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Production of laccase by *Bacillus subtilis* and *Aspergillus niger* for treatment of textile effluent

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ABSTRACT

The improper disposal of textile effluents without effective treatment has adverse environmental, social, economic, and health impacts and as such, it is vital to find innovative technological solutions to reduce the negative consequences of textile effluents. Laccases are versatile multicopper enzymes found in plants, fungi and other microorganisms with wide applications especially in the textile and paper industry. This study examined the production of laccase from *Bacillus subtilis* and *Aspergillus niger* to remediate textile effluent. Both organisms were identified by molecular method and plate test method was used to evaluate laccase production by the two organisms. Rice bran emerged as the substrate of choice for laccase production. At optimum temperature (30°C), the highest laccase produced was 0.522 U/mL and 0.642 U/mL at 35°C for *B. subtilis* and *A. niger* respectively. The optimum pH level of 5 and 6 produced the highest laccase yield of 0.583 U/mL and 0.684 U/mL respectively. Significant improvements of laccases from *B. subtilis* and *A. niger* were observed on physicochemical analysis of TDS, pH, electrical conductivity, TSS, temperature and DO in treating textile effluent. Notably, these enzymes exhibited remarkable efficacy reduction in BOD (38 %), COD (14 %), and nitrate (23 %) levels in the effluent. The study underscores the efficacy of laccases from the microorganisms in treating textile effluent, with concentrations ranging from 10 to 30 U/mL proving effective. However, laccase produced from *B. subtilis* showed more remediation potential in textile effluent treatment compared to the one produced by *A. niger*.

1. Introduction

In the last two decades, colored wastewater generated from textile industries has been mainly treated using physical and chemical approaches. However, these approaches are often costly and generate solid waste, limiting their practicality. Biological procedures that use microorganisms that may decolorize or degrade these difficult-to-solve chemicals have gained attention due to their ability to provide a more ecologically friendly solution that may even lead to full mineralization [1,2]. Organic compounds with chromophore as well as auxochrome groups attached to a benzene ring are known as dyes, and they provide a special kind of difficulty. While the chromophore imparts color, the auxochrome facilitates electrolytic dissociation. Yet, the chromogen-chromophore structure alone may not ensure solubility or adherence to fibers. It is the auxochrome, encompassing amine, hydroxyl, carboxyl, and sulfonic radicals, that enhances bonding affinity [2]. Despite the wealth of organisms showcasing dye decolorization capabilities at the laboratory scale in recent years, their application in handling processes remains underexplored. The 1970s saw the beginning of attempts to separate bacterial cultures capable of breaking down azo dyes, and *B. subtilis* became a prominent candidate [3]. Various *Bacillus* strains isolated from soil and sludge samples have demonstrated remarkable dye decolorization abilities [4]. Textile effluent, rife with reactive dyes such as Reactive Red (RR), Reactive Black (RB), Reactive Yellow (RY), then Reactive Blue (RB), alongside acid red, blue dye, black, and yellow variants, poses significant challenges. The effluent is

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further compounded by elevated surfactant, detergent, and solvent concentrations [5,6].

Laccase, a copper-containing oxidase enzyme, has garnered significant attention for its potential in environmental biotechnology, particularly in the degradation of complex organic pollutants such as textile dyes. The enzyme is produced by various microorganisms, including the bacteria *B. subtilis* and the fungus *A. niger*, both of which have been extensively studied for their industrial and environmental applications.

B. subtilis, a Gram-positive, rod-shaped bacterium, is well-known for its capacity to produce a variety of extracellular enzymes, including proteases, amylases, and cellulases, which have diverse environmental and industrial applications [7]. Previous studies have demonstrated the ability of *B. subtilis* to produce laccase, which can degrade a wide array of synthetic dyes, particularly azo dyes, which are prevalent in textile effluents [8]. These properties make *B. subtilis* a suitable organism for bioremediation processes. *A. niger*, a filamentous fungus, is another prolific producer of industrially significant enzymes. It has been widely used in the production of citric acid and a range of enzymes including pectinases, proteases, and lipases [9]. *A. niger*'s capability to produce laccase has also been explored, revealing its potential in the biodegradation of recalcitrant pollutants like synthetic dyes [10]. The microorganism's robust enzyme production and adaptability to various substrates especially rice bran which is abundant, cost effective and rich in nutrient make them an attractive option for environmental applications.

Both *B. subtilis* and *A. niger* have been the focus of numerous studies aimed at harnessing their enzymatic capabilities for environmental and industrial applications. For instance, *B. subtilis* has been used in the degradation of textile dyes in various studies, showing promising results in terms of decolorization and detoxification of dye-laden wastewater [11]. Similarly, *A. niger* has been employed in the biodegradation of aromatic compounds, including synthetic dyes, due to its efficient laccase production [12]. Commercially, enzymes from *B. subtilis* and *A. niger* are used in detergents, food processing, and biofuel production due to their stability and effectiveness in various industrial processes. The laccase enzyme, in particular, has been commercialized for use in the textile industry for the treatment of dye effluents, in the paper and pulp industry for lignin degradation, and in bioremediation processes to degrade environmental pollutants [7]. This study therefore, investigated the production of laccase by *B. subtilis* and *A. niger* to treat textile effluent.

2. Materials and methods

2.1. Samples collection

In the pursuit of understanding and addressing the complexities of textile effluent contamination, we ventured into the heart of dyeing workplaces located at Anguwan Sarki (9.6198° N, 6.5469° E), Mechanic Junction (9.6163° N, 6.5481° E), and Paida Junction (9.6154° N,

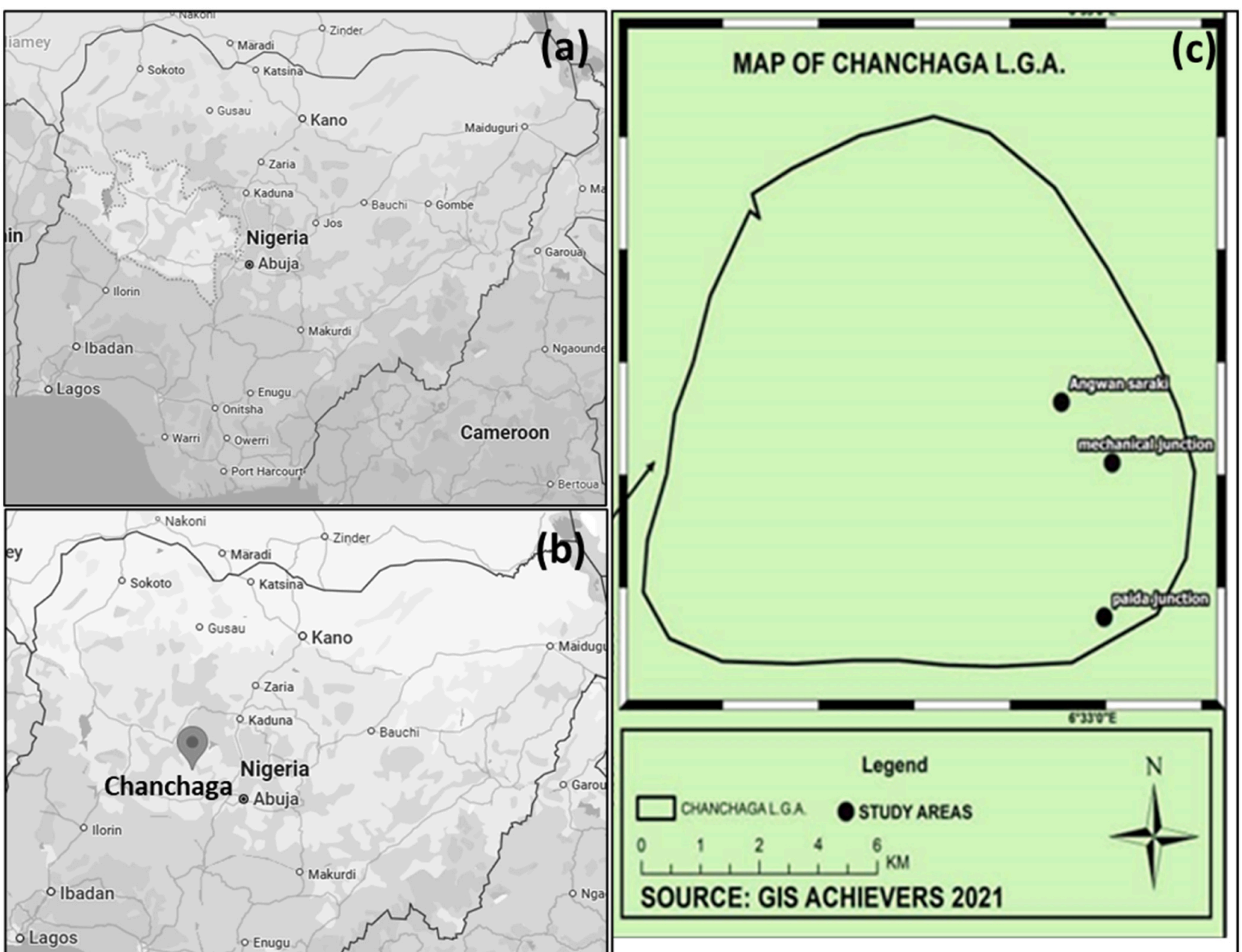

Fig. 1. The study site (Minna) within the map of Niger State.

6.5432° E) in Minna, nestled within the vibrant landscape of the Local Government Area of Chanchaga, Niger State, Nigeria (Fig. 1). Here, amidst the whirl of industrial activity, the soil bore witness to the residual effects of dyeing processes, urging us to uncover its secrets [13].

With meticulous care, soil samples were gathered from depths of 10–15 cm according to the procedure recommended by AOAC. Each sample was placed in polythene bags to ensure proper preservation. Samples were meticulously collected using clean hand trowels. These soil specimens were subsequently taken to the Federal University of Technology's renowned Microbiology Lab, FUTMinna Nigeria, where the soil samples were analyzed. Soil samples post collection were stored under refrigeration at 4 °C, till analysis. The samples were further analyzed for textile effluent contamination.

2.2. Identification and characterization of the microbial isolates

One gram of each soil sample was serially diluted with St saline from 10^{-1} to 10^{-10} and plated on NA and SA plates. For 24–48 hours, the plates had been incubated at 37 °C. To culture bacteria and fungi, a 0.1 mL sample that had been repeatedly diluted was put on Sabouraud dextrose agar (SDA) and incubated at a temperature of 28 ± 2 °C for 72 hours. While isolates of bacterial strains on NA had been selected and maintained on a sterile nutrient-agar slant, fungi were preserved for further study on a neutral SDA slant. The obtained pure culture's

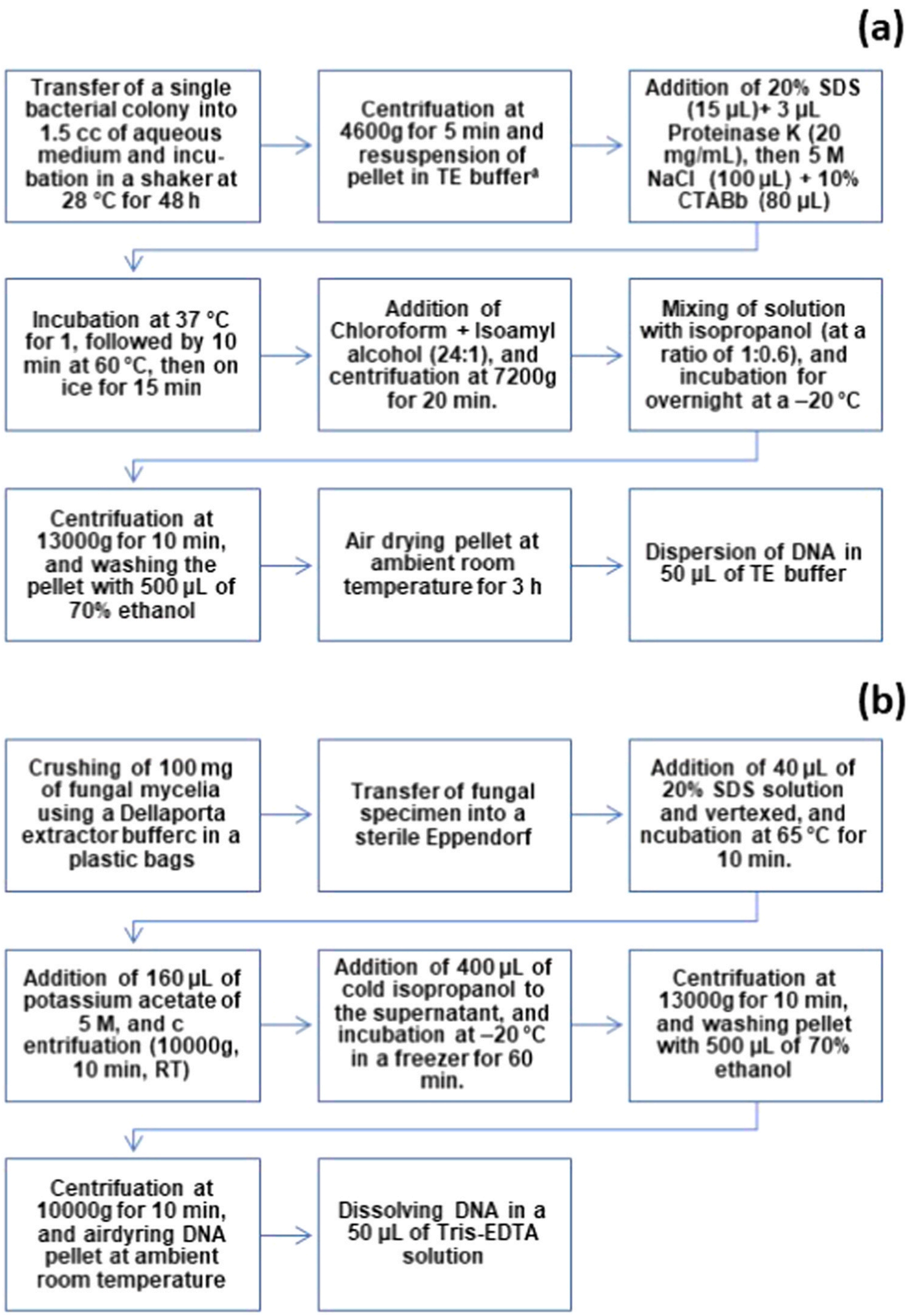

Fig. 2. DNA extraction protocol for bacteria (a) and fungi (b). ([a]TE buffer (10 mM Tris-HCl and 1 mM EDTA; pH 8.0; [b]10 % CTAB in 0.7 M NaCl; [c]EDTA pH 8, 100 mM Tris pH 8, 10 mM mercaptoethanol, and 500 mM NaCl).

microscopic characteristics, gram nature, biochemical tests (IMViC) were performed to identify the organisms isolated on NA and SA, and morphology allowed for its identification. Extraction of DNA from bacterial samples were carried out following the meticulous protocol outlined in the pioneering work [14]. Detailed were outlined in the following flow chart (Fig. 2a and b).

2.3. Laccase production by B. subtilis and A. niger

The plate test method was employed to evaluate the laccase production ability of *B. subtilis* and *A. niger* isolates. Following the protocol [15], fungal strains were inoculated onto Sabouraud's dextrose agar (SDA) plates supplemented with 0.02 % (wt/wt) 1-naphthol, five mM guaiacol, and 0.5 % (wt/wt) tannic acid to screen for laccase production and plates were incubated at 25 °C, for 24 hrs.

As for *B. subtilis*, the strain was introduced onto nutritional agar enriched with Tween 80, 0.02 % (wt/wt) guaiacol, five mM 1-naphthol, and 0.5 % (wt/wt) tannic acid, and then kept at 37 °C. The plates were monitored for color changes over several days, indicating laccase secretion, as per observations from previous studies [16]. Laccase activity was identified by distinct color changes, including deep purple in response to 1-naphthol, reddish-brown with cyanide, and brown with tannic acid.

The method outlined [17] for the bacterial inoculum was followed. A loopful of cells from a 24-hour-old culture was introduced into 50 mL of nutrient broth and Sabouraud's dextrose broth. *B. subtilis* was incubated at 37 °C, while *A. niger* was incubated at 25 °C. Both cultures were placed on a rotary shaker at 100 rpm to ensure proper growth and proliferation. After eighteen hours, one milliliter of the culture was utilized as the inoculum for solid-state fermentation (SSF) alongside a Sabouraud's dextrose broth culture containing *B. subtilis* and *A. niger*.

2.4. Crude laccase production under SSF conditions

The culture medium (nutrient agar) used was sterilized for 15 minutes using an autoclave and pH was maintained at 5, before cooling to room temperature. Subsequently, fermentation occurred in the flasks at 37 °C and pH 5, optimal for *B. subtilis*, with agitation set at 10,000 rpm.

Furthermore, a 5 mm disk containing *A. niger* fungal isolates was introduced into the growth medium to initiate the manufacturing process. *A. niger* was cultured for five days at 27 ± 2°C and pH 6, following the specified conditions [18]. The resulting supernatant was then stored in vials for further utilization as crude enzyme extracts, adhering [19] methodology.

Similarly, the impact of pH on laccase activity was explored by adjusting the medium's pH to different values (ranging from 4.5 to 8.0) using 1 N HCl and 1 N NaOH. After sterilization and cooling, *B. subtilis* was introduced into the flasks, and enzyme activity was determined in the supernatant post-centrifugation. This approach enabled an investigation into how hydrogen ions influence the biological activities of *B. subtilis* and *A. niger* enzymes.

2.5. Optimizing the amount of inoculum

In the quest to determine the optimal inoculum size, 500 mL Erlenmeyer flasks were employed for the production process. These flasks were inoculated with different volumes of *A. niger* (10 mL, 20 mL, 30 mL), alongside *B. subtilis* under optimal pH and temperature conditions. Subsequently, enzyme activity was assessed at 420 nm using a spectrophotometer [20].

2.6. Partial laccase purification

The laccase enzyme underwent partial purification using a method outlined [19], which involved a combination of ammonium sulfate precipitation and dialysis (The use of this combination was necessary because ammonium sulfate increases the ionic strength of the solution, causing proteins to precipitate out of solution and reduces protein solubility, allowing for selective precipitation of laccase) Initially, unprocessed laccase was filtered using Whatman No. 1 filter paper, and the resulting filtrate was freeze-dried, concentrated, and stored at 4°C. Following this, protein precipitation was achieved by gradually adding solid ammonium sulfate to the crude extract until reaching 70 % saturation. The mixture was gently mixed for at least an hour at 4°C and then left overnight. Afterward, centrifugation was conducted for an hour at 4°C and 8000 rpm to gather the precipitate. The supernatant was then removed, and the remaining particles were reconstituted in 0.01 M buffered phosphate solution at pH 7. Simply put, the steps involve initial purification with ammonium sulfate precipitation, enzyme concentration and removal of contaminants.

2.7. Dialysis

The pellets were dissolved in 0.01 M phosphate buffer at the optimal pH, followed by overnight dialysis against the identical buffer at 4°C.

2.8. Physiochemical examination of textile wastewater

The BOD was determined using the method of Mair and Cresser [21], where Textile effluent sample of 100 mL was measured and pour into rubber containers one milliliter (1 mL) of manganese sulphide and alkalide oxide solution were added into the sample. It was then mixed by shaking and incubated at 200^0 C for 5 days. After incubation, the top clear sample were carefully decanted, leaving the solid deposit at the foot and 2 mL of orthophosphoric acid was added into the deposit. The dirty deposit was pour into conical flask and titrated using sodium thiosulphate. End point was obtained by change in the colour from dirty to dirt free textile effluent.

The dissolved oxygen (DO), pH, temperature, chemical oxygen demand (COD), total suspended solid (TSS) (Eq. (2), total dissolved solid (TDS) (Eq. (4)), nitrogen, and electrical conductivity [22] were among the physiochemical parameters that were also analyzed.

$$Dissolved\ oxygen = \frac{V \times N \times 8 \times 1000}{V2V3} \tag{1}$$

Where V= volume in mL:N = normality at (0.025)

$$TSS(mg/L) = \frac{A-B}{V} \tag{2}$$

where: A = mass of filter + dried residue (mg)B = mass of filter (preweight) (mg)V = volume of sample filtered (L)

$$COD, mgO_2/L = \frac{(A-B) \times M \times 8000}{mL\ of\ sample} \tag{3}$$

whereA = FAS used for blank, mLB = FAS used for sample, mLM = Molarity of FAS

$$TDS(g/L) = \frac{A-B}{V} \times 100 \tag{4}$$

WhereA = final weight of the evaporating dish in g.B= initial weight of the evaporating dish in g.V = volume of the sample taken in mL

2.9. Utilizing laccase for textile effluent decolorization

The components listed below were taken in a 100 mL conical flask (Table 1) and mixed with 50 mL of textile effluent.

Before *B. subtilis* was autoclaved at 121°C for 15 minutes, the pH and temperature of the textile effluent were adjusted to optimal values of 5 and 30 °C, respectively. Similarly, laccase made from *A. niger* was

Table 1
Composition of medium.

S.No.	Chemical	Concentration (g/L)
1	Peptone	3.0
2	Dextrose	1.0
3	K_2HPO_4	0.4
4	KH_2PO_4	0.6
5	$MnSO_4$	0.5
6	$FeSO_4$	0.0005
7	ZnsO4	0.001
6	$ZnSO_4$	0.01

separately incubated at optimal pH 6 and temperature of 35 °C in an incubator shaker at 10,000 rpm for three (3) weeks, respectively, to treat the textile effluent. Following sterilization and room temperature cooling, 10 mL, 20 mL, and 30 mL amounts of crude laccase were added towards the textile effluent in the flasks. [23] found that no additional crude laccase generated by *B. subtilis* and *A. niger* was introduced during the manufacture of the control textile effluent. The treatments lasted one to three weeks. At the conclusion of each week, the samples were taken out and crude laccase was used to evaluate the absorbance level under ultraviolet light. Spectrophotometer operating at textile effluent's maximum wavelength of 579 nm. Decolorization is expressed as a percentage (%).

$$D(\%) = \frac{(\text{Absorbance}A_o - \text{Absorbance}A_1)}{\text{Absorbance}A_o} \times 100 \quad (5)$$

Where D denote the decolorization rate, with Absorbance AO representing the initial absorbance or the absorbance of the control, and Absorbance A1 representing the final absorbance or the absorbance of the test samples, as described [20].

2.10. Data analysis

Analysis of Variance (ANOVA) was used using SPSS version 26 to perform statistical analysis of the data while GraphPad prism was used to arranged the data into figures.

3. Results

3.1. Cultural and biochemical characteristics of fungal isolate

A dark brown color, long conidiophores, and globule vesicles completely coated with biseriate phialides which are transported by brown metulae are all visible in the microscopic structure as shown in Fig. 3a. *A. niger* was confirmed to exist based on these findings. A standard morphological strain produced is compared in Fig. 3a.

3.2. Molecular identification of Bacillus subtilis and Aspergillus niger

The significant amplification of the 16S rRNA region in the isolated bacterium is shown by Agarose gel electrophoresis in Fig. 3b, and the altered sequence's identity is also shown by DNA NCBI blast. Fig. 3c and d show the phylogenetic trees of identified *B. subtilis* and *A. niger.*

Table 2 presents the summary of sequencing of *A. niger* and *B. subtilis*

3.3. Optimum temperature for laccase production by Aspergillus niger and Bacillus subtilis

The optimal temperature as well as pH for *A. niger* and *B. subtilis* laccase production were shown in Fig. 4a. At the optimal temperature of 35 °C, *A. niger* produced the maximum quantity of laccase enzyme (Fig. 4a), while *B. subtilis* generated the highest percentage of laccase at the optimal temperature of 30 °C (0.522 μ/mL). The data is shown as mean ± standard error. The optimal pH for culture condition of laccase by *B. subtilis and A. niger* are shown in Fig. 4b. The optimum pH was 5 and 6 with laccase production of 0.583 u/mL and 0.684 U/mL respectively. Jadhav et al. [25] and Das et al. [26] in their research, recorded a similar optimal pH and temperature of 6–10 and 35^0 C respectively during degradation of textile dyes using *Pseudomonas* sp. Fig. 5b displays the impact of inoculum size on the generation of laccase by *A. niger* and *B. subtilis*. Also, this figure displayed the absorbance levels of laccase generated by *B. subtilis* and *A. niger* at various time periods (0, 1, 2, and 3 weeks) with variable inoculum sizes (10 mL, 20 mL, and 30 mL). The results indicate that *A. niger* has higher absorbance (0.2311 μ/mL) than *B. subtilis* (0.3541 μ/mL). The results of the investigation showed a negative association between the absorbance level and the weekly rise in the amount of laccase generated by *B. subtilis* and *A. niger*.

3.4. Effluent's physicochemical characteristics both before and after treatment

Fig. 5 presents the physicochemical characteristics of the effluent, both pre-treatment and post-treatment with crude laccase from *B. subtilis* and *A. niger*. Additionally, Fig. 5a displays the temperature of the textile effluent before and after treatment with laccase from both organisms. The pre-treatment samples for *A. niger* and *B. subtilis* were found to have higher temperatures (34 ºC, 34 ºC), but the samples treated with laccase generated by *A. niger* and *B. subtilis* had lower temperatures (33 ºC, 32 ºC) in comparison to the pre-treatment samples.

Fig. 5a illustrates the pH levels of textile effluent both before and after treatment with laccase from *A. niger* and *B. subtilis*. Initially, the pH of the untreated sample was alkaline (13.1, 13.1). Following treatment, the pH values decreased, with the sample treated with *A. niger*-produced laccase showing a neutral pH (7.02), particularly during the second and third weeks. In contrast, the sample treated with *B. subtilis*-produced laccase exhibited an acidic pH (3.14).

Fig. 5a illustrates the changes in various physicochemical parameters of textile effluent with laccase produced by *B. subtilis* and *A. niger*. Before treatment, the total dissolved solids (TDS) were high (39.53 mg/L, 39.53 mg/L), but upon treatment with increased volumes of crude laccase, the TDS decreased (19.09 mg/L, 9.20 mg/L) for both *A. niger* and *B. subtilis*. Similarly, the total suspended solids (TSS) were lower (3.0, 3.0) before treatment, but increased with higher laccase activity (7.0, 5.6) for both organisms after treatment.

Regarding electrical conductivity, the pretreatment samples exhibited high values (5.90 Ps/cm, 5.90 Ps/cm), which decreased after treatment with laccase produced by *A. niger* (2.84 Ps/cm) and *B. subtilis* (1.37 Ps/cm). Furthermore, dissolved oxygen (DO) levels were initially high (7.0 mg/l, 7.0 mg/l) but decreased after treatment with laccase from *A. niger* (6.28 mg/l) and *B. subtilis* (6.27 mg/l). Additionally, the biological oxygen demand (BOD) and chemical oxygen demand (COD) were high before treatment (5.2 mg/l, 5.2 mg/l for BOD; 6.1 mg/l, 6.1 mg/l for COD) but decreased after treatment with laccase (3.24 mg/l, 3.20 mg/l for BOD; 5.38 mg/l, 5.37 mg/l for COD). Moreover, nitrate levels were high before treatment (18.0 mg/l, 18.0 mg/l) but decreased after treatment with laccase from *B. subtilis* (14.78 mg/l) and A. niger (14.58 mg/l).

Based on the data presented in Fig. 5b, it can be inferred that laccase produced by *B. subtilis* and *A. niger* effectively decolorizes textile wastewater. The results indicated that the 10 mL concentration of laccase produced by both *B. subtilis* and *A. niger* exhibited lower decolorization potential compared to the higher concentrations (20 mL and 30 mL). However, it was observed that *B. subtilis*-produced laccase demonstrated a higher degree of decolorization (0.72 nm) than that produced by *A. niger* (0.65 nm). Moreover, the extent of decolorization increased over time, from week 0 to week 3.

Significantly, the decolorization efficiency notably increased at the 20 mL concentration of laccase produced by both *A. niger* and *B. subtilis*, with *B. subtilis*-derived laccase exhibiting superior performance. Similarly, the application of 30 mL concentrations of laccase resulted in the highest degree of decolorization, which continued to increase from week

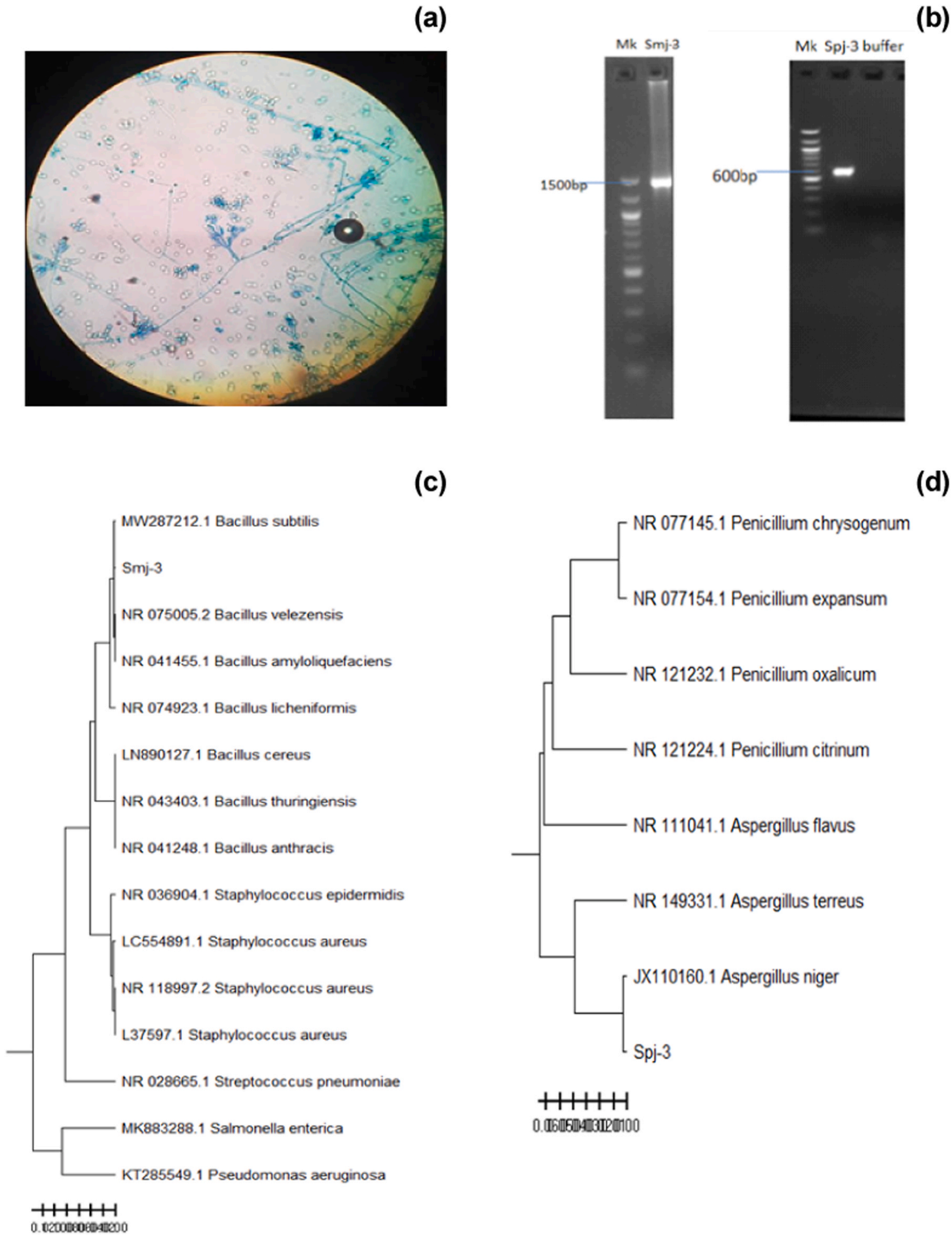

Fig. 3. Details of microorganisms used in this investigation. (a) *Aspergillus niger* morphological structure under 40 × (a) and 250 × (b) magnification (modified from Islam and Mostafa [24]). (b) Agarose gel electrophoresis of SMJ (*Bacillus subtilis*) and SPJ (*Aspergillus niger*). (c) Phylogenetic tree of *Bacillus subtilis*. (d) Phylogenetic tree of of *Aspergillus niger*.

Table 2
Summary of sequencing of *Bacillus subtilis* and *Aspergillus niger* accession numbers.

Sample ID	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Smj−3	*Bacillus subtilis*	2652	2652	99 %	0	99.59 %	OQ686745
Spj−3	*Aspergillus niger*	1011	1011	99 %	0	99.82 %	OQ686964

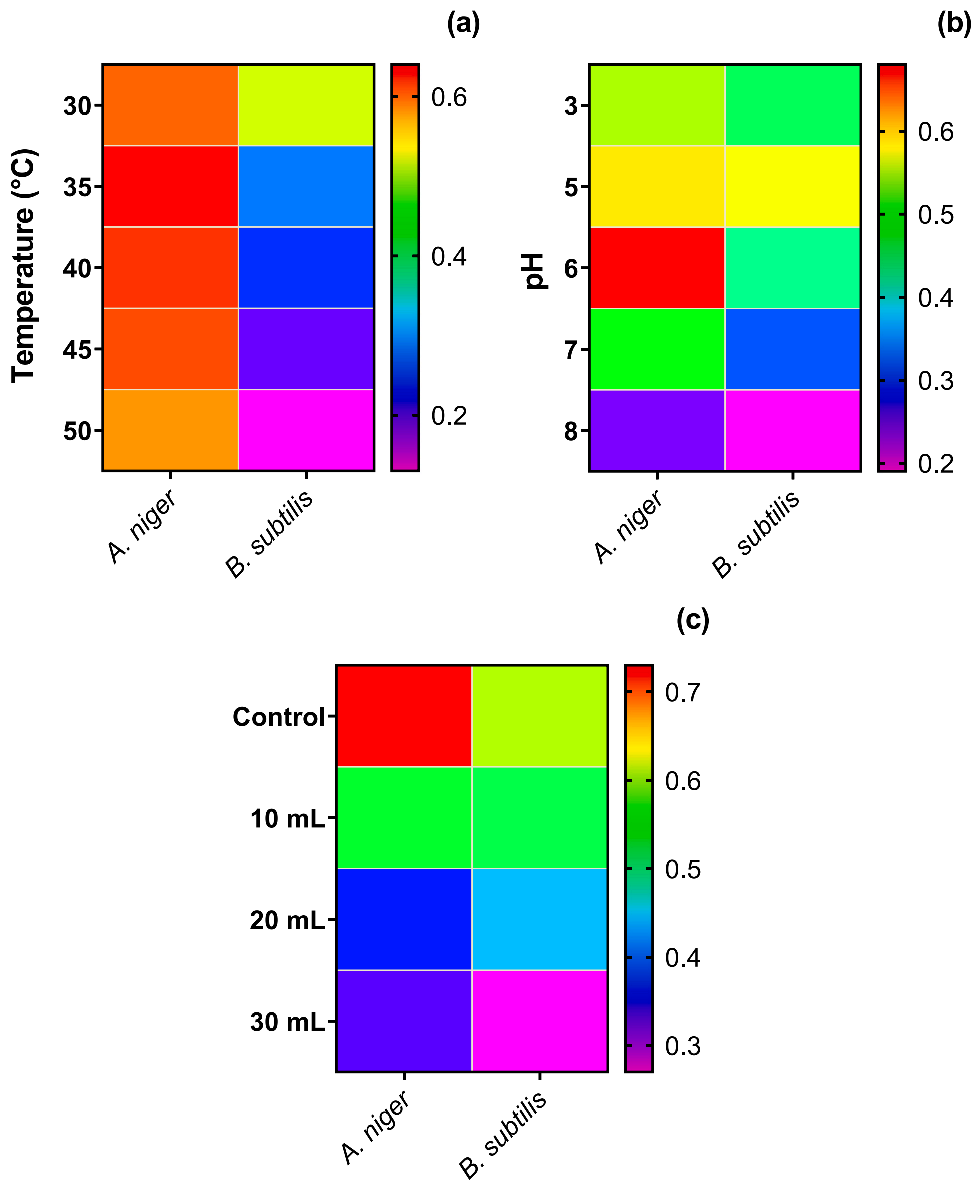

Fig. 4. Mean Effect of Temperature (a), pH (b) and inoculum size (c) on the production of laccase by *Aspergillus niger* and *Bacillus subtilis*.

0 to week 3. Notably, *B. subtilis*-derived laccase outperformed that of *A. niger* in terms of decolorization efficiency. The proportion of laccase generated by *A. niger* and *B. subtilis* that decolorizes textile effluent during a period of 0–3 weeks is shown in Fig. 5b. From Fig. 5b, it was observed that the sample containing laccase produced by *B. subtilis* showed *a* higher percentage of decolorization of textile effluents compared to the samples containing laccase produced *by A. niger,* especially at higher concentrations of *B. subtilis.* In the figure, the decolorization percentage *for B. subtilis* is represented by the color blue*,* while the decolorization percentage for *A. niger* is represented by the color red. Fig. 5b illustrates that as the concentration *of B. subtilis* increases, the blue bars (representing *B. subtilis)* show a significant increase in decolorization percentage, indicating its superior effectiveness in breaking down the textile dyes compared to the red bars *(*representing *A. niger).* This breakdown process of textile effluent is driven by the enzymes laccase. It was reported by Das et al. [26] that, microbial enzymes, such as laccase, peroxidase, azoreductase played a major role in the biodegradation of synthetic dyes which are prominently a components of textile effluents, these enzymes offer promising alternatives to conventional methods for dye removal due to their ability to efficiently break down complex dye molecules.

4. Discussion

The production of laccase for the treatment of textile effluent by *B. subtilis* and *A. niger*, isolated from soil, was the main focus of this study. The crude laccase produced was optimized with respect to pH and temperature.

4.1. Biochemical and molecular characterization of the isolates

The results of the investigation into the molecular traits (Table 1 and Fig. 1) and biochemical identification (Fig. 3) of the isolates showed that *A. niger* and *B. subtilis* were identified. This study, which examined the isolation and screening of laccase-producing fungus from sawdust-contaminated areas in Ado-Odo Ota, Ogun State, is comparable to that carried out by Akinyemi et al. [27] who also identified similar organism through similar process.

4.2. Optimization of pH and temperature for the production of laccase

Temperature and the pH were varied in this study. *B. subtilis* recorded maximum laccase production of 0.522 U/mL at optimal temperature of

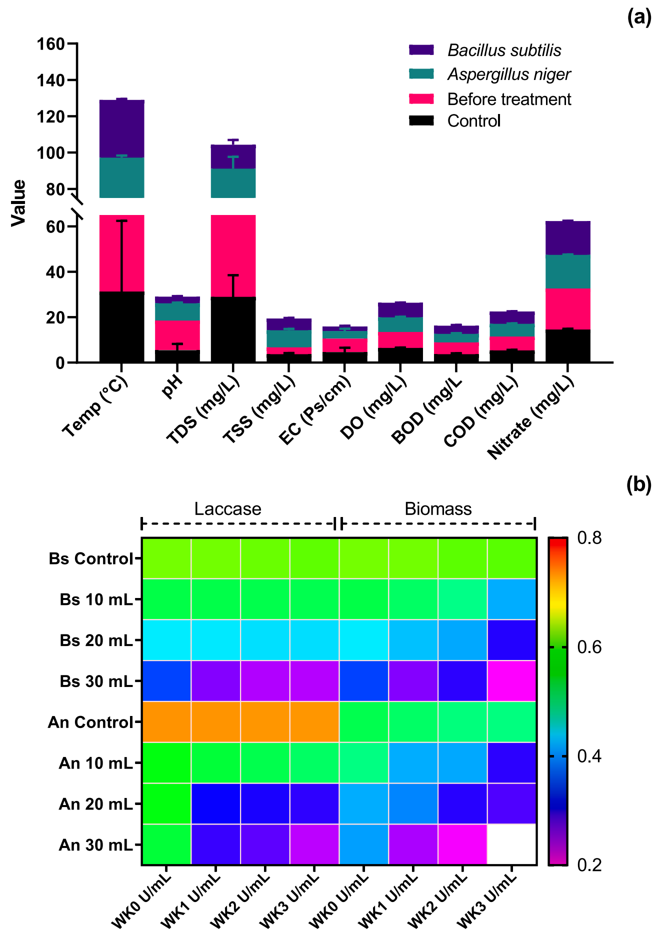

Fig. 5. Characteristics of textile effluents and action of laccase. (a) Physicochemical characteristics of textile effluents before and after treatment with laccase produced by *Bacillus subtilis* and *Aspergillus niger*. Untreated samples were considered as control. (TDS = total dissolved solid, TSS= total suspended solid, EC = electrical conductivity, DO = dissolved oxygen, BOD = biological oxygen demand, COD = chemical oxygen demand) (b) Decolorization of textile effluent by laccase produced by *B. subtilis* (Bs) and *A. niger* (An) based on enzyme concentration, shown by mosaic heatmap.

30 ºC, while *A. niger* produced 0.642 U/mL volume of laccase enzyme at optimal temperature of 35 ºC. Following the temperature (Fig. 4a) and pH (Fig. 4b) optimization on laccase production ability, 30 ºC and 35 ºC were recorded as the best respectively. *B. subtilis* and *A. niger* have the capacity to produce a significant quantity of laccase at optimum temperature. Laccase was produced at optimal pH values of 5 and 6 for *B. subtilis* and *A. niger* respectively. These values are similar with the result presented [28], whereby the scientists assessed the impact of temperature and pH on the activity of laccase produced by *Trichoderma* sp. For the phenolic substrate guaiacol, the ideal pH and temperature were found to be 4.9 at 55 ºC. In the acidic pH range of 3.0–6.5, the enzyme remained stable. Its thermal stability was also established, and it remained stable up to 40 ºC.

4.3. Decolorization of textile effluent by laccase produced by Bacillus subtilis and Aspergillus niger

The decolorization of textile effluent treated with laccase produced by *A. niger* and *B. subtilis* as shown in Fig. 5b reveals that, sample treated with laccase produced by *B. subtilis* has high absorbance value than the laccase produced by *A. niger* most especially at higher concentration of laccase in the treatment, hence it tends to have a strong decolorizing effect. This is in line with the findings of Khaleed et al. [5] who investigated laccase producing bacteria, and it was asserted that the laccase influenced high decolorization of textile azo dyes. It was concluded in their findings that the *Bacillus cereus* and *Pseudomonas parafulva* laccases are very important in azo dye decolorization.

This study is also supported by the findings of Ikram et al. [29] who conducted a study on *B. subtilis* as an efficient bacterial strain for the reclamation of water loaded with textile azo dye, orange II. The authors opined that laccase produced by *B. subtilis* should be used as a potential strain for the treatment of textile effluents containing orange II and other toxic azo dyes. Similarly, the study of Majumdar and Bhowalet [30] on production and evaluation of bio-pigment and synthetic dye decolorization capacity of laccase produced by *A. oryzae* cultivated on agro-waste, also agreed with the findings of the present study on fungi ability for treatment of textile effluent. The dye decolorization efficiency of crude *A. oryzae* laccase was studied and their findings concluded that the fungus has potential for decolorization of textile azo dye. Furthermore, the findings were found to support the opinion of Kathing and Saini [31], the authors conducted a review of various treatment methods for the removal of dyes from textile effluent, and it was asserted that adsorption and biological methods are better than other techniques due to their ability to degrade diverse classes of dye, less accumulation of harmless sludge, and cost-effective and safer approach for the disposal of textile effluent. The physical and chemical methods are expensive and generate toxic sludge, which is difficult to decompose.

4.4. Physicochemical characteristics

Fig. 5a presented the changes in the physicochemical parameters of textile effluent upon treatment with laccase produced by *A. niger* and *B. subtilis*, the temperature showed a slight decrease from 34.0°C to 33°C and 32°C after the addition of laccase from *A. niger* and *B. subtilis*, respectively. Despite variations between weeks, both laccases had a similar effect on temperature, indicating a consistent temperature maintenance during wastewater treatment. Similarly, the pH decreased from 13.1 to 7.02 and 3.14 after treatment with *B. subtilis* and *A. niger* laccase, respectively. Notably, *B. subtilis* laccase led to an acidic pH, while *A. niger* laccase tended to neutral in the wastewater.

The total dissolved solids (TDS) measurements revealed higher levels in the pre-treatment of the textile effluent, which was decreased in the post-treatment with laccase from both microorganisms. Notably, textile effluent treated with laccase produced by *A. niger* had higher TDS compared to that of *B. subtilis*. Over time, laccase production by both microorganisms contributed to the reduction in TDS, indicating the efficacy of laccase in treating the effluent.

Equally, total suspended solids (TSS) tended to decrease in the pre-treatment of the effluent but increased post-treatment with the inclusion of laccase from both microorganisms. *A. niger* laccase treatment resulted in higher TSS compared to *B. subtilis* laccase treatment, possibly it could be due to particle suspension caused by laccase action. Furthermore, increasing laccase concentration led to decreased effects on electrical conductivity and dissolved oxygen (DO), supporting previous findings by Abioye et al. [32], indicating the active role of laccase in wastewater treatment.

Similarly, treatment with laccase from both microorganisms significantly reduced biological oxygen demand (BOD) compared to untreated samples, likely due to decreased organic pollutants in the effluent. Additionally, reductions in chemical oxygen demand (COD) and nitrate levels were observed post-treatment with laccase, further indicating the efficacy of laccase in treating textile effluent. Furthermore, significant differences in pH were observed pre- and post-treatment, with *B. subtilis* laccase treatment resulting in the lowest pH compared to *A. niger* laccase treatment.

5. Conclusion

This study investigated laccase production by *B. subtilis* and *A. niger* for textile effluent remediation. Both organisms exhibited significant potential for laccase production. The study revealed that optimal laccase production occurred at a temperature of 30°C and a pH range of 5–6 for both *A. niger* and *B. subtilis*. Furthermore, the investigation highlighted the impact of laccase produced by *B. subtilis* and *A. niger* on various physicochemical parameters of the textile effluent, including temperature, pH, TSS, TDS, electrical conductivity, and DO. Additionally, the laccase treatment notably influenced parameters such as BOD, COD, and nitrate levels in the textile effluent. It was evident from the study that laccase generated by both microorganisms, particularly when applied at concentrations ranging from 10 to 30 mL, could effectively treat textile effluent. A comparative analysis between the treatment efficacy of laccase produced by *A. niger* and that of *B. subtilis* revealed a higher potential for treatment with the latter.

CRediT authorship contribution statement

Aransiola Sesan Abiodun: Data curation, Conceptualization. **Umaru Suleiman:** Writing – original draft, Validation. **Abioye Olabisi Peter:** Validation, Supervision. **Oyewole Oluwafemi Adebayo:** Visualization, Software, Resources. **Prasad Ram:** Validation, Resources, Project administration. **Maddela Naga Raju:** Writing – review & editing, Data curation.

Declaration of Competing Interest

The authors have no competing interests to declare that are relevant to the content of this article.

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Data availability

Data will be made available on request.

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