Received: April 30, 2023

Revised: September 28, 2023

Revised: September 28, 2023

NILE UNIVERSITY OF NIGERIA
HONORIS UNITED UNIVERSITIES

Biodecolourization of Textile Effluents using Lignolytic Enzymes Produced from Selected Bacterial and Fungal Isolates from Waste Dump Site

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ABSTRACT

Textile effluent contains enormous chemicals with detrimental environmental effects on both fauna and flora due to its chemical compositions. In this study, the effect of lignolytic enzymes produced by microorganisms for the treatment of textile effluent was examined using standard microbiological techniques. The potential of the isolates to produce laccase (L), lignin peroxidases (LiP) and manganase peroxidase (MnP) was investigated using streak plate method and assay methods. The L, LiP and MnP enzymes produced with the optimal processing parameter were used to decolorize textile effluent singly and as consortia for ten (10) days. Fourteen (14) microbial isolates which include eight (8) bacterial and four (4) fungi were isolated from soil contaminated with textile effluents. *Aspergillus terreus* and *Aspergillus niger* showed higher production of laccase with 8.0 mm diameter zone of inhibition. *Bacillus licheniformis* and *Bacillus subtilis* had the widest zone of inhibition (12.0 and 8.0 mm) respectively. Only *Aspergillus flavus* however had the potential to produce lignin peroxidase (with 10 mm zones of clearance) of all the fungi isolated in this study. Laccase recorded the highest decolourization (72.5%) comparable to 71.1% observed for the three-enzyme combination while LiP has 57.0%. This finding established the potential use of bacterial and fungal ligninocellulolytic enzymes for the decolourization of textile effluent.

KEYWORDS: ligninolytic enzyme, peroxidase, textile effluents, decolourization

DOI: *https://doi.org/10.5455/NJEAS.151030*

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1 | INTRODUCTION

Around the world, an estimated 2.8 10^5 tons of textile dyes are released into industrial wastewater for the textile industry each year, according to Jin et al. (2007) The release of these dyes into the environment is dangerous not only because of their color but also because many azo dyes and the breakdown products of those dyes (colorless amines) are poisonous or mutagenic to living things (Xu et al., 2007, Weisburger, 2002,). It is believed that azo dyes are electron deficient xenobiotic compounds because they feature electron withdrawing groups, which lead to an electron deficit in the molecule (dyes), making them resistant to destruction (Singh et al., 2014). The constantly expanding application of enzymes is creating a growing demand for biocatalysts that display improved or new properties (de Carvalho and Carla, 2011). By individually acting on certain recalcitrant pollutants, enzymes can remove them by precipitation or transformation to other products. Biological (enzymatic) processes have an added benefit over traditional chemical/physical methods as they are considered as clean and green (Mutambanengwe et al., 2008). The various physicochemical treatments like chemical precipitation, coagulation, flocculation, floatation, membrane filtration offer advantages like ease of operation and control, flexibility to change in temperature and are rapid but their benefits, however, are outweighed by several drawbacks such as their high operational costs due to the chemicals used, high energy consumption and handling costs for sludge disposal (Kurniawan et al., 2006). Stringent government policies regarding permitted levels of pollutants, high costs of specialized chemical treatments for pollutant removal, and the fact that some of these treatments create additional solid waste have led to the development of many practical yet simple biological methods. Enzymes are highly specific and exceptionally efficient catalysts (Nelson and Cox, 2004). They selectively degrade a target pollutant without affecting the other components in the effluent. Further, they can operate under mild reaction conditions, especially temperature and pH. In this respect, enzymes outperform the regular catalysts (transition elements like Cu, Ni). Enzymes have been designated for the decolorization and degradation of dyes, and due to their biodegradability, they are preferred from an environmental perspective. These enzymes include azoreductase, laccases, catalases, oxidases, and peroxidases. (Dalby, 2007 and Ali 2009). In the occasion of reactions wherein the target pollutant is oxidized, the protein receives one or more electrons from the substrate and donates these electrons to an electron acceptor. Hence, at the completion of the reaction, the enzyme is regenerated and is available for the next catalytic cycle. The biological origin of enzymes reduces their adverse impact on the environment, thereby making enzymatic wastewater treatment an ecologically sustainable technique. This study is aimed at using crude ligninolytic enzymes to biodecolourize textile effluents.

2.0 | MATERIALS AND METHODS

2.1 Collection of Sample

The nine (9) soil contaminated with textile effluents samples were collected, three each per waste dump sites of three textile companies in Sharada industrial area in Kano State, Nigeria. The soil samples were kept in commercially obtained sterile polythene bag and transported to Microbiology laboratory, Federal University of Technology, Minna, for analysis. Samples were preserved by refrigeration at 4°C without any preliminary treatment.

2.2 Isolation, Characterization and the Screening for lignin peroxidase producers among the Isolates

The soil contaminated with textile waste were collected into screw cap bottles. One millimeter (1 ml) of the serially diluted sample was plated in triplicates on nutrient agar for growing bacteria and Sabrouard

Dextrose Agar (SDA) for fungi. The Nutrient agar plates were incubated at 370C for 48 hours and SDA plates at 250C temperature for 3-5 days. The isolates obtained were purified to get pure cultures, subcultured on the same media, stored on slants, and maintained at a temperature of 40C for further use. The isolates were identified, screened for their ability to produce ligninolytic enzymes using the method described by Omotosho et. al. 2019.

2.3 Crude Enzyme Production

Production of laccase (L), lignin peroxidase (LiP) and manganese peroxidase (MnP) by selected microorganisms was done in Liquid Basal Medium (LBM) amended with the following ligninolytic inducers: 1 mM ABTS (2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid)), 2.5 mM veratryl alcohol (3,4- dimethoxybenzyl alcohol), 2.16 mM ferulic acid, 3.38 mM guaiacol, 0.05% Tween 80 and 0.1% Kraft lignin (Orth et al., 1993). Selected isolates were inoculated into 25 mL of each autoclaved medium (250 ml Erlenmeyer flasks) with a 1.0 cm3 of the corresponding culture. Flasks were incubated at 30º C and 120 rpm for between 5 – 10 days. Aliquots (3 ml) of culture supernatants centrifuged (10,000 rpm, 4º C, and 5 min) before the quantification of ligninolytic enzymes. Lac, MnP, and LiP were spectrophotometrically estimated according to the methods described by Orth et al. (1993), Enzyme activities were calculated using the formula below:

Enzyme Activity (units/mL) =

$$\frac{\text{Absorbance}}{\text{Time}} \times \text{Dilution factor}$$

2.4 Decolourization of the Effluents Decolourisation of the effluents was determined by monitoring the decrease in absorbance at the maximum wavelength of effluents (λmax. 523nm) by using a UV-Visible spectrophotometer. The decolorisation activity (%) was then calculated by the following formula and the entire assay done in triplicate. 850mL of untreated textile effluent was used against 150mL of each of the three enzymes, 75ml of consortia and 50ml each of the three enzymes. Thus, the absorbance calculated using the equation as described by Saratale et al. (2009).

% Decolourisation =

$$\frac{\text{Final Absorbance - Initial absorbance}}{\text{Initial Absorbance}} \times 100$$

3 | RESULTS AND DISCUSSIONS

Laccase activity (μU/S) of fungal and bacterial isolates Laccase activities at an optimum incubation time 72 hours were 44.3. 36.8 and 32.2 μU/S respectively for A. terreus, T. harzianum and A. niger respectively (Table 1 and Figure 1). The bacteria isolates show an optimum incubation time of 60 hours for B. licheniformis and 72 hours for B. subtilis with an enzyme activity of 18.3 and 17.2 μU/S respectively (Table 1 and Figure 1)

Table 1: Laccase activity (μU/S) of fungal isolates

Time (Hours)	*A. terreus*	*T. harzianum*	*A. niger*
48	21.2	7.83	10.3
72	44.3	36.8	32.2
96	18.5	20.3	5.17

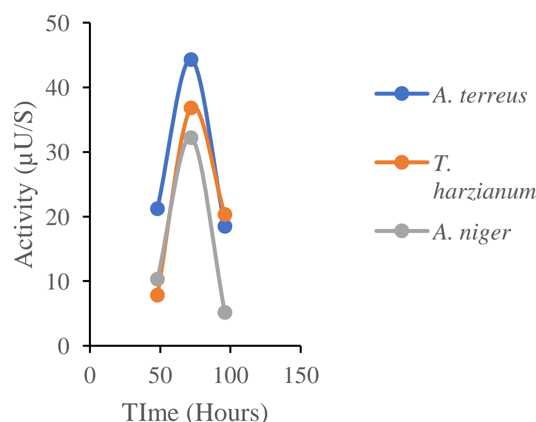

Figure 1: Laccase activity (μU/S) of fungal isolates

Table 2: Laccase activity (µU/S) of bacterial isolates

Time (Hours)	*B. subtilis*	*B. licheniformis*
24	0.73	1.33
36	12.5	17.2
48	13.5	27
60	13.7	18.3
72	17.2	9.3
84	12.8	6.35
96	8.25	3.12

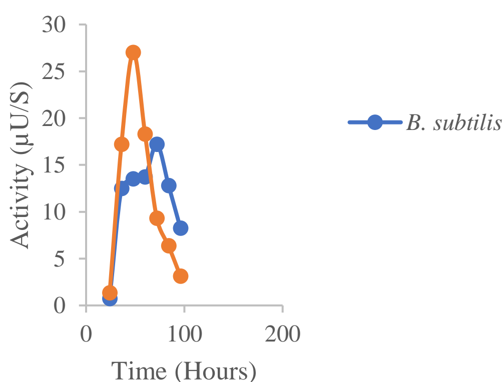

Figure 2: Laccase activity (µU/S) of bacterial isolates

Table 3: Effect of the enzymes on the colour of textile effluent

Time (Days)	Control	L+MnP +LnP	L	LiP	L+MnP
2	1.42	0.72	0.74	0.75	0.76
4	1.42	0.55	0.55	0.73	0.73
6	1.42	0.41	0.45	0.71	0.69
8	1.42	0.4	0.43	0.68	0.64
10	1.42	0.41	0.39	0.55	0.62
% D	35	71.1	72.5	61.3	56.3

Time (Days)	Control	MnP	L+ LnP	MnP + LnP
2	1.42	0.76	0.73	0.88
4	1.42	0.55	0.7	0.68
6	1.42	0.67	0.67	0.63
8	1.42	0.48	0.63	0.59
10	1.42	0.45	0.61	0.54
% D	35	68.3	57.0	61.9

Key: %D = Percentage decolourization,
L = Laccase,
MnP = Manganase Perosidase,
LnP = Lignin perosidase

3.1 Discussion

The colour is the primary and most apparent indicator of water pollution, and it is the primary indicator of pollutants in wastewater. The discharge of highly coloured synthetic effluents is aesthetically displeasing and can damage the receiving water body by impeding the penetration of light (Khehra et al., 2006). The effluent selected for the present study is a brownish blue colour. The effluent was highly coloured, indicating the high content of different dyes and pigmentproducing compounds (Elango and Govindasamy, 2018). The high intensity may be the combined result of pH that does not allow the chromophore group of dye to disintegrate during the dyeing process and making the effluent highly coloured. Colour reduces the photosynthesis activity of aquatic life, and it affects other parameters such as DO, BOD.

In the present study, colour removal efficiency indicated clearly that the respective enzyme treatment stages exhibit some degree of dye removal and hence a reduction in colour. This may be because of the activity of the microbial enzymes which decomposed the toxic pollutants present in the effluent and made the change in colour of the effluent. This is supported by the work of (Leonowicz et al., 2001) who reported that ligninolytic enzymes like lignin peroxidase, manganese peroxidase, and laccase efficiently catalyzed the oxidation of phenolic, non-phenolic and polycyclic aromatic hydrocarbons as well as a variety of recalcitrant aromatic compounds (Leonowicz et al., 2001), thus making them a valuable agent in textile wastewater treatment (Gianfreda et al., 2006).

Table 3, shows that Laccase recorded the highest decolourization of 72.5%. This finding explains why laccase and lignin peroxidase are commonly used in textile finishing, to improve the whiteness in conventional bleaching of cotton (Tzanov et al., 2003) or to bleach indigo dyes denim fabrics to lighter shades (Pazarlioglu et al., 2005; Ollikka et al., 1993; Verma and Madamwar, 2002). Kirby et al. (2000) demonstrated that laccase from Phlebia tremellosa causes 96% decolourization of eight synthetic textile dyes. Kalme et al. (2009) reported that a laccase from Pseudomonas desmolyticum efficiently decoulorised Direct Blue 6, Green HE4B, and Red HE7B dyes from textile effluent. The combinations of the three enzymes gave 71.1% decolourisation, manganese peroxidase, and lignin peroxidase cause 68.3% and 61.3% decolourizations, respectively. In contrast, a combination of laccase and manganese peroxidase caused the least effluent decolourisation of 56.3%. The differences in the textile effluent decolourizations potentials of these enzymes, as reported in this study and also in comparison with the previous studies, could be attributed to the differences in the enzyme source and the type/structure of contaminant in the effluent. Nyanhongo et al. (2002) showed that dyes are not equally susceptible to enzymatic degradation. Palmieri et al. (2005) have demonstrated that the extent of dye decolourization also depends on enzyme concentration.

4 | CONCLUSION

The textile effluent treated with microbial enzymes, laccase, lignin peroxidase, manganese peroxidase, and their combinations causes increase decolourization of the effluent with increase remediation time. Laccase recorded the highest decolourization of 72.5%, the combinations of the three enzymes gave 71.1% decolourization, manganese peroxidase, and lignin peroxidase recorded 68.3% and 61.3% decolourization respectively while the combination of laccase and manganese peroxidase caused the least effluent decolourization of 56.3%.

Acknowledgement: I like to extend my heartfelt gratitude to the Microbiology laboratory staff of the Federal University of Technology Minna, whose support played an integral role in the accomplishment of this research work. Without their expertise and willingness to go extra mile, this research work would not have been successful.

Funding Statement: “The author(s) received no specific funding for this study”.

Author Contributions: Study conception and design: Omotosho Azeez Olalekan.; data collection: Ezeagu Gloria, analysis and interpretation of results: Mohammed SSD. All authors reviewed the results and approved the final version of the manuscript

Conflicts of Interest: “The authors declare that they have no conflicts of interest to report regarding the present study”.

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Author Contributions:

Study conception and design: Omotosho Azeez Olalekan.; data collection: Ezeagu Gloria, analysis and interpretation of results: Mohammed SSD. All authors reviewed the results and approved the final version of the manuscript.

Availability of Data and Materials: Due to the nature of this research, participants of this study did not agree for their data to be shared publicly, so supporting data is not available.

Conflicts of Interest: "The authors declare that they have no conflicts of interest to report regarding the present study".