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Biodegradation potential of bacterial isolates from dye wastewater at Marina, Sokoto metropolis

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Abstract

This study was carried out to determine the biodegradation potential of bacterial isolates from dye wastewater at Marina Area, Sokoto. Three (3) of the bacteria were identified; *Bacillus subtilis*, *Luteimonasaestuarrii* and *Bacillus cereus* and were used for further studies based on their degradation ability of green, red, yellow and blue dyes. *Bacillus subtilis*, *Luteimonas aestuarii* and *Bacillus cereus* have the potential to degrade all the four dyes used during the present investigation. However, efficacy of the various bacterial strains was found to vary at different concentration levels in degradation of a particular dye. Thus, by this study it can be concluded that *Bacillus cereus*, *Bacillus subtilis* and *Luteimonas aestuarii* can be used as a good microbial source for dye waste water treatment. The selected bacterial species represent a promising tool for application in biodegradation of dye waste water and the potential observed would allow for the application of the bacterial isolates for treatment of dye effluents before disposal.

Keywords: effluents, isolates, biodegradation, dye and bacterial.

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Introduction

Effluents from textile industries contain different types of dyes, which because of high molecular weight and complex chemical structures, show low level of biodegradability (Olayinka and Alo, 2004). Hence, direct deposition of these effluents into sewage networks, produce disturbances in biological treatment processes (Babu *et al*., 2000). On the other hand, these types of effluents produce high inorganic salts, acids and bases in biological reactors leading to the increase of treatment costs. Dyes are mostly stable in light and heat. The classic and conventional treatment methods for these types of effluents are based on chemical precipitation, activated sludge, chlorination and adsorption on activated carbon.

Several methods for dye removal from the wastewater are available. These techniques are physical, chemical and biological (Cooper, 1993; Vandevivere *et al*., 1998; Robinson *et al*., 2001; Blumel *et al*., 2002).The physicochemical techniques include Membrane filtration, coagulation/flocculation, precipitation, flotation, adsorption, ion exchange, ion pair extraction, electrolysis, Fenton oxidation, advanced

oxidation (ozonization) and chemical reduction. The biological remediation techniques include bacterial and fungal biosorption and biodegradation in aerobic/anaerobic treatment process

Extensive research on dye degradation using microbes has shown that biological approaches alone and or in combination with physical-chemical treatments can offer low cost alternative treatment (Willmott *et al*., 1998; Pearce *et al*., 2003). Microbial strategy is cost effective as well as environmentally friendly technique (Dubrow *et al*., 1996; Robinson *et al*., 2001). Khalid *et al*., (2009) reported that many microorganisms are capable of decolorizing the azo dyes including Gram positive as well as Gram negative bacteria, fungi and yeast.

Biological degradation processes detoxifies the original dye stuff and enables its conversion into normal nutrient in nature. Additionally, microorganisms utilized in such treatment technologies can be grown using cheap and abundant carbon and nitrogen wastes derived from agro industry (Khehra *et al*., 2005). Therefore, this study is aimed at investigating the biodegradation potential of bacteria isolated from dye wastewater. To determine the biodegradation potential of the bacterial isolates from dye wastewater on different dyes. To carry out polymerase chain and sequencing of the 16srRNA for bacterial isolates that had biodegradation potential of the dyes.

Materials and Methods

Sample Collection

Ten litres (10L) of dye wastewater sample was collected from drains at different locations of dyeing sites, Marina area of Sokoto North Local Government. The sampling sites were selected on the basis of discharge of the dye wastewater. The sampling sites were at the point of discharge and where the flow stops which are 20 meters apart. The wastewater samples were collected in sterile plastic containers and transported to laboratory at room temperature for bacteriological analysis.

Isolation and Identification of Bacteria from Dye Waste Water Sample

Serial dilution of the dye wastewater sample was made and 0.2 mL each of the respective dilutions was plated onto Nutrient Agar using the spread plate technique. The plates were incubated at 37ºC for 24 hours before enumeration of colonies. The isolates were purified and sub cultured on Nutrient agar, stored on slants and was maintained at 4ºC for further usage. Identification of the isolates was achieved by subjecting the isolates to catalase test, indole test, urease test, methyl-red VogesPrauskauer test (MRVP), triple sugar iron agar test (TSI), citrate test**,**starch hydrolysis test, oxidase, gram staining reaction and motility test.

Screening of Bacterial Isolates Based on Degradation Activity

Each isolate was enriched with 10 mg (0.01g) of the four reactive dyes (Blue, Red, Green and Yellow) into 50mL of nutrient broth in 100mL conical flask. It was incubated at 37ºC for 48hours. Then, 1mL of each culture broth was appropriately diluted and plated out by streaking on nutrient agar containing 10 mg (0.01g) of the four reactive dyes labeled B, R, G and Y respectively. The plates were incubated at 37°C for 24 hrs. The respective dye incorporated agars without any inoculum was used as control. Decolorization activity was judged by the presence of clear zones surrounding the colonies and comparing the inoculated plates with the control plates. After incubation, morphologically distinct bacterial isolates showing clear zones was selected for further screening (Shah, 2014).

Genomic DNA Isolation

The genomic DNA was obtained from 500µL of overnight culture of the isolated bacteria (B, C and D isolates) and pipetted into 1.5 mL micro centrifuge tube. It was then centrifuged at 13,000 rpm for 1 minute after which the supernatant from the tube was discarded into the biohazard waste container. Sterile deionized water 250µL was added to the tube and shaken to resuspend the cell in the water. The tube was then placed in dry ice bath for 3 minutes. This was repeated twice before centrifuging at 13,000 rpm for 1 minute. The quality of DNA was checked by running on 0.8% of agarose gel stained with ethidium bromide. A single intense band with slight smearing was documented.

PCR Amplification of 16SrRNA

For polymerase Chain Reaction, 49µL of mastermix (containing forward and reverse primers, dNTPs, Taq polymerase, $MgCL_2$ and PCR reaction buffer) in ice bucket was pipetted into 0.2 ml micro centrifuge tube. One microliter (1µL) of the DNA template was pipetted into 0.2 ml micro centrifuge containing the PCR mix. 16SrRNA gene of the isolates was amplified using 789bp forward primer (1F-GGACTACAGGGTATCTAAT) and 789bp of the reverse primer (2R- AGAGTTTGATCCTGG). Amplification was done by initial denaturation at 94ºC for 5 minutes at 1st cycle, followed by 40 cycles at 94ºC for 1 minute, 52ºC for 1 minute (annealing), followed by 25 cycles at 72ºC for 1 minute and 72ºC for 5 minutes (elongation).

PCR Product Sequencing

The purification of the PCR product was done using the QIA quick gel purification kit according to manufacturer's instructions (QIAGEN, Germany). Sequencing was done by Sanger method using 96 capillary high through put sequencer, ABI 31730XL (Xcelris, India). The 16srRNA sequence was uploaded on the NCBI database and BLAST was used for sequence comparison (www.ncbi.nlm.nih.gov/BLAST).

Biodegradation of Dyes using Bacterial Species

Dye decolorization experiment was carried out in 100mL flasks containing minimal salt medium with the following composition in g/50 mL (NaCl 0.05, $CaCl_2$ 0.005, $MgSO_4.7H_2O$ 0.025, KH_2PO_4 0.05, Na_2HPO_4 0.05, yeast extract 0.05, peptone 0.25) and four different concentrations of each of the four reactive dyes effluents which were 25mg (0.025g), 50 mg (0.05g), 75 mg (0.075g) and 100 mg (0.1g). Each of the four different concentrations was replicated thrice having a control for each concentration. It was then autoclaved at 121ºC for 15 minutes. Aliquot (0.5 mL) of the culture broth was pipetted into each concentration representing the selected isolates except for controls. Initial absorbance was measured at wavelength maxima (ʎm) of 600nm for each of the dye concentration before sterilization. The selected isolates were incubated at 37ºC with shaking at 150rpm for 7 days. After incubation, 10 mL of the broth was centrifuged at 500rpm for 15minutes and the supernatant collected. Biodegradation/ Decolorization was assessed by measuring the final absorbance with the help of spectrophotometer at wavelength maxima (λm) of 600nm for each of the respective dye concentration (Shah, 2014). The decolorization activity was measured in terms of percentage degradation/decolorization and calculated using the following equation:

$$\% \text{ Degradation/Decolorization} = \frac{\text{Initial absorbance} - \text{Final absorbance}}{\text{Initial absorbance}} \times 100$$

Results and Discussion

Nine bacterial isolates were identified using morphology and biochemical characterization which indicated that they belong to the genera *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Luteimonas aestuarii* , *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris*, *Klebsiella pneumonia* and *Proteus mirabilis* as shown in Table 1. Contaminated dye waste water site has been identified to be the primary sources of these isolates with the ability to degrade or remove pollutants in the dye waste water. This resultagree with the findings of Khehra *et al*. (2005) and Suraj *et al.,* (2010) that identified *Bacillus subtilis*, *Pseudomonas aeruginosa, Staphylococcus aureus* and *Eschericia coli* in sludge samples collected from waste disposal sites.

However, observation made on bacterial degradation potential revealed that out of the nine bacterial isolates selected and screened, only three showed high clear zones at 10 mg/L in Table 2. The experimental result for the screening test indicated that the three bacterial isolates tested positive for all the four reactive dyes. *Bacillus subtilis* was highly positive to Blue, Green and Yellow reactive dyes showing clear zones. *Luteomonasaestuarii* was also positive to Blue, Red, Green and Yellow reactive dyes. *Bacillus cereus* also showed high positive reaction to Red and Yellow reactive dyes. These results are similar with the findings of Sivaraj *et al*. (2011) and Tripathi and Srivastara (2011) where *Bacillus subtilis* and *Bacillus cereus* emerged as a potent decolorizer strain of syntheticdyes. These findings also coincided with the result of Gurar *et al*. (2011) where *Bacillus spp*. has been proven as a better decolorizer.

Molecular Identification of the Three Bacterial Isolates

The PCR product of the screened isolates amplified using 16s rRNA primer gene 640bp, 609bp and 411bp fragments of the rRNA component of the small (30s) subunit when electrophoresed with 100bp ladder marker. The sequence analysis using 16s rRNA primer revealed that the dye effluent isolate ' B' has 94% homology with *Bacillus subtilis* strain $CMHC_2$, isolate 'D' has 100% homology with *Bacillus cereus* strain HR120 and isolate 'C' has 65% homology with *Luteimonas aestuarii* strain PR140. This could be attributed to the textile dye effluents as a rich medium capable of supporting the growth of potential dye degraders. This result is similar to that obtained by Stolz (2001) who characterized *Bacillus cereus* and *Bacillus subtilis* using 16s rRNA primers.

Biodegradation/Decolorization of Textile Dye Effluents

Table 3-6 showed all the three bacterial isolates; *Bacillus subtilis*, *Luteimonas aestuarii* and *Bacillus cereus* have potential to degrade all the four dyes used during the present investigation. However, efficacy of the various bacterial strains was found to vary at different concentrations in degradation of particular dye.

Table 1: Morphological and Biochemical Characterization of Identified Bacterial Isolates

Isolate Code	GR	Cat	Ind	Cit	Ur	Ox	MR	VP	Glu	Sta	Lac	Suc	H_2S	Mot	Agar Slant Characteristics	Identified Organisms
A	-	+	-	+	+	+	-	-	-	-	-	-	-	+	Mucoid Colonies medium turn green	*Pseudomonas aeruginosa*
B	+	+	-	+	-	+	-	+	+	+	-	+	-	+	Dry flat irregular lobate margin	*Bacillus subtilis*
C	-	+	-	-	-	+	-	-	+	+	-	-	+	-	Smooth, circular yellow colonies with entire edges	*Luteimonas aestuarii*
D	+	+	-	+	+	-	-	+	+	+	-	+	+	+	White irregular large colonies	*Bacillus cereus*
E	+	+	-	+	+	-	+	+	+	-	+	+	-	-	Abundant opaque golden growth	*Staphylococcus aureus*
F	-	-	+	-	-	-	+	-	+	-	+	+	-	+	Abundant thick, white glistering	*Escherichia coli*
G	-	+	+	-	+	-	+	-	+	+	-	+	+	+	Thin blue-grey growth	*Proteus vulgaris*
H	-	+	-	+	+	-	-	+	+	-	+	+	-	-	Shiny, transparent raised growth	*Klebsielliapneumoniae*
I	-	+	-	+	+	-	+	-	+	-	-	+	+	+	Large, circular, grey, smooth colonies	*Proteus mirabilis*

KEY: GR = Gram Reaction, Cat = Catalase, Ind = Indole, Cit = Citrate, Ur = Urease, Ox = Oxidase, MR = Methyl Red, VP = VogesProuskeur, Glu = Glucose, Sta = Starch Hydrolysis, Lac=Lactose, H_2S = Hydrogen Sulphide,
Mot = Motility

Table 2: Screening of Bacteria for Degradation/Decolorization Potential Showing Clear Zones

Isolate	**Reactive Dyes**			
	Blue 10 mg/L	**Red 10 mg/L**	**Green 10 mg/L**	**Yellow 10 mg/L**
Pseudomonas aeruginosa	-	-	-	-
Bacillus subtilis	++	+	++	++
Luteimonas aestuarii	+	+	+	+
Bacillus cereus	+	++	+	++
Staphylococcus aureus	-	-	-	-
Escherichia coli	-	-	-	-
Proteus vulgaris	-	-	-	-
Klebsiella pneumonia	-	-	-	-
Proteus mirabilis	-	-	-	-

K**EY:** + = Positive, ++ = Highly Positive, - = Negative

Degradation of Green Dye

It is clear from Table 3 that *Bacillus subtilis*had higher decolorization potential of textile dye effluents(37.36%) at 25 ppm concentration than *Luteimonas aestuarii* and *Bacillus cereus* while *Luteimonas aestuarii* resulted in higher decolorization potential of textile dye effluents of Green dye at 50ppm (47.22%) and 100 ppm (3.27%) than *Bacillus subtilis* and *Bacillus cereus*. But *Bacillus cereus* at 75 ppm produced higher decolorization potential of textile dye effluents of Green dye (46.52%) than *Bacillus subtilis* and *Luteimonas aestuarii*. Similar results were reported by Chang *et al*. (2004) and Kumar *et al*. (2005) that these bacteria are effective in degrading dyes.

Table 3: Biodegradation/Decolorization (%) of Green Dye at Different Concentrations

Organism	**25 ppm**	**50 ppm**	**75 ppm**	**100 ppm**
Bacillus subtilis	**37.36**	15.96	2.98	1.09
Luteimonas aestuarii	35.61	**47.22**	4.41	**3.27**
Bacillus cereus	36.64	12.30	**46.52**	2.00

Degradation of Red Dye

It was observed from Table 4 that *Bacillus subtilis*had higher decolorization potential of red textile dye effluentsat 25 ppm (45.36%) and 50 ppm (40.50%) concentrations than *Luteimonas aestuarii* and *Bacillus cereus* while *Luteimonas aestuarii* resulted in higher decolorization potential of textile dye effluents of red dye at 100 ppm (3.13%) than *Bacillus subtilis* and *Bacillus cereus*. But *Bacillus cereus* at 75 ppm produced higher decolorization potential of red textile dye effluents (13.90%) than *Bacillus subtilis* and *Luteimonas aestuarii*. Similar results were reported by Chang *et al*. (2004) and Kumar *et al*. (2005) that these bacteria are effective in degrading dyes.

Table 4: Biodegradation/Decolorization (%) of Red Dye at Different Concentrations

Organism	**25 ppm**	**50 ppm**	**75 ppm**	**100 ppm**
Bacillus subtilis	**45.36**	**40.05**	6.40	2.02
Luteimonas aestuarii	39.32	17.91	9.15	**3.13**
Bacillus cereus	43.63	28.23	**13.90**	2.36

Degradation of Yellow Dye

In Table 5, *Luteimonas aestuarii* was observed to have the highest degrading potential as compared to other organisms isolated with 52.09% degradation potential of yellow dye at 25 ppm, 49.23% at 50ppm, 7.90% at 75ppm and 3.6% at 100ppm. The result showed that *Luteimonas aestuarii* was more effective in degradation of yellow dye as compared to *Bacillus cereus* and *Bacillus subtilis*. This result agrees with the findings of Stolz (2001) who

reported that *Luteimonas aestuarii* was more effective in degrading yellow dye than *Bacillus subtilis.*

Table 5: Biodegradation/Decolorization (%) of Yellow Dye at Different Concentrations

Organism	25 ppm	50 ppm	75 ppm	100 ppm
Bacillus subtilis	10.61	40.00	7.84	2.89
Luteimonas aestuarii	**52.09**	**49.23**	**7.90**	**3.56**
Bacillus cereus	51.55	31.69	7.33	1.58

Degradation of Blue Dye

*Bacillus subtilis*was observed in Table 6 to have the highest degradation potential of blue dye at 75ppm (13.85%) and at 100ppm (8.25%) when compared to *Luteimonas aestuarii* and *Bacillus cereus*. *Luteimonas aestuarii* had the highest degradation potential of blue dye effluent at 25ppm (53.81%) and 50ppm (43.02%) when compared to *Bacillus subtilis* and *Bacillus cereus*. This result agrees with the findings of Saraswathi and Balakumar (2009) who reported higher degradation potential of blue dye effluent with *Bacillus laterosporous* and *Luteimonas aestuarii* .

Table 6: Biodegradation/Decolorization (%) of Blue Dye at Different Concentrations

Organism	25 ppm	50 ppm	75 ppm	100 ppm
Bacillus subtilis	48.27	35.89	**13.85**	**8.25**
Luteimonas aestuarii	**53.81**	**43.02**	0.46	1.55
Bacillus cereus	44.48	17.55	3.94	2.81

Conclusion

Biodegradation of wastewater is animportant process to both textile industries and wastewater treatment analysts. The result of this study suggests a great potential for bacteria to be used to remove pollutants from textile effluents. *Bacillus subtilis*, *Luteimonas aestuarii* and *Bacillus cereus* have the potential to degrade all the four dyes used during the present investigation. However, efficacy of the various bacterial strains was found to vary at different concentrations in degradation of a particular dye. Thus, by this study it can be concluded that *Bacillus cereus*, *Bacillus subtilis* and *Luteimonas aestuarii* can be used as a candidate for dye waste water treatment.

Recommendations

From the findings, it is recommended that the tested bacteria used in the study should be further used in large scale as an alternative treatment system for dye effluent before discharging to appropriate channels. Also, application of the study to more dyes and identification of end products of the dyes using mass spectrometry is required to confirm the fate of aromatic amines.

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