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Evaluation of Microbial Systems for Biotreatment of Textile Waste Effluents in Nigeria: Biodecolourization and Biodegradation of textile Dye

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ABSTRACT: The evaluation of some microbial species for the decolourization and degradation of textile dye has been investigated. Six microbial strains were isolated from soil contaminated with textile waste effluents using the spread plate technique and the isolates were identified as bacterial isolates (*Pseudomonas fluorescence, Pseudomonas nigificans,* and *Pseudomonas gellucidium*) and fungal isolates (*Aspergillus niger, Proteus morganii* and *Fusarium compacticum*} based on gram staining, morphological and biochemical tests. They were evaluated for their capability to remove colour and degrade dye, reduce chemical oxygen demand (COD) and biological oxygen demand (BOD) levels of textile waste effluents. The results revealed that all the bacterial and fungal isolates have a good potential to remove colour and degrade dye, reduce the COD and BOD levels of the textile waste effluents with percent colour removal, COD and BOD reductions between 39 and 48%, 74 and 97% and 77 and 95%, respectively. Binary mixed culture of *Pseudomonas fluorescence* and *Aspergillus niger* was efficiently utilized for the removal of different initial concentration (10, 15, 20, 25 and 30 mg/l) of dye from textile waste effluents. It had a higher percent decolourization than individual isolates. The stirred tank bioreactor was found to be very effective for efficient biotreatment of textile waste effluents. @JASEM

Textile waste effluents contain several types of chemicals such as dyes, dispersants, leveling agents, acids and alkali (Cooper, 1995; Olukanni *et al*., 2006) that are discharged into water bodies by textile industries without any effective treatment. This increases the chemical oxygen demand (COD) and biochemical oxygen demand (BOD), alters the pH and gives the water bodies (rivers) intense colourations. Coloured wastewater from textile industries is rated as the most polluted in almost all industrial sectors (Andleeb *et al*., 2010). Tremendous amount of dyes in textile sectors are continuously being exhausted in wastewater streams due to their poor adsorb ability to the fiber (Wagner, 1993; McMullan *et al*., 2001). The presence of very small amounts of dyes in water is highly visible and affects the aesthetic merit, water transparency and gas solubility in lakes, rivers and other water bodies (McKay, 1979) and degradation products of these dyes are often carcinogenic (Kim *et al*., 2003). The treatment of textile waste effluents is still a major environmental concern because of synthetic dyes which are difficult to be removed by conventional physical and chemical technologies (Zhang *et al*., 2004) such as membrane filtration, coagulation, precipitation, flotation, adsorption, ion exchange, chemical reduction, ultrasonic mineralization, electrolysis and advanced chemical oxidation (Gogate and Pandit, 2004; Kang *et al*., 2010). Some of these methods are effective but have inherent drawbacks such as high cost, intensive energy requirements, formation of hazardous by-products and generation of sludge which causes secondary pollution (Do *et al*., 2002; Verma *et al*., 2003; Maier *et al*., 2004; Ramya *et al*., 2007; Dayaram and Dasgupta, 2008). Current available treatment technologies have been reviewed by Robinson (Robinson *et al*., 2001) and specific attention is given to biological treatment processes because they are cost- effective and environmentally friendly.

The most widely studied class of microorganisms in regard to dye degradation and decolourization are the fungi (Bochart and Libra, 2001; Maxima and Costa-Ferreira, 2004; Mechichi *et al*., 2006; Singh *et al*., 2007; Revankar and Lele, 2007; Ramya *et al*., 2007, Dayaram and Dasgupta, 2008; Andleeb *et al*., 2010). A great number of these fungi (especially white-rot fungi) have been shown to excrete extracellular enzymes like lignin peroxidase, manganese peroxidase and laccase (Hatakka, 1994) which catalyze the formation of activated oxygen so that the process of attack on the stable structure of dyes can be initiated. The role of some bacterial and algal species for the decolourization and degradation of textile dyes has also been reported (Jumarkar *et al*., 2006; Olukanni *et al*., 2006; Pourbabaee *et al*., 2006; Togo *et al*., 2008; Cheriaa *et al*., 2009). Dyes are removed by biosorption (Fu and Viraraghavan, 2000) and enzymatic mineralization (degradation) by lignin peroxidase, manganese peroxidase, manganese independent peroxidase and laccases (Raaghukumar *et al*., 1996; Duran *et al*., 2002; Wesenberg *et al*., 2003; Svobodova *et al*., 2006).

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The development of efficient dye degradation and decolourization require a suitable strain and its use under favourable conditions to realize the degradation potential. Therefore, it is important to explore the possibilities of isolating efficient aerobic degraders for use in decolourization and degradation of textile dye waste effluents.
The objective of this study was to isolate microorganisms from soil contaminated with textile dye waste effluents and evaluates their remediation potential to decolourize and degrade dye or reduce the COD and BOD of textile waste effluents. The study is aimed at discovering isolates with efficient biodecolourization and biodegradation potential for use in the biotreatment of textile waste effluents.

MATERIALS AND METHODS

Microorganisms: The microorganisms used in this study were isolated from soil contaminated with textile waste effluents discharged from one of the textile industry located at Oshodi in Lagos, Nigeria.

Textile waste effluents: The textile waste effluents sample used for this study was obtained from the same textile industry located at Oshodi in Lagos, Nigeria.

Characterization of textile waste effluents: The textile waste effluents was analyzed in triplicates for pH, chemical oxygen demand (COD), biochemical oxygen demand (BOD), total solids (TS), total suspended solids (TSS), total dissolved solids (TDS), chlorides and dye using standard methods (APHA-AWWA, 1985).

Determination of pH: pH of the samples was determined using pH meter which has been initially standardized by using buffer solutions of known value before analysis

Determination of Total Solids: A clean dish was taken and dried at 103 – 105^{o}C in an oven to a constant weight (W1). Twenty-five millilitre of thoroughly mixed effluent sample was accurately pipette into a dish, weighed and evaporated to dryness on a steam bath. The residue was dried in an oven for about 1 h at 103 – 105^{o}C and re-weighed after cooling to room temperature. The cooling was done until the weight of the dish plus residue was constant (W2). The weight of the dish was subtracted to obtain the weight of the total solids.

Determination of Total Suspended Solids: Twenty-five millilitres of the effluent sample was withdrawn into a conical flask with a pipette. It was filtered in Gooch funnel fitted with glass fibre filter paper which has been pre-dried at 103 – 105^{o}C. The glass fibre was carefully removed from the Gooch and dried to a constant weight at 103-105^{o}C and the weight subtracted from the weight of the filter paper to obtain the weight of the suspended solids.

Determination of Total Dissolved Solids: Total dissolved solid was obtained by the difference between total solids and suspended solids.

Determination of Chlorides: The Chloride was determined by Mohr's titration. Twenty millilitres of sample was placed in a conical flask and pH adjusted to between 6 and 8 with small amount of (0.1 M) calcium carbonate solution. One millilitre of potassium chromate solution prepared by dissolving 50 g of potassium chromate in a minimum of distilled water was added and the solution was titrated with (0.0141 M) silver nitrate solution with constant stirring.

Determination of Total dye: Textile sample effluents (50 mg/L) were taken and their light absorbance was measured at wavelengths of 400 –700 nm. UVVIS spectra were determined on a Spectronic Helios alpha spectrophotometer operated with VISION32, and 1 cm×1 cm quartz cuvettes (Starna) were used as sample cells.

The values of the various parameters for the untreated textile waste effluents are presented in Table 1.

Table 1: Characterization of the untreated textile waste effluents

Parameter	Concentration (mg/L)
pH	10[a] ± 1
Biological oxygen demand (BOD)	5200 ± 100
Chemical oxygen demand (COD)	6410 ± 230
Total suspended solids (TSS)	2820 ± 110
Total dissolved solids (TDS)	4560 ± 160
Total solids (TS)	7380 ±270
Chlorides	916 ± 25
Total dye	30 ± 4

a = no unit for pH

Isolation and characterization of microorganisms: The contaminated soil sample (10 g) was diluted serially (10^{-1} to 10^{-7}). One mililitre from dilution 10^{-2}

and 10^{-3} were plated in triplicate on sterile potato dextrose agar plates while dilutions of 10^{-4}, 10^{-5} and 10^{-7} were plated in triplicates on sterile nutrient agar plates using the pour plate method. Incubations were carried out at room temperature for the potato dextrose agar plates for 7 days and 24 h for the nutrient agar plates, respectively. Plates yielding 30 to 300 colonies were enumerated for bacterial isolates. Plates with fungal colonies were also enumerated. The colonies of bacteria and fungi were picked randomly using a sterile inoculating loop and sub cultured (to purify) by streaking on nutrient agar plates and potato dextrose agar plates, respectively. The plates were incubated at room temperature for 24 h (bacterial isolates) and 72 h (fungal isolates), respectively, to obtain pure colonies. The isolates were identified by gram staining, morphological and biochemical tests using the taxonomic scheme of Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1994).

Preparation of bacterial and fungal inoculums: In order to meet the nutritional requirement of the microorganisms for proper growth, the textile waste effluents was supplemented with mineral salts medium containing the following compositions g/L: glucose 1 g; KH_2PO_4 2 g; K_2HPO_4 7 g; $MnSO_4$ 0.1 g; $Na_3C_6H_5O_7$ 0.5 g; $(NH_4)_2SO_4$ 1.0 g (Ramya *et al*., 2007). All chemicals were of analytical grade. Colonies were transferred from the agar plates containing the isolates to 100 ml medium (containing both the mineral salt medium and the waste effluent samples in ratio 4:1) in 250 ml Erlenmeyer conical flasks. The flasks were incubated on New Brunswick gyratory shaker (G25-R model, New Jersey, USA) at 120 rpm and 30^{o}C for 24 h. The pH of the medium was adjusted to 7 using 0.1 M HCl and 0.1 M NaOH. Between 5 – 10% (v/v) of these grown bacterial cultures were used to inoculate fresh flasks and were cultivated at the same conditions for 24 h.

Mycelium and spore suspension of fungal cultures was prepared. Disc of 0.5mm containing mycelium and spores (0.4 mg dry weight) were removed from the borders of fungal colonies on the potato dextrose agar plate and added to 100 ml of medium (containing both the mineral salt medium and the waste effluent samples in ratio 4:1) in 250 ml Erlenmeyer conical flasks. Streptomycin (0.05%) was used as antibacterial agent. The flasks were incubated on New Brunswick gyratory shaker (G25-R model, New Jersey, USA) at 120 rpm and 30^{o}C for 72 h. The pH of the medium was adjusted to 6 using 0.1M HCl and 0.1M NaOH. Between 5 – 10% (v/v) of these grown fungal cultures were used to inoculate fresh flasks and were cultivated at the same conditions for 72 h.

Batch microbial treatment studies: Different biodegradation runs were carried out on a 7.5 L capacity New Brunswick Microferm Twin Fermentor designed for mass cultivation of microorganisms in batch and continuous culture fermentation. The reactor is equipped with a control panel which permits the regulation of agitation and temperature. An autoclaved mineral salt medium (0.8 L) was measured into the bioreactor and 3 L of textile waste effluents was added. The prepared inoculum (200 ml) was added aseptically to make up 4 L of working volume. The reactor was operated at room temperature and agitation speed of 300 rpm. Aeration was effected with compressed air at a flow rate of 2.0 volume of air per volume broth per minute (vvm). The fermentation was carried out for 14 days after which samples were taken for decolourization, COD and BOD analysis. All samples were filtered through Whatman paper No 1 and centrifuged for 10 min. The pellet was discarded and clear solution was analyzed.

Determination of colour removal: Decolourization of the textile waste effluents was determined by measuring the absorbance at the pre-determined absorbance maximum (600 nm) using a UV-visible spectrophotometer (Labomed, USA). The decolourization efficiency was expressed as per the following equation:

$$\% \text{ Decolourization} = \left[\frac{A-B}{A}\right]x100$$

Where, A and B are the initial and final absorbance, respectively.

Determination of chemical oxygen demand: COD analyses of the untreated and treated samples from bioreactor were determined by a standard colorimetric method (APHA-AWWA, 1985). Ten millilitres of the sample was poured into a 250 ml conical flask, and 5 ml of concentrated sulphuric acid and about 1 g of copper sulphate were added. Three millilitres of prepared N/40 potassium permanganate solution was then added to the contents in the flask and subsequently immersed in boiling water for 30 min. Three millilitres of prepared N/40 sodium oxalate was then added and immediately titrated with N/40 potassium permanganate until a violet colour was obtained. This procedure was repeated for the blank using ten millilitres of distilled water instead of ten millilitres of sample.

Determination of biochemical oxygen demand: BOD analyses of the untreated and treated sample from bioreactor were performed by using 5– day BOD test. The BOD test was carried out using a BOD track instrument which is a refrigerated incubator that has chassis on which amber sample bottles are placed and the bottles are connected by caps and tubing to the instrument's pressure sensor. The waste water samples were cooled to 20^{o}C by dipping the sample container in ice bucket. Using a clean measuring cylinder, 200 ml of the sample was poured into each amber sample bottle. A magnetic stirring bar was put into each of the sample bottles as well as the content of one package of BOD Nutrient buffer solution pillow to allow for optimum bacteria growth. Stopcock grease was then applied to the sealed lips of the bottles and to the top of the sealed cups. Each seal cup was then put into the neck of the bottle. One Lithium hydroxide powder pillow was added through a polyethylene funnel into each of the seal cups. This removes the carbon-dioxide produced during the oxidation of the organic matter within the sample. The bottles were then placed on the chassis of the BOD Track. Appropriate tubes were then connected to each bottle and tightened firmly to the cap. The BOD Track was then placed in the incubator and left for 5 days. Pressure sensors monitor air pressure within the sample bottles and when the air pressure drops, the pressure changes are converted to mg/L BOD. The BOD result was read directly from the control panel display by pressing the key corresponding to each sample bottle.

HPLC analysis of degraded products: The textile dye degradation was monitored by HPLC as the decolourization progressed. Ten millilitres of samples were taken at the start of the experiment and daily (24 h), centrifuged and filtered through 1.2 µm filter paper. The filtrate was extracted three times with methylene chloride and evaporated in rotary evaporator with 45 – 50^{o}C water bath, after which the residue was dissolved in 2 ml methanol. The extracted samples were analyzed using 60% acetonitryl and 40% water (mobile phase) at a flow rate of 0.5 ml/min. The elution of the samples was done isocratically using a C 18 reversed phase column (RPC – 18 phenomenex) and the UV–VIS detector was set at 285 nm.

RESULTS AND DISCUSSION

A total of six bacterial isolates that are capable of utilizing textile dye as a source of carbon and energy were obtained from the soil contaminated with textile waste effluents. The gram staining, morphological and biochemical characterization of the isolates revealed the following bacterial species: *Pseudomonas fluorescence, Pseudomonas nigificans* and *Pseudomonas gellucidium*; and the fungal species, which are *Aspergillus niger, Fusarium compacticum* and *Proteus morganii*.

Figure 1 shows the percent dye decolourization by the different microbial isolates. It could be seen that all the isolated microorganisms has a percent dye decolourization between 39 and 48%. Comparatively among the bacterial isolates, *Pseudomonas fluorescence* demonstrated a higher percent dye decolourization (46%) than *Pseudomonas nigificans* (41%) and *Pseudomonas gellucidium* (39%), respectively. Also, in comparison among the fungal isolates, *Aspergillus niger* showed a higher percent dye decolourization (48%) than *Proteus morganii* (44%) and *Fusarium compacticum* (42%), respectively. Generally, the order of percent dye decolourization among all the isolates is as follows: *Aspergillus niger* > *Pseudomonas fluorescence* > *Proteus morganii* > *Fusarium compacticum* > *Pseudomonas nigificans* > *Pseudomonas gellucidium.*

The efficient use of different *Aspergillus* species (*A. niger, A. foetidus, A. fumigates* and *A. terreus*) for decolourization of different types of dye has been reported (Sumathi and Manju, 2000; Ali *et al*., 2007; Jin *et al*., 2007; Andleeb *et al*., 2010). The results of HPLC analyses (not shown) taken for the untreated and treated samples proved the role of degradation mechanisms in the dye colour removal phenomenon. It has been reported that decolourization of dye effluents is as a result of dye degradation mechanism (Glenn and Gold, 1983; Wesenberg *et al*., 2002; Ramya *et al*., 2007).

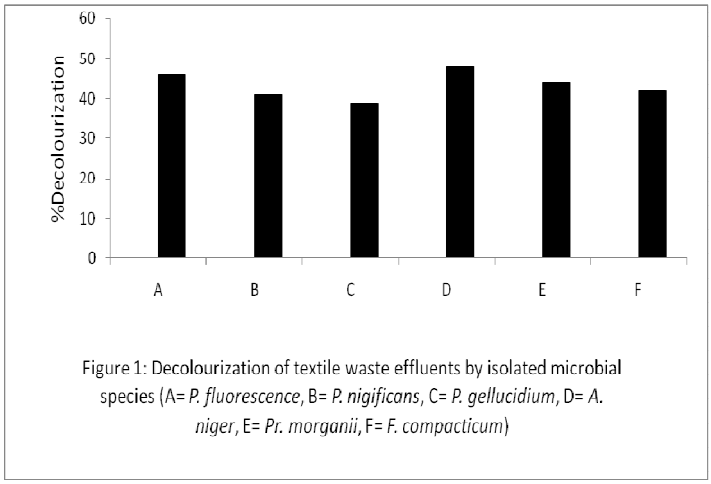

Figure 1: Decolourization of textile waste effluents by isolated microbial species (A= *P. fluorescence*, B= *P. nigificans*, C= *P. gellucidium*, D= *A. niger*, E= *Pr. morganii*, F= *F. compacticum*)

The percent COD and BOD reductions of the treated textile waste effluents by the different microbial isolates is shown in Figure 2 and Figure 3, respectively. It is seen that all the isolates demonstrated a COD reduction of between 74 and 97% and a BOD reduction of between 77 and 95%, respectively. In comparison among the bacterial isolates, *Pseudomonas fluorescence* depicted a relatively higher COD and BOD reductions (95% and 93%) than *Pseudomonas nigificans* (88% and 82%) and *Pseudomonas gellucidium* (74% and 77%), respectively. Among the fungal isolates, the *Aspergillus niger* revealed a relatively higher % COD and BOD reduction (97% and 95%) than *Proteus morganii* (93.5% and 91%) and *Fusarium compacticum* (92% and 88%). Nevertheless, *Aspergillus niger* had relatively higher % COD and BOD reductions than other bacterial and fungal isolates. Pourbabaee *et al*. (2006) and Andleeb *et al*. (2010) observed similar reductions in COD and BOD in their study of aerobic biotreatment of Terasil black in textile effluent by a newly isolated *Bacillus* sp. and sulfur black by *Aspergillus terreus* SA 3, respectively. High COD reduction in the treatment of textile effluent by *Pseudomonas* species has also been reported (Olukanni *et al*., 2006).

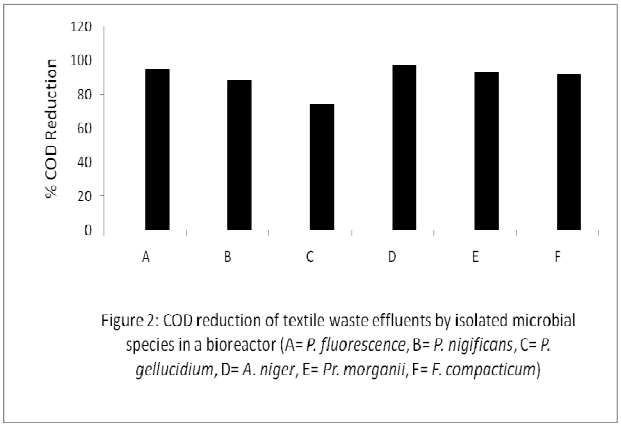

Figure 2: COD reduction of textile waste effluents by isolated microbial species in a bioreactor (A= *P. fluorescence*, B= *P. nigificans*, C= *P. gellucidium*, D= *A. niger*, E= *Pr. morganii*, F= *F. compacticum*)

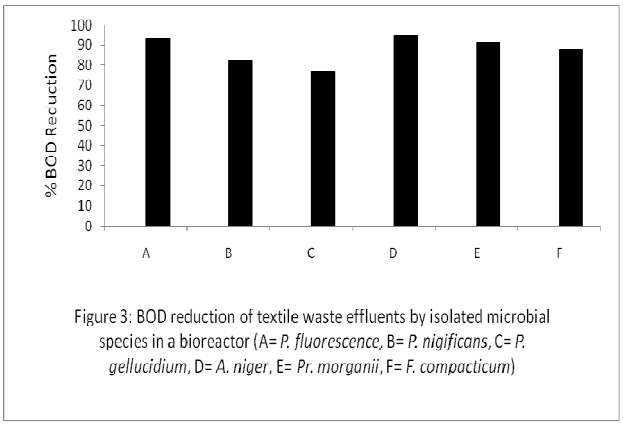

Figure 3: BOD reduction of textile waste effluents by isolated microbial species in a bioreactor (A= *P. fluorescence*, B= *P. nigificans*, C= *P. gellucidium*, D= *A. niger*, E= *Pr. morganii*, F= *F. compacticum*)

Since *Pseudomonas fluorescence* and *Aspergillus niger* relatively had a higher percent colour removal, COD and BOD reductions than other bacterial and fungal isolates respectively, they were further used as binary mixed culture to study the biodecolourization of dye in textile waste effluents under the same experimental conditions. The textile dye waste effluent with 30 mg/L dye concentration was diluted with sterile distilled water to obtain waste effluents with varying dye concentration of 10, 15, 20 and 25 mg/L, respectively. The textile waste effluents with dye concentration of 10 to 30 mg/L were introduced into the bioreactor and the percentage decolourization was monitored as shown in Figure 4. Maximum colour removal of 100%, 95%, 88%, 79% and 72% by the binary mixed culture of *Pseudomonas fluorescence* and *Aspergillus niger* was observed when the bioreactor was operated for 14 days for 10, 15, 20, 25 and 30 mg/L textile dye removal in the textile waste effluents (Figure 4). This indicates that the percent decolourization decreased with increased dye concentration. Thus, the rate of decolourization decreased with increased dye concentration. A similar observation has been reported for the colour removal of Reactive blue dye by *Polyporus rubidus* (Dayaram and Dasgupta, 2008) and the removal of sulfur black by *Aspergillus terreus* SA 3 (Andleeb *et al*., 2010), respectively. Moreover, the mixed culture had a higher percent decolourization (72%) of textile waste effluents with 30 mg/L dye concentration than the individual isolates. The advantages of employing mixed cultures (microbial consortium) as opposed to pure cultures in bioremediation have been demonstrated (Agarry *et al*., 2008).

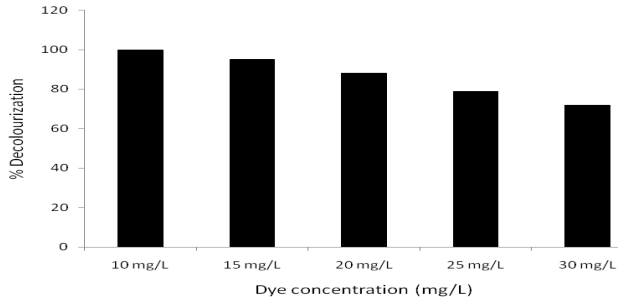

Figure 4: Effect of dye concentration on decolourization by binary mixed culture of *P. fluorescence and A. niger*

Conclusions: Several microorganisms may seem to have a potential for dye decolourization and degradation. However, it has been reported that very few strains can withstand the conditions of dyeing effluents (Maier, 2004). Thus, as a preliminary step in the development of textile waste effluents biotreatment process involving indigenous microbial species, the present study have shown that *Aspergillus niger, Pseudomonas fluorescence, Proteus morganii, Fusarium compacticum, Pseudomonas nigificans* and *Pseudomonas gellucidium,* respectively, has a significant potential for dye decolourization and degradation. Therefore, they are promising material for the removal of dyes from textile waste effluents. The present bioreactor system due to its cost effectiveness and easy

maintenance has great potential to be used for the biotreatment of textile dye waste effluents.

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