

Full Length Research Paper

Aerobic Bacterial degraders in effluent from Itoku textile industry, Abeokuta

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The local textile industry in Itoku village is one major textile industry in Abeokuta Ogun state, known for “adire” production whose processes are not maintained at regulatory standards. This study involves isolating and identifying aerobic microorganisms in waste water effluents from this textile Industry and screening for aerobic degraders among the isolates. Fifty milliliter (50 ml) of three dye effluents; purple, brown and green were collected into sterile bottles and designated (Ef₁), (Ef₂) and (Ef₃). Tap water was used as control (Cont). Isolation, identification and screening of isolates for percentage decolorization were done using standard method. Colony forming units showed the trend Ef₃ > Ef₂ > Ef₁. Bacterial isolates included *Bacillus megaterium*, *Staphylococcus simulans*, *Micrococcus varians*, *Bacillus niacinni*, *Lysinibacillus* sp. and *Bacillus carbonificus*. *Aneurinibacillus aneurinilytus* alone was isolated from the control sample. *Bacillus megaterium* and *Lysinibacillus* sp. were the most predominant species in the effluents. *S. simulans* was the most efficient degrader and its use could be exploited.

Key words: Aerobic bacteria, textile effluent, dye, Itoku.

INTRODUCTION

Every day, industrial, commercial and domestic activities produce wastes; some of which are hazardous to the health of the general public and the ecosystem. USEPA (2005), listed seven major hazardous constituents of dye as aniline, o-anisidine, 4-chloroaniline, p-cresidine, 1,2-phenylenediamine, 1,3-phenylenediamine, and 2,4-dimethylaniline.

Dyes are synthetic aromatic compounds with various functional groups. They are used extensively in textile industries (for dyeing clothing materials), paper printing, colour photography, pharmaceutical industries, food industries, cosmetic industries, leather and other Industries (Rafi et al., 1990; Ola et al., 2010). Approximately 10,000 different dyes and pigments are used industrially, and almost one million tons of synthetic dyes are produced annually, worldwide (Ollgaard et al., 1999; Adebayo et al., 2004). Coloured dye effluents are enormously toxic to the aquatic ecosystem. They change the symbiotic process by disturbing the natural balance

through reduced photosynthetic activity due to the colouration of the water in streams and other water bodies. With the increased use of a wide variety of these dyes, pollution by dye wastewater is becoming increasingly startling hence, serious environmental damages are inevitable (Cunningham and Saigo, 2001; Padmavathy et al., 2003; Asamudo et al, 2005).

Biodegradation is the breakdown of contaminating (toxic) compounds into simpler, less toxic or non toxic forms using microorganisms (Prescott et al., 2008). These microorganisms (usually fungi and bacteria) often use contaminants as their food source, thereby, completely eliminating toxic compounds breaking them into basic (non toxic) elements such as carbondioxide and water (mineralization). Incomplete degradation may also occur; which is the partial breakdown of the original contaminant to a less complex form. Typically, biodegradation provides an efficient and economic way to reduce environmental hazardous wastes using indige-

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nous or introduced microorganisms that naturally degrade these contaminants. Several members of Algae, Fungi and Bacteria are known to be able to degrade various compounds and toxic wastes. These microorganisms are capable of transforming or degrading a variety of organic and inorganic contaminants such as perchlorate, radionuclides, lead, mercury, petroleum products, arsenic, etc., at levels below possible health risk standards (Wu et al., 2001; Okeke et al., 2002; Wang, 2008, 2009).

Bacillus spp. and *Paenibacillus* spp. have been isolated as degraders from dye effluent (Shanooba et al., 2011). *B. brevis*, *B. formosus*, and *B. chosinensis* strain is also reported to be good degraders of Toluidine blue dye (Alhassani, 2007).

Bacillus spp. was reported by Modi et al. (2010) to be very effective in the decolorization of sulfonated dye after 72 h. *B. cereus* was reported to be potent for the decolorizing sulfonated azo dyes while *B. megaterium* had shown degradation of red 2G by 64.89% percentage (Khan, 2011). *Staphylococcus simulans* amongst other *Staphylococcus* species and microbial flora of the human body had caused degradation and decolorization of various dyes. *S. simulans* (NCH298) reportedly decolorization of Methyl red and Orange II dye effectively (Stingley et al., 2010).

The local textile industry in Itoku village is a major textile industry in Abeokuta south local government area, known for “adire” production and sales. The production processes (majorly manual) as well as effluent treatment are not maintained at regulatory standards. Their operations pose a potential risk to the community as some of the dye constituents are carcinogenic to human beings and toxic to the aquatic habitat, therefore, the degradation and/or decolorization of these azo dyes have become a necessity.

The research into bioremediation of pollutants is receiving plausible response in recent times and information on degraders among bacterial isolates has not been reported from this site, an industry without regulatory standards. The aim of this study was to isolate and identify aerobic microorganisms in waste water effluents from Itoku Textile Industry, Abeokuta, Ogun State and to screen for degraders among the isolates. The objectives of this study were to isolate and enumerate aerobic microorganisms present in dye samples as well as determine the degraders among the isolates.

MATERIALS AND METHODS

Sample collection

Fifty milliliter (50 ml) wastewater of different colours; purple (EF₁), brown (EF₂) and green (EF₃) were collected aseptically into 3 sterile sample bottles from three different points at Itoku Textile Industry Abeokuta. The bottle were then placed in a brown envelope and transported to the laboratory; and tap water was used as control (Cont). Serial dilutions of the solution (10⁻¹ to 10⁻⁹) were done

before screening.

Bacterial cultures

One milliliter (1.0 ml) from diluents 10⁻¹, 10⁻², 10⁻³, 10⁻⁴ and 10⁻⁵ were aseptically inoculated unto nutrient agar (NA) plates using the spread plate method and incubated at 37°C for 24 h. Un-inoculated NA plates were also incubated to serve as the controls. This was done in duplicate and distinct colonies were enumerated using colony counter and distinct colonies were sub-cultured to obtain pure cultures. Pure cultures were further subjected to identification processes. Pure cultures were transferred into slants of Nutrient agar in Bijou bottles and preserved at 4°C in the refrigerator.

Microscopic and biochemical characterization of bacterial isolates

This included standard identification procedure like Gram-staining procedure described by Brown (2007) and biochemical tests such as including, oxidase test (Cheesbrough, 2006a), catalase test, (Cheesbrough, 2006b), coagulase test (Goldstein and Roberts, 1981), motility test (Perilla, 2003), Indole test, (Maria, 2010), Urease test, hydrogen sulphide production (triple sugar iron) (TSI) test, methyl red vogues proskauer test and Citrate Utilization test (Claus, 1989). Final identification of isolates was done by software (ABIS online- www.abis.online.com).

Screening for aerobic dye degrader

0.015 g of each dye RB 13(Blue), RY 45 (Yellow) and RR58 (Red) was put in a 500 ml conical flask, 500 ml of distilled water was added and the dye mixture was designated (BYR). The dyes were allowed to dissolve properly, shaken and autoclaved at a temperature of 121°C for 15 min. A sterile pipette was used to dispense 5 ml of the dye into sterile test tubes, including the control. The initial absorbance (A₀) was determined using the UV visible spectrophotometer machine. Afterwards, 5 ml of each pure bacteria grown overnight in nutrient broth was aseptically transferred into the tubes of dye mixture (RYR); each tube was sealed with sterile cotton wool and labeled accordingly. The test tubes were incubated for 24 h after which they were centrifuged and the absorbance of the supernatant was measured (A_t) using UV visible Spectrophotometer machine (Olukanni et al., 2010), experimental set-ups were in triplicate.

Data analysis

Data collected are presented with descriptive statistics.

RESULTS AND DISCUSSION

Bacterial count

The colony forming units were counted and calculated for the various dye samples EF₁, EF₂ and EF₃ (Figure 1). EF₃ showed the highest cfu /ml, followed by EF₂ and the EF₁ was the least. All effluent samples had higher colony forming unit than the control.

Identification of bacterial isolates

Pure isolates obtained from effluent samples collected

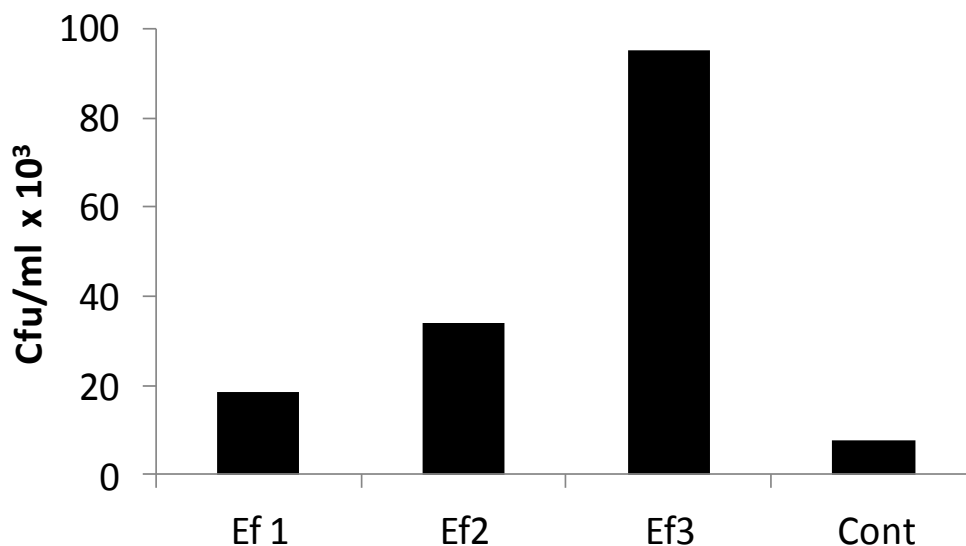


Figure 1. A bar chart representing the number of colony forming unit in effluents samples and control. Ef1 = Purple; Ef2 = Brown; Ef3 = Green; Cont = Tap water control; Cfu/ml = Colony forming units/milliliters.

from Itoku textile industry Abeokuta were identified. Each isolate was given a sample number. Gram stain and biochemical results for all samples are presented in Table 1. Table 2 shows bacteria isolated in the different effluent samples and the control sample.

The organisms isolated from the samples were *B. megaterium*, *S. simulans*, *M. varians*, *B. niacinni*, *Lysinibacillus* sp., *B. carbonificus*. *A. aneurinilytus* alone was isolated from the control sample. *B. megaterium* and *Lysinibacillus* sp. were the most predominant species in the effluents. Most bacteria isolated from effluent have been reported in literatures as degraders of dye except *A. aneurinilytus*.

Three species of *Bacillus*; *B. megaterium*, *B. carbonificus* and *B. niacinni* were reported in this study. Findings from Khan (2011), indicated that *B. megaterium* isolated from marina beach decolorized red 2G by 64.89%. Moreover, *B. megaterium* NCIM 2087 and *P. desmolyticum* NCIM 2112 showed 70% decolorization of textile dye Vat Blue 66. *Bacillus* spp. had earlier been isolated from dye effluent (Shanooba et al., 2011) and three strains of *B. brevis*, *B. formosus*, and *B. chosinensis* strain had earlier been reported to be good degraders of Toluidine blue dye (Alhassani, 2007). *Bacillus* sp. was reported to be more effective for decolorization of sulfonated dyes; 95% of reactive red 195 dye (Modi et al., 2010). However, *B. megaterium* identified in this study showed only 11.3% decolorization in the dye mixture (RYR) in 24 h, and this could be due to the mixture of dye used. *Lysinibacillus* spp. showed the lowest degradation (0.27%) from this study, although Dawker (2007) had reported that *Bacillus* spp., and other *Lysinibacillus* spp were used for the degradation of dye. *A. aneurinilyticus*, among other bacteria were isolated

from hot spring waters of Kharaghan (in Gazvin province) and Mahallat (in Arak). *A. aneurinilyticus* was however isolated in this study from the control (tap) water sample.

S. simulans was isolated in this study and it could be as a result of the manual method of textile processing employed in this industry which involves the use of hands; this bacterium amongst other *Staphylococcus* species which are microbial flora of the human body had been demonstrated to degrade and decolorize various dyes (Ref.).

Percentage decolorization of dye mixture

The percentage decolorization of dye mixture (RYR) is presented in Table 3. The result indicates that *Lysinibacillus* sp. showed the lowest percentage decolorization (0.27%) followed by *M. varians* (8.5%) while the highest decolorization was recorded by *S. simulans* (88.9%) followed by *B. niacinni* (23.3%). *S. simulans* which showed 88.98% decolorization of dye mixture (BYR) in 24 h from this study caused 100% degradation in 1.25 h of MR (Methyl red) and 86% deduction in 24 h of OrII (Orange II) (Stingley et al., 2010). Variation in the percentage decolorization of dye with *S. simulans* could be due to the mixture of dyes used. This study therefore supports that *S. simulans* is a good degrader of dye.

Conclusion and recommendation

Seven aerobic bacteria were isolated from the Itoku textile effluent an indication of the hostile condition for the

Table 1. Identification of Isolated bacterial organism.

Isolate number	Catalase	Oxidase	Methyl red	Motility	Voges -Proskauer	Citrate	Urease	Indole	Gram reaction	Shape	Coagulase	Suspected organism
1	+	-	-	+	-	+	+	-	+	Rods	-	<i>Bacillus megaterium</i>
2	+	-	-	+	-	-	+	-	-	Cocci	-	<i>Staphylococcus simulans</i>
3	+	+	+	-	-	+	+	+	-	Rods	-	<i>Aneurinibacillus aneurinilytus</i>
4	+	-	-	+	-	+	-	-	+	Cocci	+	<i>Micrococcus varians</i>
5	+	-	-	-	-	-	-	+	+	Rods	-	<i>Bacillus carboniphilus</i>
6	+	-	-	-	-	-	-	+	-	Rods	-	<i>Bacillus niacinni</i>
7	+	-	+	+	-	-	+	+	+	Rods	-	<i>Lysinibacillus sp.</i>

Table 2. Bacterial isolates from the effluent samples and control.

Sample	Species found (micro organism)
Ef ₁	<i>Micrococcus varians</i> , <i>Bacillus megaterium</i>
Ef ₂	<i>Lysinibacillus</i> , <i>Bacillus megaterium</i> , <i>Bacillus carboniphilus</i>
Ef ₃	<i>Staphylococcus simulans</i> , <i>Bacillus niacinni</i> , <i>Lysinibacillus</i>
Control	<i>Aneurinibacillus aneurinilytus</i>

Ef₁ = Purple; Ef₂ = Brown; Ef₃ = Green.

Table 3. Percentage decolorization of dye (BYR) using isolates.

Isolate	Average absorbance	% Decolorization
<i>Micrococcus varians</i>	0.68±0.01	8.514
<i>Bacillus carbonificus</i>	0.58±0.01	21.081
<i>Bacillus niacinni</i>	0.57±0.03	23.378
<i>Lysinibacillus sp</i>	0.74±0.02	0.270
<i>Bacillus megaterium</i>	0.66±0.03	11.351
<i>Staphylococcus simulans</i>	0.08±0.02	88.986

$$\% \text{ Decolourization} = \frac{A_0 - A_t}{A_0} \times 100$$

Where A₀ is the initial absorbance of the dye mix at its λ_{max} (0.740 nm) and A_t is the final absorbance.

normal flora of water. *S. simulans* proved to be the most efficient degrader of the dye mixture (BYR), its mechanism and best condition of degradation could be researched into and its use as a degrader could be exploited. The textile industry in Itoku though informal has great potential to be exploited by government and should be institutionalized. With government intervention, monitoring the industry's activities and processes could be embarked on and policy which ensures proper treatment of their effluents before discarding into neighbouring environments could then be enacted and executed.

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