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Microbial Decolourization of Cibacron Scarlet and Remazol Blue Dyes

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Abstract

Eleven microorganisms that include six bacteria: Proteus mirabilis (LS), Proteus vulgaris (LE), Bacillus alvei (LS), Proteus mirabilis (AS), Bacillus polymyxa (AS), Bacillus alvei (AE), and five fungi: Penicillium antrovenetium (LS), Rhizopus oryzae (LEM), Penicillium funiculosum (LE), Rhizopus oryzae (LS) and Candida valida (LEM) were isolated from the effluent and soil samples collected from the premises of a textile manufacturing and local dyeing industries. The BOD and COD of the effluents collected from these industries were found to be relatively higher than the Nigeria Federal Environmental Protection Agency permissible level.

Increase in decolourization of Cibacron Scarlet was observed for all the isolates on day 5, seven of which showed decolourization greater than 50%. The highest decolourization of 85.7% was observed with P. mirabilis (AS) and R. oryzae (LS) had the lowest decolourization of 50.7%. B. alvei (LS) showed 74.8% decolourization of Remazol Blue on day 3, but decrease in the decolourization was observed for this organism on day 5. All the isolates showed decrease in the decolourization of Remazol blue at day 5 with the exception of three isolates; P. mirabilis (LS), B. polymyxa (AS) and R. oryzae (LS) with 46.3% and 64.1%, 54.1% and 67.8%, and 54.1% and 58.8%, respectively, on day 3 and day 5. Decolourization pH for Cibacron Scarlet by the bacterial isolates was slightly alkaline (8.5 – 8.8) and acidic (3.4 – 4.4) for the fungal isolates. Increase in bacterial count and fungal mycelia weight was observed as the decolourization progress. Thus, results obtained from this study revealed the decolourization potential of indigenous effluent and soil adapted microorganisms in decolourization of textile dyes.

Keywords: *Effluent, Cibacron Scarlet, Remazol Blue, FEPA limits, decolourization.*

Introduction

Water pollution control is presently one of the major areas of scientific activity. While coloured organic compounds generally impart only a minor fraction of the organic load to wastewater, their colour renders them aesthetically unacceptable.

Coloured effluent discharged from dye consuming industries and and dye manufacturing industries to neighbouring water bodies and dye manufacturing industries to neighbouring water bodies and wastewater treatment systems is currently causing significant health problem and this has become a matter of concerns to environmental regulatory agencies (Banat *et al*. 1996).

In most developed countries, government legislation is becoming more stringent with regard to the removal of dyes from effluents which is becoming an increasing problem for textile industries. The new environmental regulations concerning textile products have

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banned the discharge of coloured waste into natural water bodies (Máximo *et al*. 2003). Therefore an effective and economic treatment of effluents from textile industries has become a necessity for clean production technology in textile industries.

During the past two decades, treatment systems based on physical and chemical methods (physicochemical decolourization techniques) for removal of dyes from effluents have been widely used (Robinson *et al*. 2001). However, few textile industries have accepted these methods due to high cost, low efficiency and inapplicability to a wide variety of dyes. Also, these procedures have inherent drawback as they generate a significant amount of sludge and cause secondary pollution due to formation of hazardous byproducts (Zhang *et al*. 2004).

The new guidelines for effluent and sludge disposal have generated interest in the use of biological treatment of textile processing industries effluent, as they are known to achieve complete mineralization with detoxification without production of any toxic sludge (Kalyani *et al*. 2009). The ability of microorganisms to carry out decolourization and degradation (Chang and Kuo 2000; Ogunjobi *et al*. 2012) is seen as cost effective method for removing these pollutants from the environment. This study therefore investigates the potential of bacteria and fungi isolated from textile effluent in decolourizing Remazol Blue and Cibacron Scarlet dyes.

Materials and Methods

Collection of Samples:

Textile effluents and soil samples were collected from effluent discharging site of a textile manufacturing company in Lagos, Lagos State, Nigeria, and from the vicinity of a local textile dyeing centre in Abeokuta, Ogun State, Nigeria, by random sampling into sterile sample bottles. The textile dyes used were obtained from United Nigeria Textile Industry, Kaduna, Kaduna State, Nigeria.

Determination of Physico-chemical parameters of the effluents:

The pH and the temperature of the effluents were determined using digital pH meter and Thermometer (*in situ*). The Biological Oxygen Demand (BOD) was determined using BOD Trak instrument and the Chemical Oxygen Demand (COD) was determined using the standard titration method of potassium dichromate with ferrous ammonium sulphate solution, at the Federal Institute of Industrial Research Oshodi (FIIRO), Lagos, Lagos State, Nigeria.

Isolation of microorganisms:

Isolation of microorganisms from the textile effluents and soil samples was carried out using standard dilution method and pour plate techniques on Nutrient agar and Potato Dextrose Agar for bacterial and fungal isolation, respectively. The morphologically distinct colonies were subculture to obtain pure isolates and were stored on an agar slant.

Screening for dye decolourizing isolates and Characterization:

The pure microbial isolates were screened for their dye decolourizing ability by culturing them on dye-agar medium with the composition: $MgSO_4H_2O$ 0.1%, KH_2PO_4 0.1%, $CaCl_2$ 0.1%, $FeSO_4$ 0.05%, NH_4NO_3 0.1%, Glucose 0.05% and Agar 2% according to Marchant *et al*. (1994).

The cultures capable of growth on this medium were further purified; these were latter characterized and identified using their morphological, physiological and biochemical properties.

Preparation of Dye Stock Solution:

The dye stock solutions were prepared by dissolving 5 mg of each dye in 0.09% (w/v) Sodium Hydroxide. The preparations were filter sterilized using membrane filtration method. The dyes maximum absorbance was determined spectrophotometrically from the prepared stock solution using Scanning Ultra Violet Spectrophotometer (GENESYS 10 UV).

Decolourization Experiment:

The decolourization experiment was set up in 50 ml conical flasks corked with cotton wool, containing 30 ml of the dye decolourizing medium and 13 ml of the dye stock solution, inoculated with 7 ml of the 24

hours old broth culture of the isolated microorganisms, to make up a total volume of 50 ml. Uninoculated flasks were also set up for each of the dye to serve as control.

The flasks were incubated aerobically at room temperature for five days; samples were withdrawn on the third and fifth day to determine the degree of decolourization. The residual dye content (the degree of decolourization analysis) was determined by taking the absorbance reading of the withdrawn samples supernatant after centrifugation at 4,000 rpm for 20 minutes to remove microbial cells. The bacterial count and fungal mycelia weight were also determined alongside.

Analytical Method:

The degree of dye decolourization was measured spectrophometrically from the residual dye content and calculated from the absorbance reading obtained against the uninoculated control, using the formula:

% *Decolourization* =
(*Absorbance of uninoculated dye broth* - *Absorbance of residual dye broth*) /
(*Absorbance of uninoculated dye broth*) × (100/1).

Determination of the Fungal Mycelia Weight:

This was carried out by taken out 5 ml of the decolourization experiment samples and their subsequent filtration through a constant weight filter paper. The filtrate was later dry in oven at 40^{o}C until a constant weight is obtained.

Results

The maximum absorbance of the dyes used for this study (Table 1) showed that Remazol Blue has the maximum absorbance of 595 nm while Cibacron Scarlet has 511 nm.

Table 2 revealed the physicochemical properties of the textile dye effluents with the Abeokuta local effluent having higher BOD and COD of 260 mg/L and 395,200 mg/L, respectively, compared to 108 mg/L and 544.32 mg/L for Lagos Industrial effluent which has pH of 12.1 and temperature of 41^{o}C.

A total of eleven isolates (Table 3), six bacteria and five fungi were obtained from the screening on dye decolourizing medium. The six bacterial isolates include *Proteus vulgaris*, *Bacillus polymyxa* and two each of *Proteus mirabilis* and *Bacillus alvei*. Out of the five fungi, two are *Rhizopus oryzae* while the rest are *Penicillium antrovenetium, Penicillium funiculosum* and *Candida valida.*

Table 1. The maximum absorbance of the dyes taking spectrophotometrically.

Dye	Type	Maximum Absorbance (λ_{max}) (nm)
Remazol Blue	Diazo	595
Cibacron Scarlet	Reactive	511

Table 2. Physico-chemical properties of the textile dye effluents.

Para-meter	BOD (mg/L)	COD (mg/L)	pH	Temperature (^{o}C)
LAG	108.0	544.32	12.1	41
ABK	260	395,200	ND	ND

Note: LAG - Effluent from textile manufacturing industry in Lagos; and
ABK - Effluent from a local textile dyeing center in Abeokuta.

Table 3. Microbial isolates obtained for dye decolourization study after screening.

Isolates	Source
Proteus mirabilis	LS
Proteus vulgaris	LE
Bacillus alvei	LS
Proteus mirabilis	AS
Bacillus polymyxa	AS
Bacillus alvei	AE
Penicillium antrovenetium	LS
Rhizopus oryzae	LEM
Penicillium funiculosum	LE
Rhizopus oryzae	LS
Candida valida	LEM

Note: LS - Lagos Soil;
LE - Lagos Effluent;
LEM - Effluent from inside Lagos factory;
AS - Abeokuta Soil Sample; and
AE - Abeokuta Effluent.

The percentage decolourization of Cibacron Scarlet by the eleven isolates (Table 4) showed that all the isolates have percentage

decolourization less than 50% after day 3. However after day 5, there was increase in the colour reduction as seven of the isolates were found to have decolourization greater than 50% with *P. mirabilis* (AS) showing the highest of 85.7%, followed by *P. atrovenetium* (LS) 70.3%, *P. vulgaris* (LE) 68.4%, *R. oryzae* (LEM) 62.3%, *B. alvei* (AE) 60.5% *B. alvei* (LS) 51.5% and *R. oryzae* (LS) 50.7%. The lowest decolourization was obtained on day 5 with *B. polymyxa* (AS) (39%).

Table 4. The percentage decolourization of Cibacron Scarlet by the microbial isolates.

Treatment	Day 3	Day 5
P. mirabilis (LS)	40.1	47.8
P. vulgaris (LE)	ND	68.4
B. alvei (LS)	12.5	51.5
P. mirabilis (AS)	47.6	85.7
B. polymyxa (AS)	29.9	39
B. alvei (AE)	ND	60.5
P. atrovenetium (LS)	39.4	70.3
R. oryzae (LEM)	24.1	62.3
P. funiculosum (LE)	40.1	49.1
R. oryzae (LS)	27.4	50.7
C. valida (LEM)	26.9	43.6

The pH of the experimental set up for Cibacron Scarlet as the decolourization experiment proceeds (Table 5) revealed that the pH of the set up for bacterial isolates on day 5 was slightly alkaline (8.5-8.8) with the exception of *B. polymyxa* (AS) (4.5) whose pH is in the acidic range as observed in all the fungal isolates (3.4-4.4).

Table 5. The pH of the experimental set up for the Decolourization of Cibacron Scarlet.

Treatment	Day 0	Day 3	Day 5
Control	7.2	7.3	7.4
P. mirabilis (LS)	7.3	6.8	8.8
P. vulgaris (LE)	8.0	7.1	8.6
B. alvei (LS)	4.0	7.4	8.8
P. mirabilis (AS)	3.4	8.1	8.5
B. polymyxa (AS)	4.4	4.2	4.5
B. alvei (AE)	3.8	8.1	8.8
P. antrovenetium (LS)	5.6	3.8	3.8
R. oryzae (LEM)	5.4	4.7	4.0
P. funiculosum (LE)	5.4	3.6	3.4
R. oryzae (LS)	5.3	4.3	4.4
C. valida (LEM)	5.5	3.9	3.8

The growth of the fungi during Cibacron Scarlet decolourization showed increase in mycelial weight as the decolourization experiment progresses (Fig. 1). On day 5, P. funiculosum have highest mycelial weight of 0.13 g, C. valida 0.12 g, R. oryzae (LEM and LS) 0.10 g and P. atrovenetium (LS) had the lowest mycelia weight of 0.09 g.

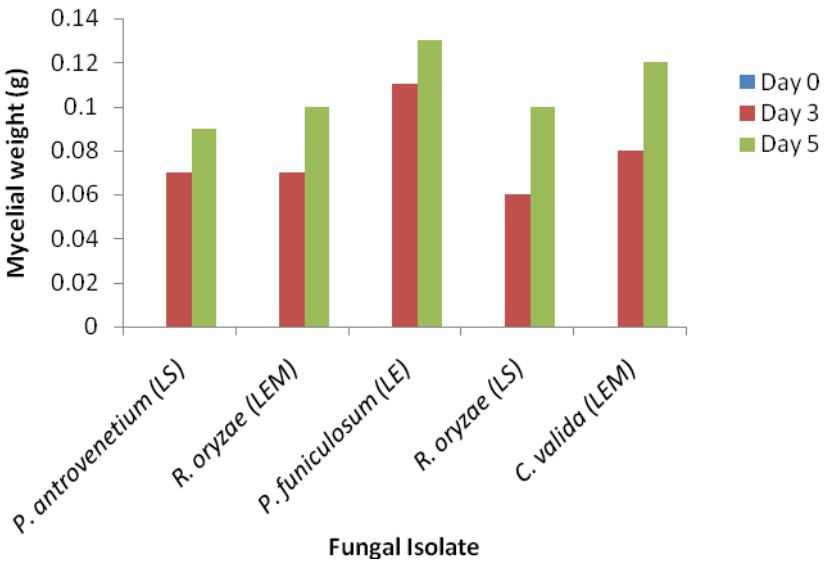

Fig. 1. The fungal mycelial weight per 5 ml of the decolourization experimental set up sample for Cibacron Scarlet dye.

The percentage decolourization of Remazol Blue by the microbial isolates as shown in Table 6 revealed that eights of the isolates had decolorization potential of greater than 50% on Day 3. The highest percentage of 74.8% was recorded with *B. alvei* (LS), followed by *P. vulgaris* (LE) with 70.7% while the least was 46.3% with *P. mirabilis* (LS). However, on day 5, reduction in the colour removal was recorded by the isolates when compared with day 3, with the exception of *P. mirabilis* (LS) and *B. polymyxa* (AS) which increased from 46.3% to 64.1% and 54.1% to 67.8% on day 3 and 5, respectively.

Table 6. The percentage decolourization of Remazol Blue by the microbial isolates.

Treatment	Day 3	Day 5
P. mirabilis (LS)	46.3	64.1
P. vulgaris (LE)	70.7	53.6
B. alvei (LS)	74.8	69.8
P. mirabilis (AS)	68.9	56.6
B. polymyxa (AS)	54.1	67.8
B. alvei (AE)	64.8	58.8
P. antrovenetium (LS)	61.5	28.5
R. oryzae (LEM)	62.6	30.3
P. funiculosum (LE)	48.2	38.6
R. oryzae (LS)	54.1	58.8
C. valida (LEM)	49.6	45.7

Table 7 shows the pH of the experimental set up for Remazol Blue during the experimental process. The result showed that the pH for the decolourization process with bacterial cultures on day 3 range between 8.1 to 8.9 with a slight increase on day 5 between 8.6-9.1. The fungal decolourization cultures also followed the same trend with pH range between 5.6-7.1 and 7.6-8.5 on day 3 and day 5, respectively.

The fungal growth during decolourization of Remazol Blue dye (Fig. 2) showed increase in mycelia weight of the fungi as the decolourization progressed on day 3 and 5. *P. antrovenetium* (LS) showed the highest mycelia weight of 0.09 g and 0.23 g on day 3 and 5, respectively.

Table 7. The pH of the experimental set up for the Decolourization of Remazol Blue.

Treatment	Day 0	Day 3	Day 5
Control	8.3	9.4	9.2
P. mirabilis (LS)	8.7	8.1	8.6
P. vulgaris (LE)	8.7	8.4	9.1
B. alvei (LS)	8.7	8.1	8.6
P. mirabilis (AS)	8.4	8.8	8.9
B. polymyxa (AS)	8.6	8.6	9.0
B. alvei (AE)	8.6	8.9	9.0
P. antrovenetium (LS)	7.3	5.9	8.0
R. oryzae (LEM)	7.1	6.2	7.8
P. funiculosum (LE)	7.2	7.0	8.5
R. oryzae (LS)	7.1	7.1	8.5
C. valida (LEM)	7.2	5.6	7.6

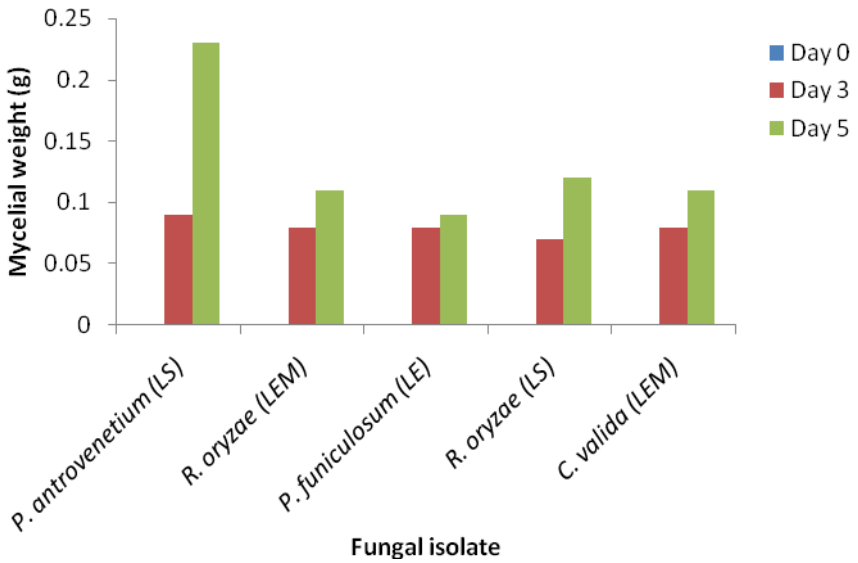

Fig. 2. The fungal mycelial weight per 5 ml of the decolourization experimental set up sample for Remazol Blue dye.

The lowest weight of 0.08 g and 0.09 g was observed in *P. funiculosum* (LE). *R. oryzae* (LEM and LS) and *C. valida* had weight of 0.08 g and 0.11 g on day 3, 0.12 g and 0.11 g, respectively, on day 5.

Discussion

The result obtained from the maximum absorbance of the Remazol Blue and Cibacron scarlet dyes as 595 nm and 511 nm, respectively, was very close to 590 nm and 515 nm reported for the same dyes by Banat *et al*. (1996).

The BOD of both effluent samples; Abeokuta effluent 260 mg/L and Lagos effluent 108.0 mg/L (Table 2) was above the 50 mg/L interim effluent limitation guidelines in Nigeria (FEPA, 1991). Likewise, the COD of the Abeokuta effluent was relatively higher (395,299 mg/L) when compared with LAG effluent (544.32 mg/L). This may basically be as a result of fact that no treatment is carried out (to the best of my knowledge) on the ABK effluent before discharge into the environment through the drainage channels, being a small scale local textile industry. The high temperature of the LAG effluent (41^{o}C) can also affect aquatic organisms.

The decolourization ability of the microbial isolates obtained from this study agreed with the earlier studies which have shown the ability of *Bacillus* sp. and *Proteus vulgaris* in metabolizing dyes (Yatome *et al*. 1991; Maier *et al*. 2004). Martins *et al*. (1999) and several authors have also reported textile dyes colour removal by *Candida* sp. This study also showed the ability of *Penicillium* sp. and *Rhizopus* sp. to decolourize textile dyes.

The fall in the percentage decolourization of Remazol Blue by some organisms might be due to the production of certain metabolites, which might have contributed to the increase in the absorbance. The extension of the incubation period for the decolourization study may or may not achieve increase in the percentage decolourization, if no toxic intermediate product is produced or if the nutrient required for the microbial growth has not been exhausted.

The ability of some isolates to remove the colour to a larger extent within three days is of interest; Sarnaik and Kanekar (1995) have reported that the incubation period of five to seven days is quite long and unsuitable for any industry for any actual application purpose, thus embracing the fact that some of the isolates from this study can be used in industrial application.

The dyes removal by the isolates revealed the decolourization process to be pH dependent. The acidic pH of 4.5 recorded for *B. polymyxa* (AS) as against what was observed in other bacterial isolates in day 5 during Cibacron Scarlet decolourization (Table 5) could have been one of the reasons why the lowest decolourization was obtained. The result revealed that neutral and basic pH values would be more favourable for the decolourization of dyes by bacteria while a slightly acidic pH would be more favourable for fungi or the same rule hold for the fungi as in the case of bacteria, depending on the nature and type of the dye. Assadi and Jahangiri (2001) supported the concept that fungal growth usually occurs at low pH values. The pH of dyes colour removal by microorganisms is isolate and dye dependent, as some dyes are known to contain inhibitory functional groups (Hu and Wu 2001). Methyl Red was completely degraded by *Klebsiella pneumonia* RS-13 between pH 6.0 and 8.0 while *Alcaligenes liquefaciens* S-1 completely degraded the same dye at pH 6.5 (Wong and Yuen 1998).

The increase in the bacteria count as the decolourization experiment proceeds (result not shown) shows that the bacterial cells are still viable in the decolourization process. The bacterial count may witness a decrease if toxic intermediate product(s) are produced or when the nutrient required for their growth is exhausted. The increase in the fungal mycelia weight all through the decolourization process (Figs. 1 and 2) may be due to either the growth of the fungi or the adsorption of the dyes to the fungal mycelia, which can also contribute to their increased weight.

Conclusion

The ability of the isolated microorganisms to decolourise textile dyes offer considerable advantages in the treatment of coloured effluents. However, there is need for the optimization of the conditions required for efficient decolourization. The potential of the cultures need to be demonstrated for its application in the treatment of real dye-bearing wastewater using appropriate bioreactors. Environmental, regulatory and monitoring agencies in Nigeria should ensure that industrial activities and waste management/ discharge practices conform to the set limits and standards with proper monitoring to ensure a safe environment.

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