zaidi JH, Akram M. Comprehensive evaluation of the effluents eluted from different processes of the textile industry and its immobilization to trim down the environmental pollution. Journal of Radio-analytical and Nuclear Chemistry. 2011; 288(3):903-910.
15. Jasińska A, Paraszkiewicz K, Słaba M, Długoński J.

Microbial Decolorization of Triphenylmethane Dyes. In microbial degradation of synthetic dyes in wastewaters. Springer International Publishing. 2015, 169-186.
16. Jyothi S, Seethadevi A, Prabha KS, Muthuprasanna P, Pavitra P. Microencapsulation: A Review. International Journal of Pharmaceutical and BioSciences. 2012; 3(1):509-531.
17. Karthikeyan, K. Phytotoxicity Analysis of untreated and bacterial consortium treated textile dye effluent. International Journal of Pharmaceutical and Biological Archive. 2015; 5(3):172-175.
18. Knapp JS, Newby PS. The microbiological decolorization of an industrial effluent containing a diazo-linked chromophore. Water Research. 1995; 29(7):1807-1809.
19. López-Grimau V, Vilaseca M, Gutiérrez-Bouzán C. Comparison of different wastewater treatments for colour removal of reactive dye baths. Desalination and Water Treatment. 2015, 1-8.
20. Mondol SK, Sarif MR, Rimi RH, Bakali B, Mobin MN. Assessment of physico-chemical characteristics of textile effluent at different stages of treatment process. Journal of Environmental Science and Natural Resources. 2015; 7(2):53-58.
21. Nasser NM, El-Geundi M. Comparative cost of colour removal from textile effluents using natural adsorbents. Journal of Chemical Technology and Biotechnology. 1991; 50(2):257-264.
22. Naveed S, Bhatti I, Ali K. Membrane technology and its suitability for treatment of textile waste water in Pakistan. Journal of Research (Science), 2006; 17(3):155-164.
23. Nigam P, Armour G, Banat IM, Singh D, Marchant R. Physical removal of textile dyes and solid state fermentation of dye adsorbed agricultural residues. Bioresource Technology. 1996; 72:219-226.
24. Nigam P, Armour G, Banat IM, Singh D, Marchant R. Physical removal of textile dyes from effluents and solid-state fermentation of dye-adsorbed agricultural residues. Bioresource Technology. 2000; 72(3):219-226.
25. Nigam P, Marchant R. Selection of the substratum for composing biofilm system of textile decolourizing bacteria. Biotechnology Letters. 1995; 17:993-996.
26. Ogawa T, Yatome C. Biodegradation of azo dyes in multistage rotating biological contactor immobilized by assimilating bacteria. Bulletin of Environmental Contamination and Toxicology. 1990; 44(4):561-566.
27. Patil P, Patil V, Surwase S, Jadhav J. Evaluation of the efficiency of isolated bacterial consortium PMB11 in removal of colour, degradation and reduction of toxicity from textile dye effluent. Biologia. 2015; 70(1):11-18.
28. Ranade VV, Bhandari VM. Industrial wastewater treatment, recycling, and reuse: An Overview. Industrial Wastewater Treatment, Recycling and Reuse. 2014, 1.
29. Reddy CA. The potential for white-rot fungi in the treatment of pollutants. Current opinion in Biotechnology, 1995; 6(3):320-328.
30. Robinson T, Chandran B, Nigam P. Removal of dyes from synthetic textile dye effluent by biosorption on apple pomace and wheat straw. Water Research. 2002; 36(11):2824-2830.
31. Robinson T, McMullan G, Marchant R, Nigam P. Remediation of dyes in textile effluent: a critical review on current treatment technologies with a proposed alternative. Bioresource Technology. 2001; 77(3):247-255.
32. Singh L, Singh VP. Textile Dyes Degradation: A microbial approach for biodegradation of pollutants. In microbial degradation of synthetic dyes in wastewaters, 2015, 187-204.
33. Singh VN, Mishra G, Panday KK. Removal of congo red by wallastonite. Indian Journal of Technology. 1984; 22(2):70-71.
34. Stolz A. Basic and applied aspects in the microbial degradation of azo dyes. Applied Microbiology and Biotechnology. 2001; 56(1-2):69-80.
35. Tehrani MM, Assadi MM, Rashedi H. Biodecolorization of textile effluents by autochthonous fungi. Journal of Applied Biotechnology Reports. 2015; 1(4):161.
36. Zeng X, Guo X, Su G, Danquah MK, Zhang S, Lu Y *et al*. Bioprocess considerations for microalgal-based wastewater treatment and biomass production. Renewable and Sustainable Energy Reviews. 2015; 42:1385-1392.
37. Zhao Y, Zhao Y, Xu H, Yang YA. Sustainable slashing industry using biodegradable sizes from modified soy protein to replace petro-based poly (vinyl alcohol). Environmental Science and Technology. 2015; 49(4):2391-2397.