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Decolourisation of Reactive Dyes Using Bacterial Isolates Recovered from Textile Effluent in Lagos State

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Abstract-A serious problem have been posed to the ecosystem due to untreated or partially treated waste water and industrial effluent discharges into our natural ecosystem and the life forms. Effluent samples were collected from three industrial textile industries in Lagos State, Nigeria. The total heterotrophic count of bacteria was determined using pour plate method on Plate Count Agar (PCA). Pure isolates were subjected to the solid phase screening medium. Pure isolates from the solid phase were subjected to liquid screening phase and potential isolates from the liquid screening phase were used for the decolourisation experiments. For the decolourisation experiments, potential isolates were inoculated into Mineral Salt Medium (MSM) supplemented with 0.1mg/L of reactive black, reactive red and reactive yellow dye. Decolourisation activity was determined by spectrophotometer. The result of the Total Heterotrophic Bacterial Count (THBC) of textile effluents showed that NHT at Ikorodu had the highest bacterial count of 2.85×10^8 CFU/mL while SWL at Ikeja had the lowest count of 1.13×10^8 CFU/mL. Fourteen isolates were able to grow on Luria- Bertani Agar medium (LB) supplemented with 0.1g/L in the solid phase method. Four isolates were chosen as potential isolates from the liquid phase and were used for decolourisation experiments. In the decolourisation experiments, *klebsiella oxytoca* after 5 days also had the highest decolourisation potential of 70% for reactive black while *Bacillus firmus* had the least decolourisation activity of 25%. Decolourisation of reactive red Her dye after 5days showed that *Staphylococcus aureus* had the highest decolourisation of 45% while *Bacillus firmus* had the lowest decolourisation potential of 14%. *Bacillus macerans* after 5 days showed the highest reactive yellow He4g dye decolourisation of 21% while *Klebsiella oxytoca* had the least decolourisation activity of 13%. The present showed the potential of different isolates to decolorize dye at different levels which would help in solving the problem of pollution

Keywords- *Dye, Decolourisation, Spectrophotometer*

I. INTRODUCTION

Control of pollution is one of the problem concerns of society today. With economic constraints on pollution control processes, affordable and effective methods have become a necessity [1]. Safia *et al*. [2] reported that there are more than 7×10^5 metric tons of dyes that are commercially available and are intensively use in textile processing, paper printing, pharmaceuticals, food and other industries.

Dyes, particularly reactive dyes are stable and difficult to biodegrade due to their synthetic origin and complex aromatic molecular structures [3]. Reactive dyes differ from all other dye classes due to their ability to bind to textile fibres, such as cellulose and cotton through covalent bonds [4]. Reactive dyes are typically azo-based chromophores combined with various types of reactive groups that show different reactivity [5].

The waste water generated by different textile industries are with different characteristics and vary according to the process employed [6].

Various waste liquor coming out of the operations in wet processing such as desizing, scouring, bleaching, mercerizing, dyeing, printing and finishing [7]. Concentration of dye contained in the effluent varies depending on the dyeing process but it is generally in the range of 10-200 mg/L. Many dyes and pigments are hazardous and toxic at the concentration discharged to receiving water for human as well as aquatic life [8].The water pollution caused by the textile mill effluent is hazardous for aquatic ecosystem. Many water borne diseases and increase BOD of the receiving water because of their complex structure and largest molecular size. Dyes present in the water on contact can causes ulceration of skin, and mucous membrane, dermatitis, perforation of nasal septum and severer irritation of respiratory track, vomiting, pain, hemorrhage and sharp diarrhea can all be caused by high concentration of dyes [9]. The solution to the environmental problems caused by the textile dye effluent is being sought by physical, chemical and biological treatment processes. The physico-chemical methods include adsorption, chemical precipitation, flocculation, electro floatation, oxidation via chlorine, peroxide, electrolysis and ozone treatment, reduction, electrochemical destruction and ion-pair extraction [10].

However, these physical and chemical methods are not always effective, costly, produces large amount of secondary metabolites, timely, requires high technical skills, labourious hence arise the need for biological methods which are very effective and not costly.

Biological methods of removal involve the use of microorganism 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, have low cost and are easy to use [11]. Various bacteria and fungi are effective in decolourisation and in many cases, adsorption of dyes to the microbial cell surface is the primary mechanism for decolourisation [12]. This study however, investigates the potential of bacteria isolated from textile effluent to decolourise dye.

II. MATERIALS AND METHODS

Effluents sample were collected from three industrial textile industries in Lagos State, Nigeria. Physical and chemical parameters of samples effluent (pH, temperature, biochemical oxygen demand (BOD), chemical oxygen demand (COD) and electrical conductivity) were analyzed using [13] .The total heterotrophic count of bacteria were determined using pour plate method on Plate Count Agar (PCA). Effluent samples were serially diluted up to 10^{-6} and 0.1ml from the aliquot was plated on Plate Count Agar (PCA) and was incubated for 24 hours. Pure bacterial isolates capable of growth on PCA were streaked on Luria-Bertani Agar medium (LBA) supplemented with 0.1g/L of dye for the solid phase screening method. The plates was incubated for 72hours and bacterial isolates capable of showing clear zone around colonies were picked as potential isolates[13]. Isolates that possessed zone of clearance around colonies were subjected to the second screening medium on the liquid phase using 100ml of Mineral Salt Medium (MSM) supplemented with 0.1mg/L of reactive black (WNN). The medium was incubated in the rotary shaker (Gallenkamp) for 4 days. Isolates were selected on the basis of their ability to grow and reduce colour under these conditions using UV-Vis Spectrophotometer. Isolates with higher decolourisation potential were chosen for decolourisation experiment.

Maximum absorption wavelength of each dye was determined within the visible region of 340-700nm by observing the dye solution at different wavelength within the visible region and the wavelength where the dye showed maximum absorption which was taken as absorption maximum of the dye according to [14].

Decolourisation experiment was carried out as described by [15].

Experiments were carried out in 50 ml of MSM containing 0.1g/L of reactive black, red and yellow dye. The pH was adjusted to 7. Flasks were autoclaved at 121°C for 15mins and were inoculated with 5mls of inoculum of each microorganism. They were incubated for 5 days and after the end of this period. Decolorisation activity was determined by monitoring the decrease in absorbance on a spectrophotometer at the absorbance maximum of each dye. Decolourising activity was analyzed and was expressed according to [16] in terms of percentage decolourisation. Decolourisation activity (%) was calculated by the formula:

% Decolourisation = Initial Absorbance - Final Absorbance / Initial Absorbance × 100.

III. RESULTS

Table 1 shows the physical and chemical properties of the effluents. Effluents colour were deep colours which included brown and black. Odour of the effluents varied from pungent, foul and rotten egg smell. The pH ranged between 8.4 to 9. NHT had the highest pH value of 9 while SWL had the lowest pH of 8.4. The highest temperature at collection was 45ºC from SWL and NHT and lowest temperature of 40ºC was from SFL. Electrical conductivity (E.C) ranged between 189.45 µS/cm to 289.18µS/cm. NHT had the highest E.C. of 289.18 µS/cm while SWL had the lowest E.C. of 189.45µS/cm. The highest BOD of 53.34 was at NHT while the lowest BOD of 48.30 was at SWL. The highest and lowest value of COD was 78.43 and 69.98 at SWL and SFL respectively.

TABLE I. ANALYSIS OF CONSTITUENT OF THE EFFLUENT

S/No	Sample sites codes	(WHO permissibe limits)	pH (6.5 to 8.5)	Temp(ºc) (< 35ºC)	E.c(µs) (2500)	BOD (mg/L) (<500)	COD (mg/L) (<1000)	Colour (colourless)	Odour (No odour)
1	SWL		8.4	45	189.45	48.30	78.43	Brown	Pungent
2	SFL		8.7	40	198.43	52.86	69.98	Black	Rotten egg
3	NHT		9	45	289.18	53.34	76.33	Black	Foul

Key: SWL – Ikeja, Lagos, NHT – Ikorodu, Lagos, SFL – Ilupeju, Lagos, E. C – Electrical Conductivity, COD – Chemical Oxygen Demand

Table 2 showed the result of the Total Heterotrophic Bacterial Count (THBC) of textile effluents at Ikeja, Ilupeju and Ikorodu. NHT at Ikorodu had the highest bacterial count of 2.85×10^8 CFU/mL while SWL at Ikeja had the lowest count of 1.13×10^8 CFU/mL.

TABLE II. TOTAL HETEROTROPHIC BACTERIAL COUNT (THBC) IN EFFLUENT SAMPLES

Sample sites	THBC (x 10^6) cfu/mL
IND 1: Ikeja	1.13 ± 12
IND 2: Ilupeju	2.18 ± 9
IND 3: Ikorodu	2.85 ± 14

Fourteen isolates were able to grow on Luria- Bertani Agar medium (LB) supplemented with 0.1g/L of reactive dyes. Nine belonging to the following genera: *Klebsiella oxytoca, Providencia rettgeri, Pantoea spp, Proteus mirabilis, Citrobacter fameri, Enterobacter sakazakii, Enterobacter aerogene, Salmonella arizonae and Salmonella salamae*

Two belong to the genus *Staphylococcus* and three belong to the genus *Bacillus.*

Screening test for ability to decolourize reactive dyes on the liquid medium showed that Klebsiella *oxytoca* and *Bacillus macerans* had the highest decolourisation of 0.2850 and 0.2900 ^{after} 96hrs while *Bacillus subtilis* had the least decolourisation of 0.400 after 96 hrs. *Bacillus firmus, Staphylococcus aureus* and *Staphylococcus cohnii spp urealyticum* showed high decolourization values of 0.200, 0.3350 and 0.3505 after 96 hrs respectively (Table 3). Mean values with the same superscript are not significantly different using Duncan Multiple Range test at ($P < 0.05$).

TABLE III. SCREENING RESULTS OF LIQUID PHASE SHOWING THE ABSORBANCE OF REACTIVE BLACK WNN DYE

Isolates	24hrs	48hrs	72hrs	96hrs
Klebsiella oxytoca	0.3980 ^{d}	0.3890 ^{e}	0.3675 ^{c}	0.2850 ^{a}
Staphylococcus cohnii spp urealyticum	0.3575 ^{a}	0.3535 ^{a}	0.3515 ^{a}	0.3505 ^{c}
Bacillus firmus	0.4280 ^{h}	0.4210 ^{g}	0.3840 ^{e}	0.3200 ^{b}
Providencia rettgeri	0.4270 ^{h}	0.3940 ^{e}	0.3890 ^{f}	0.3595 ^{c}
Pantoea spp	0.3785 ^{b}	0.3700 ^{bc}	0.3685 ^{c}	0.3630 ^{c}
Proteus mirabilis	0.3765 ^{b}	0.3765 ^{cd}	0.3700 ^{c}	0.3665 ^{c}
Bacillus subtilis	0.4270 ^{h}	0.4115 ^{fg}	0.4200 ^{g}	0.4000 ^{d}
Citrobacter fameri	0.3905 ^{c}	0.3535 ^{ab}	0.3515 ^{a}	0.3530 ^{c}
Enterobacter sakazakii	0.4175 ^{g}	0.3945 ^{e}	0.3875 ^{f}	0.3550 ^{c}
Staphylococcus aureus	0.4055 ^{ef}	0.384 ^{de}	0.3785 ^{d}	0.3350 ^{b}
Enterobacter aerogene	0.4120 ^{fg}	0.4075 ^{f}	0.3825 ^{e}	0.3600 ^{c}
Salmonella arizonae	0.4055 ^{de}	0.3850 ^{de}	0.3710 ^{c}	0.3620 ^{c}
Salmonella salamae	0.4305 ^{h}	0.4090 ^{f}	0.3685 ^{c}	0.3640 ^{c}
Bacillus macerans	0.4150 ^{g}	0.3700 ^{bc}	0.3505 ^{a}	0.2900 ^{a}
Control	0.4855 ^{i}	0.4850 ^{h}	0.485 ^{h}	0.4850 ^{e}

IV. DECOLOURIZATION EXPERIMENT REACTIVE DYES

Klebsiella oxytoca after 5 days also had the highest decolourisation potential of 70% for reactive black Wnn while *Bacillus firmus* had the least decolourisation activity of 25% (Figure 1).

Figure 2 shows the decolourisation of reactive red Her dye after 5days. *Staphylococcus aureus* had the highest decolourisation of 45% while *Bacillus firmus* had the lowest decolourisation potential of 14%.

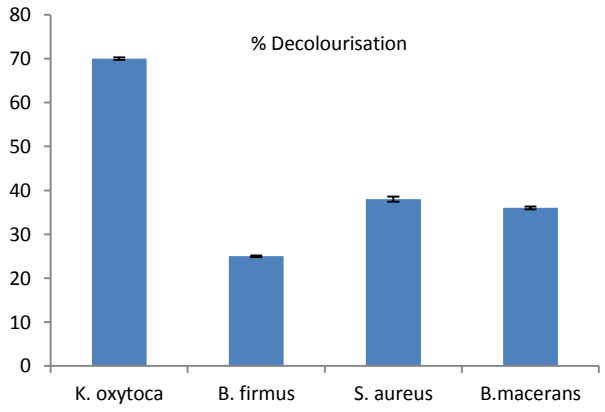

Figure 1. Spectrophotometric analysis of decolourization of reactive black at day 0 and after 5 days

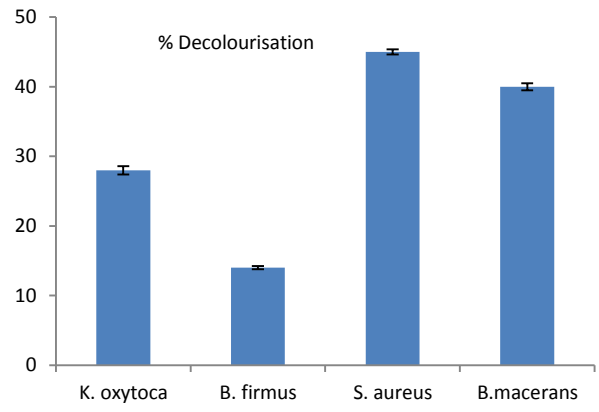

Figure 2. Spectrophotometric analysis of aerobic decolourization of reactive red at day 0 and after 5 days

Bacillus macerans after 5 days showed the highest reactive yellow He4g dye decolourisation of 21% while *Klebsiella oxytoca* had the least decolourisation activity of 13% (Fig. 3).

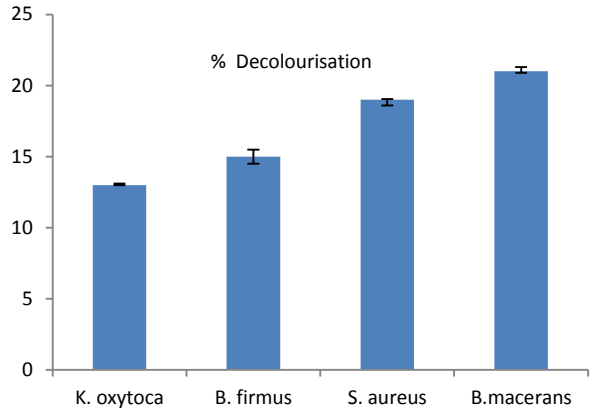

Figure 3. Spectrophotometric analysis of aerobic decolourization of reactive yellow after 5 days

V. DISCUSSION

The physical and chemical parameters of the textile effluent sample collected from local and industrial textile mills revealed a high load of pollution indicators. Odour of the effluent sample disagrees with WHO rules that states that it must be odourless. All effluent from the different sites had colour. Colour of the effluents may be due to high quantity of dissolved solids present in the effluents. Colour reduces light penetration into water which in turn affects aquatic life and algae leading to reduction in rate of photosynthesis. The pH affects the physical and chemical attributes of water which in turn adversely affect aquatic life, plant, animals, population and activities of microorganisms. The alkaline pH values of the effluents can be due to the use of carbonate, bicarbonate, hydrogen peroxide and sodium hydroxide during bleaching process in the textile mills [17]. Electrical Conductivity (E.C) values were lower compared to [18] where a range of 14815.0 µs/cm to 16200 µs/cm was reported. E. C values of sample exceed the permissible levels of WHO standard. Higher E.C. indicates presence of higher concentration of ions in effluent samples.

The genera of bacteria isolated and screened for dye decolourizing bacteria has been reported by several authors to be associated with dye effluent [19, 20]. Olukanni *et al*. [21] isolated bacteria from textile dye effluent belonging to the genera, *Bacillus* sp *and Staphylococcus* sp. The result obtained from the sample analyzed showed that the dye waste effluent obtained from the different industries had higher value of total heterotrophic bacterial count (THBC) with the lowest count from ikeja (IND 1) which could be due to the pre-treatment given to the effluent sample from IND 1 before discharging the effluent to the environment. Bacterial biodegradation of dyes is often initiated by cleavage of azo bonds by azoreductases which are followed by the aerobic degradation of the resulting amines [22]. This study provides the evidence that metabolizing cells of *Klebsiella oxytoca*, *Bacillus firmus*, *Staphylococcus aureus*, *Bacillus macerans* were capable of removing colour and in the process breaking down of bonds of dyes with the highest decolourization percentage (70%) of reactive black by *Klebsiella oxytoca.*

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