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Biodegradation of Methyl Red by *Staphylococcus aureus* Isolated from Waste Dump Site

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ABSTRACT

The release of azo dyes especially methyl red into the environment is of great concern due to coloration of natural water, toxicity, mutagenicity and their biotransformation product. Economical and bio-friendly approaches are needed to remediate dye contaminated waste water from various industries. In this study, *Staphylococcus aureus* capable of degrading azo dye was isolated from waste dump site. The isolate was studied for their ability to decolorize Methyl Red (MR) using UV spectrophotometer at 430 nm at 3 different concentrations (250, 500 and 750 mL) under aerobic condition for the period of 12 days. The 62, 58 and 50% methyl red decolorizations were recorded in 750, 500 and 250 mL dilution respectively at the end of 12 day. The results of this study suggest the potential of *Staphylococcus aureus* for the treatment of waste water containing methyl red.

Key words: Azo dyes, biodegradation, methyl red, *Staphylococcus aureus,* decolorization

INTRODUCTION

Dyes contain chromophoric groups-decolorized electron systems with conjugated double bonds and auxochromes that cause or intensify the color of the chromophore. Usual chromophores are –C = C–, –C = N–, –C = O, –N = N–, $–NO_2$ and quinonoid rings. Examples of auxochromes are $–NH_2$, $–SO_3H$, –COOH and –OH (Singh *et al*., 2012). Dyes may be classified according to types of chromophores in their structure or as per the method of application. Based on their chemical structures, dyes can be classified into 20-30 different types. Over one million tonnes of synthetic dyes are produced worldwide every year for dyeing and printing. Azo dyes which are aromatic compounds with one or more -N = – groups, contribute to environmental pollution and is one of the major and most important problems of the modern world. Elimination of colour from dye bearing wastewater is one of the major environmental problems, because of difficulties in treating such wastewaters by conventional methods, as most of the dyes are stable to heat and oxidizing agents (Adowei *et al*., 2012). Colour pollution may cause potential toxicity and turbidity problems, thus contributing significantly to the pollution of aquatic ecosystems.

Since, 1856, over 105 different dyes have been produced worldwide with an annual production of over 735 metric t (Chen *et al*., 2003). Paper and pulp mills, textiles and dyestuff industries, distilleries and tanneries are some of the industries which release highly colored wastewaters (Raghukumar, 2000). Due to large scale production and extensive application, synthetic dyes can cause considerable environmental pollution and are serious health-risk factors

Table 1: Microbes use in azo dye degradation

Organisms	Strain	Dyes	References
Bacteria	*Galactomyces geotrichum*	Yellow	Govindwar *et al*. (2014)
	Enterobacter agglomerans	Methyl red	Keharia and Madamwar (2003)
	Enterocbacter sp.	Cl reactive red 195	Kalyani *et al*. (2008)
	Bacillus subtilis	Acid blue 113	Gurulakshmi *et al*. (2008)
	Brevibacillus laterosporus	Navy blue 3G	Jirasripongpun *et al*. (2007)
	Bacillus fusiformis kmk 5	Acid orange 10 and disperse blue 79	Kolekar *et al*. (2008)
Fungi	*Geotrichum* sp.	Reactive black 5, reactive red 158 and Reactive yellow 27	Kuhad *et al*. (2004)
	Shewanella sp. NTOVI	Crystal violet orange ll	Chen *et al*. (2008)
	Phanaerochaete chrysosporium		Sharma *et al*. (2009)
	Aspergillus ochraceus NCIM-1146	Reactive blue 25	Parshetti *et al*. (2006)
Yeast	*Kluyveromyces marxianus* IMB3	Ramazol black B	Meehan *et al*. (2000)
	Saccharomyces cerevisiae MTCC46	Methyl red	Jadhav and Govindwar (2006)
Actinomycetes	*Streptomyces ipomoea*	Orange II	Molina-Guijarro *et al*. (2009)
Algae	*Spirogyra rhizopus*	Acid red 247	Ozer *et al*. (2006)
	Cosmarium sp.	Triphenylmethane dye and malachite green	Daneshvar *et al*. (2007)

(Forgacs *et al*., 2004). The untreated dyeing effluents that are straightly used in agriculture have a serious impact on environment and human health (Pourbabaee *et al*., 2006). Para-Phenylene Diamine (PPD) also called 1,4-diamino benzene or 1,4-phenylene diamine, is an aromatic amine which is a major component of azo dyes. Para-Phenylene Diamine containing azo dyes are toxic and causes skin irritation, contact dermatitis, chemosis, lacrimation, exophthalmos and permanent blindness. Ingestion of PPD products leads to the rapid development of oedema on face, neck, pharynx, tongue and larynx along with respiratory distress. Sudha *et al. (*2014), Methyl red is an indicator dye that turns red in acidic solutions. It is an azo dye and is a dark red crystalline powder. Its molecular formula is $C_{15}H_{15}N_3O_2$, molar mass is 269.3 g mol^{-1}, density is 0.791 g cm^{-3} and melting point is 179-182°C. Methyl red is a pH indicator, it is red in pH under 4.4, yellow in pH over 6.2 and orange in between, with a pKa of 5.1 (Sarkar *et al*., 2011). As an emerging technique, microbial degradation is one of the best techniques to detoxify the azo dyes (Shah, 2014). Microorganisms that has been identified to be have degradation ability to decolorize azo dyes are shown in Table 1. The present study is therefore, aimed at determining the biodegradation and decolorization of methyl red dye by *Staphylococcus aureus* isolated from waste dump site.

MATERIALS AND METHODS

Dye and chemicals: Methyl red, a member of azo dye was collected from the Department of Microbiology, Federal University of Technology, Minna, Niger state. Nigeria. The dye was of commercial grade.

Isolation of organism: The organism that was used for biodegradation activities was isolated from soil. The soil was collected from waste dump site located at Emir's street, Kuntukwo village, Bosso LGA, Minna, Niger state, Nigeria. The soil was air dried in microbiology laboratory of Federal University of Technology, Minna, Niger state, Nigeria. The soil was serially diluted. Pour plate isolation technique was used for the isolation of the bacteria with dilution factor of 10^{-6} using nutrient agar.

Colonies which developed on the plates were counted and expressed as colony forming units per gram (CFU g^{-1}) of soil. Pure culture was obtained by repeated sub culturing on media used for primary isolation and incubated for 24 h at 37°C. The slant culture was preserved at 4°C as stock culture.

Characterization and identification of isolate: The isolate was identified to generic level using Bergeys Manual of Determinative Bacteriology and the Barrow and Feltham (2003). It includes colonial, morphological characteristics, Gram's staining reactions, biochemical test such as; glucose utilization, starch hydrolysis, catalase test, oxidate test, coagulase test among others. The organism was identified as *Staphylococcus aureus*.

Preparation of decolorizing medium: It is composed of 2.0 g L^{-1} yeast extracts, 0.5 g L^{-1} KH_2PO_4, 0.5 g L^{-1} NaCl, 1.0 g L^{-1} NH_4Cl, 3.0 g L^{-1} $MgSO_4$, 0.3 g L^{-1} $(NH_4)_2SO_4$, 1.0 g L^{-1} $NaHCO_3$, 0.2 g L^{-1} $CaCl_2.6H_2O$, 0.2 g L^{-1} $NaB_4O_7.10H_2O$, 0.1 g L^{-1} $MnCl_2.4H_2O$, 0.1 g L^{-1} $ZnSO_4.7H_2O$, 0.1 g L^{-1} $CuSO_4.5H_2O$.20 mg L^{-1} of Methyl red dye was added to 250, 500 and 750 mL of M9 synthetic medium, respectively. The pH was adjusted to neutral of 7.0 with 0.1 M HCl and 0.1 M NaOH using JENWAY, model 3020 pH meter. The medium containing methyl red dye was autoclaved at 121°C for 15 min. After the preparation of the mineral medium in which methyl red dye was added and the pH adjusted to neutral, the medium was sterilized by autoclaving at 121°C for 15 min. It was allowed to stay until ambient temperature is achieved before inoculating with test organism.

Decolorization of dyes by living cells: Twenty milliliter of decolorizing medium containing Methyl red in 50 mL Erlenmeyer flask was inoculated with 2 mL of standardized inoculum that was incubated for 24 h. All experiments were performed in triplicate. The flasks were plugged with sterile cotton wool and incubated at 37°C and aerobic condition was provided by shaking the flasks as described by Ren *et al.* (2006), throughout the duration of the experiment. Initial absorbance of methyl red dye was taking at 430 nm using UV-spectrophotometer (M®752 UV-spectrophotometer model Ym1208PTSL) after centrifugation at 1200 rpm for fifteen minute. The experiment lasted for 12 days and absorbance was taking at 3 days interval alongside the control in order to monitor decolorization activities. The percentage decolorization was calculated from the following equation according to Saranraj *et al.* (2010) as shown below:

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

where, A_0 and A_t represents initial and final dye concentrations in mg L^{-1}, respectively.

RESULTS AND DISCUSSION

Biodegradation of Methyl Red (MR), a textile dye was studied against *Staphylococcus aureus* isolated from waste dump site. The results presented in tables and graphs showed that, *Staphylococcus aureus* is capable of degrading MR under aerobic condition. Percentage degradation of 20 mg of MR in 250 mL dilution were 47.11, 48.07, 50.17 and 51.04% at days 3, 6, 9 and 12, respectively (Fig. 1a).

This result indicates that *Staphylococcus aureus* is capable of utilizing MR as the sole carbon source. The *Staphylococcus aureus* decolorized MR in the range of 51.04-47.11% in 250 mL dilution (Fig. 1a), 60.04-52% in 500 mL dilution (Fig. 1b) and 66.07-59.04% in 750 mL dilution between 3-12 days incubation period under aerobic condition. Similar results were reported on biodegradation of azo dyes by yeast (*Issatchenkia occidentalis*), where maximum decolourization was observed under aerobic conditions (Ramalho *et al*., 2004). They further reported that under anoxic condition decolourization was lesser due to absence of metabolic activities.

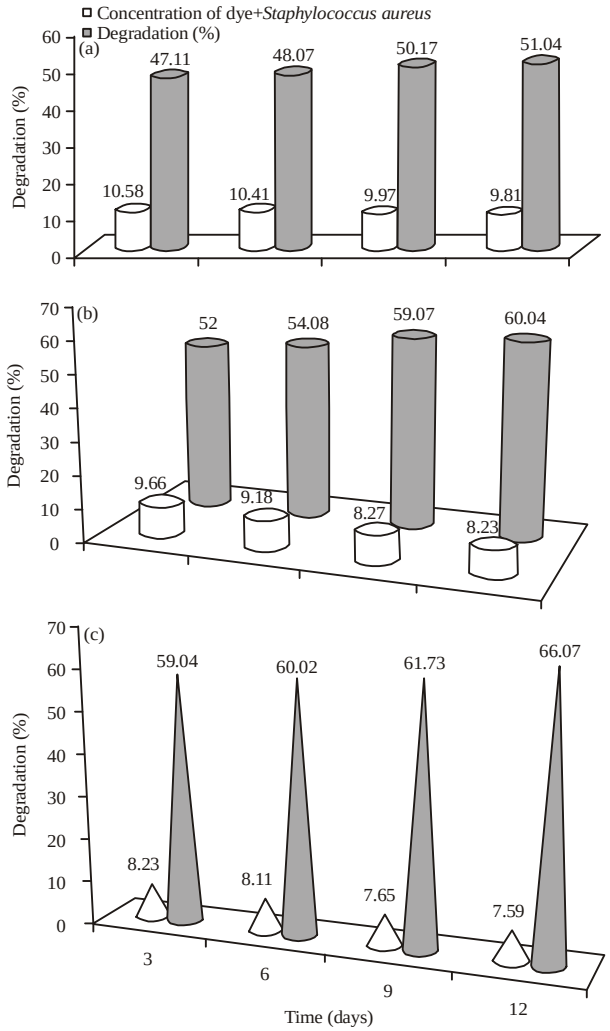

Fig. 1(a-c): Percentage degradation of 20 mg MR in, (a) 250, (b) 500 and (c) 750 mL dilution

The result in Fig. 1b shows the percentage degradation of 20 mg of MR in 500 mL dilution with 52% degradation at day three, 54.08% degradation at day six, 59.07% degradation at day 9 and 60% degradation at day 12 (Fig. 1b).

The result shows increase in percentage degradation of MR that as the number of days increases which shows that time is an important factor in degradation and this is in agreement with the work of Wang *et al*. (2009), where he reported 78.4% decolorization of Reactive Black 5 by *Enterobacter* sp. in 60 h of incubation and a maximum value of 92.6% decolorization after 108 h of incubation. The result in Fig. 1c shows the percentage degradation of 20 mg of MR in 750 mL dilution with 59.04% degradation at 3 day, 60.02% degradation at day six, 61.73% degradation at day 9 and 66.07% degradation at 12 day (Fig. 1c).

It was investigated by Deivasigamani and Das (2011) in their work on biodecolourization of basic violet-3 by *Candida crusei* isolated from textile wastewater. They reported maximum decolourizations of 74 and 100% in the media supplemented with sucrose and sugarcane bagase extract within 24 h, respectively. The result also shows that degradation was high in 750 mL

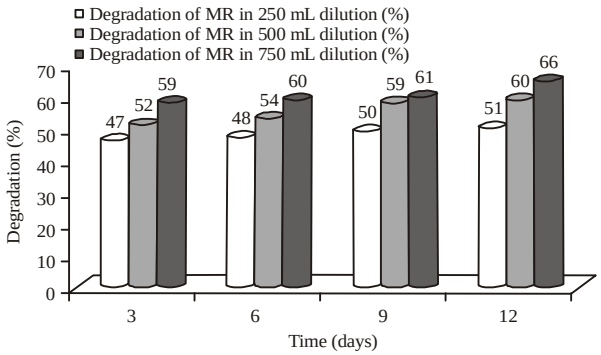

Fig. 2: Percentage degradation of MR in 250, 500 and 750 mL dilution

dilution which recorded the highest percentage of 66% followed by 500 mL dilution with 60% and lastly 250 mL dilution with 51%. These shows that concentration is an important factors in dye biodegradation. Erkurt *et al*. (2007), investigated the effect of time or incubation period including prior to inoculation, during the exponential phase of growth after 3 days, during stationary phase of growth after 5 days or after 6 days of culture. Found that incubation period greatly affect degradation percentage and this agree with the present study.

There was progressive increase in percentage degradation of the dye as the incubation period increases in all the days for all the dilutions (Fig. 2). Dye concentration was also observed to affect the rate of degradation as 750 mL dilution of 20 mg MR yielded the highest degradation percentage of 66% at day 12 as compared with 250 and 500 mL dilution that yielded 51 and 60% degradation, respectively (Fig. 2).

Findings from this current report on the effect of dye concentration or dilution may be that as dye concentration increase, the dye becomes toxic to the organism. This is in line with earlier reports on dye decolorization. Ogunjobi *et al*. (2012) reported that, the percentage decolourization by the bacterial isolates was observed to decrease gradually as the concentration of the dyes increased from 200, 300, 400 and to 500 mg L^{-1}. It has how ever been reported that dye decolorization can be strongly inhibited when a high concentration dyestuff is used to examine the poisonous effect of the dye on the degrading microorganisms (Wang *et al*., 2009). The type and concentrations of dyes and microbial concentration have been suggested to affect decolorization. Failure by the cultures to decolorize dye below certain concentration implies that there is a threshold level at which the microbial degrading mechanisms are stimulated and above which are inhibited (Adebiyi *et al*., 2011).

The result shows that, *Staphylococcus* sp. under aerobic condition can degrade methyl red dye. It agrees with Sarkar *et al*. (2011), where he reported the decolorization of Methyl red by *Staphylococcus arlettae* PF4. This study shows that bacteria have the ability to degrade dye as recorded by Ajibola *et al*. (2005), Moutaouakkil *et al*. (2003), Wang *et al*. (2009), Saratale *et al*. (2010), Agarry and Ayobami (2011), Palamthodi *et al*. (2011) and Ramya *et al*. (2010).

CONCLUSION

As an emerging technique, microbial degradation is one of the best techniques to decolorize the azo dyes, From the result obtained, it can be concluded that *Staphylococcus aureus* isolated from waste dump site has the ability to degrade methyl red under aerobic condition.

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