KRAFT LIGNIN DEGRADATION BY AUTOCHTONOUS STREPTOMYCES STRAINS ISOLATED FROM A TROPICAL LAGOON ECOSYSTEM

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ABSTRACT
Kraft lignin contributes to the toxicity of the pulping plant effluent and is known to resist microbial treatment. The lignin component must be removed from lignocellulose biomass to enhance the release of fermentable sugars for the production of biofuel and other value-added end products. Lignin-degrading bacteria provide an advantage due to their ease of isolation, wider tolerance of environmental conditions and genetic manipulations compared with their fungal counterparts. There is no documented evidence on the degradation of kraft lignin by bacteria in the tropical estuarine ecological niche in Nigeria. Bacterial growth and assessment of kraft-lignin degradation in submerged fermentation was carried out for a period of 10 days using Streptomyces spp isolated from a tropical lagoon as the inocula. The organisms utilized 23 to 99 % kraft-lignin at the rate of 2.3×103 to 9.9×103 g·d·cm⁻³ with specific growth rates of 0.020 - 0.084 h⁻¹ and doubling times of 8.3 - 35.1 h. Maximum values obtained for laccase and peroxidase activities were 9.5×10² and 400 μmol mg⁻¹ min⁻¹, respectively.

The aim of this study was to obtain evidences for Kraft lignin degradation by indigenous tropical estuarine Streptomyces species from Lagos, Nigeria. The Autochthonous bacterial species of the Lagos lagoon utilize kraft lignin as a sole carbon source and may be good candidates for biotechnological purposes. The outcome of this study has bridged an information gap in the tropical environment and will complement existing global data because the information on the degradation of kraft lignin by marine Streptomyces is not common.

Keywords: Kraft lignin, Streptomyces, biodegradation, enzyme, lignocellulose, estuarine, lagoon

INTRODUCTION
The Lagos lagoon is a wide expansive estuarine waters located in the South-Western part of Nigeria, which is used for water transportation and as a repository for myriads of industrial and domestic wastes. Apart from pollution by hydrocarbons and sewage (Akpatua and Ekundayo, 1978; Amund and Igiro, 1990), the lagoon is polluted by unquantifiable amounts of wood residues (sawdust) emanating from several sawmills fringing its shoreline (Akpatua, 1986). The trans-boundary incursion of the water hyacinth (Eichhornia crassipes) into the Lagos lagoon from the neighbouring creeks in Benin republic as well as plant litter from the mangrove forest also contribute significant amounts of lignocellulose to the lagoon.

Plant cell material is basically composed of cellulose, hemicellulose and lignin, the latter being the most difficult to biodegrade. Lignin is a polyphenolic structural constituent of wood and other plant materials that encrusts the cell wall and cement the cells together. It is a highly polymeric substance with a complex, cross-linked highly aromatic structure of molecular weight of about 10,000 D and derived from three phenyl propanoid units namely; coniferyl alcohol, coumaryl alcohol and sinapyl alcohol (Wong, 2008). Lignin is well known for resistance to microbial degradation because of its high molecular weight and presence of biologically-stable carbon-to-carbon ether linkages (Abd-Elsalam and El-Hanafy, 2009). In addition, it represents an obstacle to microbial digestion of structural carbohydrates; both because it is a physical barrier and because of the depressing effect on microbial activity by the constituent phenolic compounds (Antongiovann and Sargentini, 1991; Chandra et al., 2012). Kraft lignin (KL) is similar to natural lignin, and has been widely used for lignin-related studies. It is also the major by-product in the relatively high alkaline effluent (black liquor) generated by the pulp and paper industry (Yan, 2013).

Physical and chemical treatment methods or a combination of both exists for the breakdown of lignocellulosic compounds; however, these methods are costly and hazardous to the environment (El-Hanafy et al., 2008; Chandra et al., 2012). Currently, much attention is drawn to the biological methods because it appears to be a promising alternative due to their environmental friendliness. Despite its apparent resistance to degradation, microorganisms including fungi, bacteria and actinomycetes abound, which oxidatively degrade lignins (Tien and Kirk, 1983; Hernandez et al., 2001; El-Hanafy et al., 2008; Chandra et al., 2012). However, lignin degradation by microorganisms is well studied in the domain of aerobic fungi (Dehorger and Blondeau, 1992; Achi, 1994). Among them, the white rot fungi have received extensive attention due to the presence of efficient extracellular ligninolytic enzyme activities (Churphal et al., 2005). Practically, fungi are not stable under extreme environmental conditions such as high temperature and pH, extractives and lignin concentration. They are also difficult to cultivate in submerged condition and oxygen limitations. However, Lignin-degrading bacteria provide an advantage and have been reported to play a significant role in the decomposition of lignin due to their ease of isolation, wider tolerance of environmental conditions and genetic manipulations compared with their fungal counterparts (Chandra et al., 2007, 2008; Masai et al., 2007; El-Hanafy, 2008).

One of the best characterized lignin-degrading bacteria (Sphingomonas paucimobilis SYK-6) capable of growth on a wide variety of lignin-related compounds was reported by Masai et al. (2007). Chandra et al., 2007, 2008 investigated a good number of lignin-degrading bacteria belonging to the genera Serratia, Citrobacter, Klebsiella, Puenbacillus, Aneureinbacillus and Bacillus; some of them were positive in the treatment of black liquor (Raj et al., 2007). Also, two soil aromatic degrading-bacteria (Pseudomonas putida and Rhodococcus sp. RHA1), were found to possess lignin-degrading activity during two novel spectrophotometric assays for lignin breakdown (Ahmad et al., 2010). The role of fungi in the degradation of cellulose arising from sawdust pollution of the Lagos lagoon had received some research consideration (Akpatua, 1986). The role of estuarine bacterial species in this ecosystem for the degradation of lignin and cellulose components of sawdust was reported by Buraimoh et al., 2015a, whereas, there is no documented evidence on the degradation of kraft lignin by bacteria in this estuarine ecological niche in South-West Nigeria. Kraft lignin (KL) is the main contributor to the colour and toxicity of the pulping plant effluent and is known to resist microbial treatment because it has undergone alkaline sulphite treatment. Also, the lignin component has to be removed to enhance the release of fermentable sugars from lignocellulosic to achieve the production of biofuel and other value-added products (Chen and Dixon, 2007).
The discovery of novel marine lignin-degrading bacteria or their enzymes provide an advantage due to their ease of isolation, ability to survive extreme environmental conditions and amenability to genetic manipulation. The aim of our study was to obtain evidences for Kraft lignin degradation by indigenous tropical estuarine Streptomyces species of the Lagos lagoon, Nigeria, for future biotechnological application. It was also set to bridge a global information gap because the information on the degradation of kraft lignin by marine Streptomyces is not common.

**MATERIAL AND METHODS**

**Microorganisms**

The Streptomyces strains used in this study were isolated from a tropical estuarine ecosystem in Lagos, Nigeria. Isolation, screening and identification of strains for ligninolytic activity have been previously described in detail (Buraiamoih et al., 2015b). Five Streptomyces strains that showed the capability to break down lignin were selected and used for this study. They were identified previously on the basis of morphological and biochemical characteristics, electron microscopy and 16S rDNA gene sequencing as: Streptomyces alboegriselus strain AOB KP977545, Streptomyces aureus strain BOB KP977549, Streptomyces coelicolor strain COB KP977550, Streptomyces albus strain DOB KP977551 and Streptomyces pseudogriselus strain EOB KP977552. Phylogenetic analysis of the strains compared with other strains in the GenBank were analysed using Mega 6 software as previously described (Buraiamoih, et al., 2015a).

**Bacterial Growth Studies on Kraft Lignin**

A mineral salts medium (100 ml) in deionized water contained (gram per liter): KH2PO4 2.0; K2HPO4 2.0; MgSO4.7H2O 0.05; yeast extract 0.1g; MrCl.7H2O 0.0003; ZnCl2.7H2O 0.0003 and KNO3 2.0; pH 7.2, in 250 ml conical flasks supplemented with 0.1 % kraft lignin (Indulin AT, Mead Westvaco, Glen Allen, VA, USA) as sole carbon source was autoclaved (121°C, 15 min) and inoculated with 1 ml of pure cultures (inocula). Incubation was done on a rotary shaker (30°C, 150 rpm) for 10 days. Batch harvesting was done at intervals (48 h) and the content filtered using a pre-weighed sterile filter paper (Whatman No. 42). Growth of organisms was monitored by the dry weight method as described earlier (Ball et al., 1989). This is because Streptomyces species grow in mycellial chains or pellets; hence the turbidity method would not be suitable. The filtrates were used for the evaluation of kraft-lignin degradation.

**Analysis of Growth Kinetics**

Mean generation times (Td) and the specific growth rate (h-1) of the isolates on kraft lignin were calculated using non-linear regression of growth curves using prism version (Graph pad software, San Diego, CA).

**Assessment of Kraft Lignin Degradation in Culture Broth**

Lignin degradation was monitored as previously described (Abd-El Salem and El-Hanafy, 2009). Culture broth (1.0 ml) was centrifuged (10,000 g, 5 min). The clear supernatant (250 µl) was diluted by adding phosphate buffer (2.5mM, pH 7.5). Lignin content was measured at 280 nm using a UV-visible spectrophotometer (Beckman Coulter, DUR 800, Beckman Coulter Inc. Fullerton, CA). Graded concentrations of kraft lignin were obtained through dilution in series. Preparation of standard curve was done by preparing stock solution (1.0 g l-1) of kraft lignin using phosphate buffer (2.5mM, pH 7.5) as a diluent. Further dilutions were made as appropriate. A straight line curve with a correlation of 0.998 was obtained. Lignin values were extrapolated from the standard curve. The pH of the supernatant was determined at intervals (48 h) using the ADWA professional pH 67 water proof pH pocket tester (ADWA Instruments, Alsokkoto, Szeged, Hungary).

**Laccase Assay**

Culture filtrates obtained at intervals (24 h) from each of the culture flask were centrifuged (10,000 g, 4°C, 10 min). The supernatant was used as a source of enzymes. Laccase activity was measured as described earlier (Kizhekkedathu et al., 2007) by monitoring the oxidation of 2.2 – azino-bis-[3-ethylbenzothiazoline-6-sulfonic acid] (ABTS) (500 µmol) using a UV-visible spectrophotometer (Beckman Coulter, DUR 800, Beckman Coulter Inc. Fullerton, CA.) at 420 nm for 1 min. The reaction mixture (3 ml) contained: culture supernatant (1.0 ml), ABTS (1.0 ml) and 1.0 ml phosphate buffer (0.2M, pH 7.5). One unit of enzyme activity was defined as 1mM of ABTS oxidized per min. (The extinction coefficient = 3.6 x 10⁶ mol·cm⁻¹⁻¹).

**Lignin Peroxidase Assay**

Extracellular lignin peroxidase (Lip) activity was assayed as described earlier (Kizhekkedathu and Parukuttyamma, 2005) using 2,4-dichlorophenol (2,4-DCP,SIGMA -ALDRICH Co.,St.Lois, MO,USA) as the substrate. The reaction mixture (1.0 ml) contained 200 µl each of potassium phosphate buffer (0.1M, pH 7.0), 25 m mol 2,4-DCP, 16 m mol 4-amino antipyrine and culture filtrate. The reaction was initiated by the addition of 200 µl of 50 m mol hydrogen peroxide (H₂O₂) and was monitored for 1 min at a wave length of 510 nm using a spectrophotometer (Beckman coulter, DUR 800, Beckman coulter Inc. Fullerton, CA). An absorption coefficient of 21.64 M⁻¹cm⁻¹ was used for the calculation of enzyme activity.

**Protein Determination**

Protein content of crude extract was determined as described previously (Froud et al., 1996). Solution A for Protein assays contained: sodium carbonate (20g), sodium hydroxide (4 g) potassium sodium tartarate (0.2 g) dissolved in 1000ml of distilled water. Solution B (100 ml) contained: copper sulphate (0.5g). Solutions A and B were kept in the refrigerator (4°C). Solutions A and B were mixed in a ratio of 50:1. Just prior to use, Lowry and Folin- Ciocalteau phenol reagent (Sigma Aldrich) was diluted by adding 5ml of 2 N Folin- Ciocalteau's reagent to 6 ml of distilled water. Bovine serum albumin (BSA,0.05g) was dissolved in a volumetric flask using distilled water (500 ml). It was stirred thoroughly to dissolve (final concentration of 100 mg BSA (L stock) and prepared fresh 1h before the onset of the experiment. From this stock, graded concentrations of BSA were used as standards. Standard curve with a regression coefficient of 0.997 was obtained.

**RESULTS**

**Kinetcs of Kraft-Lignin Biodegradation**

Growth patterns of organisms are shown in Fig. 1. They exhibited exponential growth patterns in the first 2 days. The highest biomass (4.2 mg L⁻¹) was exhibited by strain COD. The Streptomyces species displayed varied capabilities to degrade kraft lignin (23 -99 %) within 10 days of exposure. Results are shown in figure 2.

**Figure 1** Biomass production by Streptomyces species growing on kraft lignin. A - Streptomyces alboegriselus strain AOB, B - Streptomyces aureus strain BOB, C - Streptomyces coelicolor strain COB, D - Streptomyces albus strain DOB, E - Streptomyces pseudogriselus strain EOB.

Uninoculated medium was used as control in all cases. All experiments were carried out in triplicates. The values were presented as mean values. SD: AOB-0.023, BOB-0.022, COB-0.070, DOB-0.024, EOB-0.027.

**Figure 2** Kraft lignin removal in growth cultures of Streptomyces species. A - Streptomyces alboegriselus strain AOB, B - Streptomyces aureus strain BOB, C - Streptomyces coelicolor strain COB, D - Streptomyces albus strain DOB, E - Streptomyces pseudogriselus strain EOB.

Spectrophotometric analysis of Kraft lignin degradation showed that at the end of the cultivation period, the maximum kraft lignin utilized was exhibited by *Streptomyces pseudogriseolus* strain EOB at the rate of 9.9 x 10⁻⁴ g d cm⁻³ with a specific growth rate of 0.084 h⁻¹ and a doubling time of 8.3 h. In the un inoculated control flask, no apparent decrease of the Kraft lignin content was observed, affirming that depletion of the substrate from the MSM was due to biodegradation by the strains and not due to abiotic losses (Table 1).

### Table 1 Growth kinetics data for utilization of kraft lignin in liquid cultures by *Streptomyces* species

<table>
<thead>
<tr>
<th>Strains</th>
<th>Kraft lignin degraded (%)</th>
<th>Rate of degradation (g d cm⁻³)</th>
<th>Specific growth rate (h⁻¹)</th>
<th>Doubling time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Streptomyces albogriseolus</em></td>
<td>40</td>
<td>4.0 x 10⁻⁴</td>
<td>0.029</td>
<td>23.5</td>
</tr>
<tr>
<td>strain AOB</td>
<td></td>
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<tr>
<td><em>Streptomyces aureus</em></td>
<td>23</td>
<td>2.3 x 10⁻⁵</td>
<td>0.020</td>
<td>35.1</td>
</tr>
<tr>
<td>strain BOB</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><em>Streptomyces coelicolor</em></td>
<td>65</td>
<td>6.5 x 10⁻⁴</td>
<td>0.059</td>
<td>11.7</td>
</tr>
<tr>
<td>strain COB</td>
<td></td>
<td></td>
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<tr>
<td><em>Streptomyces albus</em></td>
<td>70</td>
<td>7.0 x 10⁻⁴</td>
<td>0.019</td>
<td>37.3</td>
</tr>
<tr>
<td>strain DOB</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Streptomyces pseudogriseolus</em></td>
<td>99</td>
<td>9.9 x 10⁻⁴</td>
<td>0.084</td>
<td>8.3</td>
</tr>
<tr>
<td>strain EOB</td>
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### Laccase Activities

The time course of laccase production showed that the maximum laccase activity produced by *Streptomyces albogriseolus* strain AOB occurred by day two (9.5 x 10⁻⁴ μmol ml⁻¹ min⁻¹) after which a gradual reduction was observed. On the other hand, the maximum laccase activity of *Streptomyces coelicolor* strain COB (6.3 x 10⁻⁴ μmol ml⁻¹ min⁻¹) was observed by day 10. Results are shown in Fig.3.

### Peroxidase Activities

*Streptomyces albus* strain DOB produced the highest extracellular lignin peroxidase activity (400 μmol ml⁻¹ min⁻¹) within 48 h. Strain AOB showed the lowest activity of 90 μmol ml⁻¹ min⁻¹ (Fig.4).

### Protein Analysis

At the end of the incubation period, the total protein produced by strain AOB was 0.75 Mg L⁻¹ by day 2, and thereafter accumulated up to 0.90 Mg L⁻¹ of protein. The quantity of protein released by strains BOB, COB, DOB and EOB (Fig.5) fluctuated between 0.6 - 1.10 Mg L⁻¹ throughout the incubation period. The highest quantity of protein was synthesized in the culture by strain COB (1.10 Mg L⁻¹) by day 4, while the lowest quantity was synthesized by strain DOB by day 8.

### Discussion

Varying degradation rates and growth patterns were displayed by the *Streptomyces* species in this study. With respect to strain AOB, the growth and degradation pattern appeared to be exponential and rapid in the first 4 days, after which the percentage degradation and growth became slow but gradual till the termination of the experiment (supplementary Figs. 1 a-e). The degradation of kraft lignin by strain COB was gradual throughout the experimental period. Although there was a gradual build up of biomass by this isolate until day 4, a fluctuation in biomass was observed between day 4 and 8 after which a decline was observed till the termination of the experiment. Diaux-type degradation curves were observed with strains BOB, DOB and EOB. The percentage kraft lignin degraded in strain BOB appeared to be exponential up to day 2 (12 %,
with a biomass of 3.2 Mg L\(^{-1}\)), followed by a lag between day 2 and 4. There after, there appeared to be a rapid degradation till day 6 (23 %, biomass of 3.8 Mg L\(^{-1}\)). The organism then appeared to have stopped the utilization of the substrate until the experiment was terminated by day 10. For strain DOB, there was a gradual degradation of kraft lignin up to 40 % depending on lignin type, after which there is a reduction of rates degradation upon prolonged incubation. Marton, (1971), suggested that the reduced rate of kraft lignin degradation upon prolonged incubation might be due to the considerable chemical alteration (including sulfonation and condensation reactions) from their natural state during pulping. However, El-Hamamy et al. (2008) reported 76.3 and 67.1 % degradation of a compound which was found to be predominantly BahHAE8 respectively. They, attributed to a long adaptation period of 40 days. The authors pointed out that individual samples varied greatly in their overall rates of degradation and that lower molecular weight fractions of the kraft-lignin were decomposed at a significantly faster rate. This, according to the authors, is probably because kraft lignin had been fragmented considerably during pulping. In their study on the long-term stability of various waste lignins in a model aquatic ecosystem, Bouveng and Solym (1973) found that all the lignins examined contained a few fractions, one that was readily biodegradable in aquatic systems and a second which was more resistant to biodegradation. Buraimoh et al. (2015b) studied the in situ and laboratory degradation of natural lignin component of wood residues by lignocellulolytic bacteria over an incubation period of 6 Months. In their findings, the authors reported 22.49 % - 52.49 % degradation (laboratory study) and 52.33 - 82.52 % under in situ condition. The authors attributed several factors to the higher degradation rates under in situ conditions compared to the laboratory experiment. In this study however, it is worthy of note that Streptomyces pseudogriseolus strain EOB, Streptomyces albus strain DOB and Streptomyces coelicolor strain COB effectively degraded 99, 70 and 65 % kraft lignin respectively within 10 days of incubation in submersed systems. This is similar to the findings of Hassan and Amr (2009). They identified two Bacillus spp from Egyptian soil which degraded synthetic lignin (alkali lignin) as a sole carbon source and achieved a maximum degradation (81.4 %) on the sixth day. Also, Deschamps et al. (1980) found that Aeromomas sp had degraded 98 % of kraft lignin (indulin AT,1g L\(^{-1}\)) as the sole carbon source after five days of incubation. The low rate of degradation observed with Streptomyces aureus strain BOB is similar to the findings of Buraimoh et al. (2015b). Even though the authors reported an increase in the growth of the organisms when the inoculum was increased considerably as additional carbon source for lignolytic bacteria; Paenibacillus sp, Aneurinibacillus aneurinilyticus and Bacillus sp were reported to have degraded 37, 33 and 30 % kraft lignin (0.7g L\(^{-1}\)) respectively. Also, Monties et al. (1981), isolated 11 Gram negative aerobic soil bacteria and found that poplar dioxane lignin and milled wood lignin degradation rates ranged between 20-40 % of initial content after 7 days. Perestelo et al. (1989) suggested that the inhibitory effect of low molecular lignin fragments may be responsible for the decline in the culture growth rate usually observed during the 5th and 6th day of incubation with kraft lignin. In their report, S. marcescens grown in the presence of 0.1 % kraft lignin as sole carbon source declined until totally killed after 4 days of incubation. The authors however reported that this inhibitory effect was not present when the medium was supplemented with glucose. However, Buraimoh et al. (2015b) are of the opinion that apart from factors such as pH and temperature; nutrient and oxygen depletion and accumulation of toxic products of degradation may be implicated in the overall decline of the strain observed in the culture. The growth curve indicated that strain AOB may likely be in its stationary phase of growth and if the incubation period was extended it could probably enter into another exponential phase of growth or their growth may probably decline. The rate of substrate degradation was gradual and corresponded with biomass formation. The degradation of the substrate was greater when this organism started to decline by day 10, however, decrease in lignin degradation rate was not evident as at day 10 (supplementary Fig. 1c). This phenomenon observed with strain COB may not necessarily be the same for others. For example, the biomass produced by Streptomyces albogriseolus AOB was optimal at pH 6.5 – 7.0 respectively, but that optimum pH for lignin and cellulose solubilisation was 8.5. The slight fluctuation in pH observed in this study may be as a result of the release of some low molecular weight lignin fragments into the medium, within the period of incubation. The results showed that the quantity of total protein synthesized by the isolates not linearly correspond with enzyme activity. For example, when the concentration of protein was measured against laccase activity, Streptomyces albogriseolus strain AOB had its lowest quantity of protein (0.75 mg mL\(^{-1}\)) on day 2 and coincidentally, laccase activity was highest on the same day. Subsequently, laccase activity decreased gradually. A similar observation was made by Frolund et al. (1996). They found that Streptomyces albus strain BOB also had a biomass of 3.8 Mgl L\(^{-1}\) but degraded only 23 % of the substrate at the end of the incubation period. Likewise, the same biomass (3.8 mg L\(^{-1}\)) was recorded for Streptomyces pseudogriseolus strain EOB and Streptomyces albus strain DOB that degraded 99 and 70 % of substrates respectively. Streptomyces coelicolor strain COB which degraded 65 % of the kraft-lignin had a biomass of up to 4.2 mg L\(^{-1}\) at the end of the incubation period. It would deduce that the rate of enzyme production (which is responsible for substrate degradation) of each strain differ and may not be dependent on the growth phase. It is noteworthy also that the specific growth rate and doubling time of the organisms differ, and may be a contributing factor as observed with strain EOB which showed the highest percentage lignin degradation (99%) with the highest doubling time (400 h) while being the shortest in lag phase (Kirby, 2005). According to Kirky, (2005), the two major groups of enzymes involved in lignin degradation are peroxidases and laccases. The Streptomyces strains studied exhibited enzymatic degradation of lignin by oxidizing the lignin polymer. This is evidenced by laccase activities as herein reported. There was a variation in the maximum of enzyme activities and the enzymes secreted by the different strains. Different authors have expressed their opinion with respect to lignin degradation, some of which are similar to the findings made in this study. Crawford and Crawford (1980) were of the opinion that even though kraft lignins appeared more resistant to microbial attack than natural lignins, kraft lignins were degraded rapidly with two peaks during the first 100 - 200 h (4-8 days) depending on lignin type, after which there is a reduction of rates degradation upon prolonged incubation. Aneurinibacillus aneurinilyticus and Aneurinibacillus aerogeneus degrade at a rate of 50 % and 30 % respectively at pH 9. This is similar to the findings of Monteith and Singaracharya (2011). During their study on isolation, identification and screening of potential cellulase producing fungi, they found that enzyme activities did not correlate with biomass formation and were of the opinion that this may due to migration, molecular weight and temperature. Morii et al. (1995) however, are of a contrary opinion. They isolated Bacillus megaterium, Azotobacter and Serratia marcescens from compost soil capable of decolourizing or solubilizing lignin. S. marcescens and S. lutea were reported to produce acid phosphatase and solubilization. Chandra et al. (2011) studied the degradation and detoxification of pulp paper mill effluent (PPME) by mixed bacterial strains (Serratia marcescens, S. liquefaciens and B. cereus) in different ratio. They found that ratios 4:1:1 and 1:4:1 were effective for the degradation of PME, This is similar to the findings of the present study. According to the findings of El-Hamamy et al. (2008), the culture conditions and length of incubation. This is in line with the findings of Sladev and Singaracharya (2011). In their study on the biodegradation of kraft-lignin by Bacillus sp (AY392465) isolated from the sludge of a pulp and paper mill, the authors found that an increase in the pH during the growth of the strain till it reached an alkaline pH, did not alter the culture growth significantly. Pommeto III and Crawford (1986) studied the effects of pH on lignin and cellulose degradation by Streptomyces viridochromas and reported that mineralization of lignin and cellulose to CO\(_2\) was optimal at pH 6.5 – 7.0 respectively, but that the optimum pH for lignin and cellulose solubilisation was 8.5. The slight fluctuation in pH observed in this study may be as a result of the release of some low molecular weight lignin fragments into the medium, within the period of incubation. The results showed that the quantity of total protein synthesized by the isolates not linearly correspond with enzyme activity. For example, when the concentration of protein was measured against laccase activity, Streptomyces albogriseolus strain AOB had its lowest quantity of protein (0.75 mg mL\(^{-1}\)) on day 2 and coincidentally, laccase activity was highest on the same day. Subsequently, laccase activity decreased gradually. A similar observation was made by Frolund et al. (1996). They found that Streptomyces albus strain BOB also had a biomass of 3.8 Mgl L\(^{-1}\) but degraded only 23 % of the substrate at the end of the incubation period. Likewise, the same biomass
Also, the relevant proteins may lose their activity with time. Moreover, different organisms also produce different proteins with varied optimum temperatures and pH which may have effects on their activities.

CONCLUSION
The autochthonous bacterial species of the Lagos lagoon utilized kraft lignin considerably as a sole carbon source. Marine bacteria are known to produce enzymes of high biotechnological value. They could play a major role in the bioremediation of the lignin-polluted water of the lagoon, thereby solving an ecological problem. They could be enhanced for biotechnological purposes for the production of value-added products such as ethanol from lignocellulosic wastes. This work has bridged an information gap in the tropical environment and will complement existing global data. Factors such as variation of growth conditions, concentration of the substrates, the bacterial strain and their ability to synthesize the required enzyme may be implicated in the variation of lignin degradation rate, and as such further work would be carried out on the optimization and characterization of the enzymes produced from each of the organisms. In addition to this, the detection of gene mediating enzyme production in the genome of the respective strains would be examined.

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