International Biodeterioration & Biodegradation 104 (2015) 186–193

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

journal homepage: www.elsevier.com/locate/ibiod

Assessment of bacterial degradation of lignocellulosic residues (sawdust) in a tropical estuarine microcosm using improvised floating raft equipment

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ARTICLE INFO

Article history:
Received 15 October 2014
Received in revised form
10 June 2015
Accepted 10 June 2015
Available online xxx

Keywords:
Floating raft
Microcosm
Estuarine
Sawdust
Lignocellulose
Biodegradation

ABSTRACT

In situ and laboratory studies were carried out to determine the ability of bacterial strains isolated from a tropical lagoon to degrade lignin and carbohydrate components of sawdust, with a view to abating the impact of sawdust pollution on these ecosystem. A floating raft system was designed and fabricated to carry out the *in situ* biodegradation studies over a period of 24 weeks. Nine bacterial strains identified by 16S rRNA gene sequencing as species of *Streptomyces*, *Bacillus* and *Paenibacillus* isolated from the lagoon were used as seed organisms. In the *in situ* study, 59.2% of sawdust was depleted at the rate of 1.175×10^{-4} g d^{-1} cm^{-3} by the bacterial isolates, whereas the lignin component of the sawdust decreased by up to 82.5% at the rate of 1.80×10^{-5} g d^{-1} cm^{-3}. The maximum decrease in carbohydrate content was 85% at the rate of 2.192×10^{-7} g d^{-1} cm^{-3}. In a similar experiment under laboratory conditions, total weight losses ranging from 26 to 51% in the wood residues were observed.

1. Introduction

Indiscriminate dumping of sawdust into water bodies is a common practice in Nigeria. A typical example is found at the Oko Baba sawmills at the Ebute-Metta axis of the Lagos lagoon water front (Nigeria), where wood shavings and sawdust are dumped into the lagoon or destroyed by burning at the shoreline. Lagos, a commercial city in Nigeria is close to the rain forest zone which makes it a natural destination of wood obtained from felled trees. Timbers are transported along the creeks and lagoons to various sawmills on the shores of Lagos as floating rafts, while houses constructed with wood occupied by native fishermen lie within the lagoon, both serving as major sources of lignocellulose within the lagoon. Lignocellulosic "wastes" such as grasses, sawdust, paper, sugarcane bagasse and corncobs pose a threat to the environment. Sadly, much of these lignocellulosic wastes are often disposed of by burning even though the chemical properties of their components make them substrates of enormous biotechnological value (Howard et al., 2003). The high sulphur content of wood may result in the formation of sulphur dioxide during incineration, thereby aggravating air pollution and degradation of air quality in the vicinity. Other likely problems associated with this sawdust-polluted lagoon include addition of nutrients leading to algal bloom, enhancement of microbial growth leading to increased oxygen demand and development of anoxic conditions resulting in the death or migration of aquatic macroorganisms.

Sawdust originates from plant materials and is composed of cellulose, hemicellulose and lignin, hence the term, lignocellulose. The three components bind together, forming complex polymers. Hemicellulose and lignin are structural reinforcement around the cellulose which must be removed before cellulose is hydrolysed (Hamelinck et al., 2005). Lignocellulosic wastes are abundant and are renewable, hence there has been a great deal of interest in their usage for the production and recovery of many value-added products (Pandey et al., 2000; Howard et al., 2003; Alemdar and Sain, 2008; Dong et al., 2011). Fungal and bacterial communities

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http://dx.doi.org/10.1016/j.ibiod.2015.06.010
0964-8305/

produce hydrolytic enzymes capable of breaking down the major components of lignocellulose. They can be found in mesophilic as well as thermophilic ecosystems where plant matter is abundant (Himmel et al., 2010). Biodegradation of lignocellulosic feedstocks has been reported for both anaerobic and aerobic systems (Neves et al., 2006; Hu et al., 2008). However, aerobic systems are better in terms of degrading lignocellulosic wastes richer in lignin contents (Mshandete et al., 2005). An important food source contributing to the food web is the estuarine detrital biomass resulting from the biodegradation of lignocellulosic materials in the ecosystem (Benner and Hodson, 1985; Mtiu and Nakamura, 2008).

During an aerobic and anaerobic incubation of (^{14}C) labelled mangrove leaf and wood material with slurry consisting of decaying leaf material, homogenized with unfiltered water collected from the creek estuary in Andros Island, Bahamas, Benner and Hodson (1985) reported that the leachable fraction from the mangrove leaves was mineralised relatively rapidly and was assimilated into microbial biomass with an efficiency of 30%, while the rate of mineralization of the lignocellulosic component of mangrove leaves and wood was 10- fold lower. The polysaccharide component of the lignocelluloses was mineralized at a rate 2 times higher than rates of mineralization of the lignin component. The authors also reported that the lignocellulolytic degradation potential of mangrove detritus in the sediments from a mangrove swamp and a salt marsh were similar, but that lignocellulose from the mangrove is less biodegradable than lignocellulose from salt marsh plants (*Spartina alterniflora* and *Tuncus roemerianus*). Umasaravanan et al., (2011) isolated *Aspergillus tamari* from marine drift wood pieces with the ability to ferment sugarcane baggase and rice straw. The authors also reported that the fermentation of unfortified baggase by *A. tamari* for 21 days resulted in 12.63% utilization of cellulose and 13.2% of lignin, while fermentation of unfortified rice straw over the same period resulted in 6.2% utilization of cellulose and 29.5% of lignin. Also, Padmavathi et al. (2013) studied the degradation of lignocellulose biomass using marine microorganisms collected from Tamil Nadu and Karnataka seacoast. The authors reported that *Bacillus pumilus* was capable of oxidizing lignin from 12 substrates including sugarcane, rice straw, paper and maize leaf.

In nature, the most abundant aromatic compounds are the lignins. Attention is currently drawn to lignins as natural resources because their decomposition generates a diversity of monomers that have many applications in the food, pharmaceutical, cosmetics and other chemical industries. The enzymes for the degradation of cellulose and hemicellulose belong predominantly to the hydrolases which cleave glycosidic bonds, while the major groups of enzymes involved in lignin degradation are peroxidases, phenol oxidases, including the polyphenol oxidases and laccases, most of which are co-factor-dependent oxidoreductases. (Kirby, 2005; Martínez et al., 2005).

Major lignin biodegradation metabolites include coumaryl, coniferyl and sinapyl alcohols, most of which are degraded by side chain shortening to yield protocatechuic acid or catechol, that are further broken down via specific ring cleavage pathways. For example, *Pseudomonas, Acinetobacter*, *Rhodococcus* and *Streptomyces* species have been found to utilize the β-ketoadipate pathway, initiated by intradiol catechol dioxygenase, protocatechuate 3, 4-dioxygenase (Nishimura et al., 2006; Glazer and Nikaido, 2007).

Reports are available on the degradation of lignocellulose biomass by marine fungi (Mtiu and Nakamura, 2008; Umasaravanan et al., 2011) but the degradation of lignocelluloses by marine bacteria is little known. Akpata (1980), isolated four fungal spp (*Aspergillus flavus*, *Aspergillus giganteus*, *Cladosporium oxysprum* and *Trichoderma aureoviride*), from the Lagos lagoon, which were tested for spore germination in aqueous sawdust extract of different hardwood species (*Khaya ivorensis*, *Mitragyna ciliata* and *Triplochiton scleroxylon*). Also, Chinedu (2007) isolated 8 cellulolytic microfungi from wood wastes obtained from Okobaba sawmill, Ebutte-Metta fringing the lagoon. The author reported that *Aspergillus niger* 1 was the best cellulase producer when sawdust and sugarcane pulp were used as the sole carbon source. However, there has been no report on the bacterial degradation of lignocellulosic wastes in the Lagos lagoon. This study reports the use of floating rafts for the assessment of lignocellulose-degrading potential of tropical lagoon bacteria with a view to abating the impact of sawdust pollution on these ecosystem.

2. Materials and methods

2.1. Collection of samples

The sawdust of *Uapaca heudelotii* (local name: Akun) obtained from a sawmill at OkoBaba, Ebute–Metta, Lagos, Nigeria (Grid Co-ordinates: N6° 29′ 22.3″; E 003° 23′ 24.8″) was stored in polythene bags. The sawdust was rinsed with distilled water, dried in an oven (60 °C for 48 h) after which it was ground with a hammer mill, sifted with a fine wire mesh (0.2 mm) and stored in clean sterilized containers. This was used for *in situ* and laboratory experiments.

For the isolation of bacterial cultures, decomposing sawdust was collected from the lagoon at Oko Baba sawmill at Ebute–Metta axis of Lagos Mainland fringing the lagoon (Co-ordinates: N 6° 29′ 21.8″; E 003°, 23′ 29.3″) in sterile sample bottles, carefully labelled and stored in the refrigerator at 4 °C, before processing within 24 h. Water samples (1.0 L each) were collected on the 28th day of every month (January to December, 2011) from the lagoon experimental site at the University of Lagos lagoon end for physicochemical analysis.

2.2. Isolation of microorganisms

Decomposing sawdust (1.0 g) was serially diluted. Aliquots (0.1 ml) were inoculated on sterilized (121 °C, 15 min) Starch-Casein agar (pH 7.2) containing: soluble starch (1.0 g), K_2HPO_4 (2.0 g), KNO_3 (2.0 g), $MgSO_4.7H_2O$ (0.05 g), $CaCO_3$ (0.02 g) Casein (0.3 g), NaCl (2.0 g), $FeSO_4.7H_2O$ (0.01 g), Agar (15.0 g), Cycloheximide (100.0 mg), deionised water (1000 ml) and aerobically incubated (28 °C) for at least 5 days. Pure isolates were stored on Starch-Casein agar slants at 4°C.

2.3. Screening for lignocellulose degraders

Sterile filter papers were aseptically placed on the surface of freshly prepared sterile Starch-Casein agar plates. Each pure culture was then streaked on the surface of the filter paper. The plates were incubated (28 °C) for at least 5 days. Isolates were selected based on their abilities to breakdown the filter paper. Screening for the utilization of aromatic acids was carried out on minimal agar medium (pH 7.2) containing 1.0 g L^{-1} aromatic acids (vanillic or veratric acid), trace elements (1.0 ml), phosphate buffer and the pH indicator bromothymol blue as described previously (Nishimura et al., 2006). The catabolism of the aromatic acids resulted in increased pH of the medium, which can be seen visually by a change of colour from green (pH 7.2) to blue (pH > 7.2).

2.4. Cultural and morphological characteristics of bacterial isolates

Cultural attributes of isolates were observed visually on Starch-Casein agar plates and using a hand lens. Cellular morphology was observed by the use of an epiflourescence light microscope.

2.5. Scanning electron microscopy

The samples used for scanning electron microscopy (SEM) were prepared by the method of Bozzola and Russell (1999). The surfaces of spores and spore chains were examined by (SEM) using a Hitachi S-3500N model electron microscope (ThermoNaran, Hitachi technologies, American Inc).

2.6. Biochemical characteristics of organisms

Catalase reaction, oxidase test, nitrate reduction, starch, cellulose and casein hydrolysis, and liquefaction of gelatin were studied as described by Lanyi (1988). Pure cultures of bacterial isolates were identified according to the identification scheme of Bergey's Manual of Determinative Bacteriology (Holt et al., 1994).

2.7. Amplification and identification of bacterial isolates by 16S rDNA gene analysis

Total bacterial genomic DNA from each primary isolate was extracted from 1.0 ml of 48 h culture in Luria Bertani broth using the master pure Gram positive DNA kit (www.epicentre.com). 16S rDNA gene sequences of isolates were amplified by PCR using bacterial universal primers (Relman, 1993) and the protocol of Schuller et al. (2010). The universal primers supplied by integrated DNA technologies (IDT) were: Forward primers, universal 1 (UI) 5′ − ACG CGT CGA CAG AGT TTG ATC CTG GCT - 3′, universal 2 (U2) 5′ − CGC GGA TCC GCT ACC TTG TTA CGA CTT - 3′, universal 3 (U3) 5′ − AGT GCC AGC AGC CGC GGT AA - 3′, universal 4 (U4) 5′ − AGG CCC GGG AAC GTA TTC AC − 3′, universal 5 (U5) 5′ − TCA AAK GAA TTG ACG GGG GC − 3′ (K = G + T).

Reverse primer, short universal 1 (UIR) 5′ − CGA CTA CCA GGG TAT CTA AT - 3′. For culture isolates, primers were used in the following combinations to amplify the l6S rRNA gene − U1 + U2, U3 + U4, U5 + U4. The DNA amplification was performed using a T-gradient thermocycler. Initial denaturation was at 95 °C for 15 min, followed by 30 cycles of denaturation at 96 °C for 15 s, an annealing temperature of 55 °C for 1 min 30 s, elongation at 72 °C for 2 min and a final extension step at 72 °C for 10 min.

2.8. Agarose gel electrophoresis of DNA fragments

PCR-amplified DNA segments were separated by electrophoresis in a 2% agarose gel, using 100 bp DNA marker (Promega, USA) as DNA standard, *Pantoe agglomerans* DNA (positive control), Millipore water (blank) was used as negative control and ethidium bromide was used as the stain. Gel was run for 80 min at 100 V. The amplified products were observed under the Kodak fluorescent imaging equipment, model IS 4000R (Kodak image station, care stream molecular imaging health inc. Rochester, NY, USA).

2.9. 16S rDNA sequencing

Purification of PCR products was carried out using PCR clean-up system wizard SV Gel (Promega, Madison, W1, USA). Quantification of the nucleic acid was carried out using a Nanodrop 1000 Spectrophotometer (Nanodrop technologies, Wilmington, DE, USA.). Sequencing of the 16S rDNA was performed using the dye terminator cycle capillary sequencing (ABI 3100x1) at the Molecular and Cellular Imaging Centre, Ohio Agricultural Research Development Centre (OARDC), Wooster, Ohio, USA. The 16S rDNA gene sequences of isolates were edited (bio edit), aligned (mega align) and analyzed using a BLAST.

2.10. Physicochemical analysis of lagoon water

The physicochemical properties of lagoon water were measured over a twelve-month period, using standard methods. The pH, temperature, electrical conductivity and total dissolved solids were measured using the ADWA Professional IP67 Waterproof Pocket Testers (ADWA instruments, Alsokikoto, Szeged, Hungary). Salinity, dissolved oxygen, biological oxygen demand, phosphate concentration, total solids and heavy metal ions were analysed using standard methods as described earlier (Ademoroti, 1996).

2.11. In situ floating raft microcosm studies

A three-dimensional box with a volume of 18,000 cm^3 (L = 52 cm B = 34 cm H = 10 cm) was constructed with Perspex glass with drilled perforated holes on the upper and lower plates. The diameter of the holes were to size and hold the sterilizable plastic, screw-cap sample bottles which contained weighed amounts (1.0 g) of sawdust. The space between the upper and lower plates was stuffed with polystyrene foam (Styrofoam) to achieve floatation of the Perspex box in the lagoon; hence the floating raft experimental design. The base of the screw cap at the open end of the bottle was removed and replaced with a membrane filter (0.2 micron) to allow movement of lagoon water and ions in and out of the tube chamber while retaining the sawdust and microbial inocula in the bottle and thus preventing influx of microbes from the environment. Each bottle contained 1.0 g of sawdust of *U. heudelotii* (Akun), sterilized by autoclaving at 121 °C for 15 min before inoculation with 1.0 ml of microbial culture fluid (10^9 cells ml^{-1}) followed by insertion of the bottle in three-dimensional box. The experimental site was an inner creek, of the Lagos lagoon behind the Faculty of Science complex, at the University of Lagos. Inoculated sawdust was exposed to the environment over a period of 24 weeks. Total weight, lignin and carbohydrate losses in the sawdust residues harvested from the set-up were measured at intervals of 2 weeks. Uninoculated bottles served as controls.

2.12. Laboratory degradation studies on wood dust

Sawdust samples (2.0 g) were weighed into separate 250 ml conical flasks containing 100 ml of mineral salts medium [KH_2PO_4 (2.0 g), K_2HPO_4 (2.5 g), $MgSO_4.7H_2O$ (0.05 g), NaCl (2.0 g) $MnCl.7H_2O$ (0.0003 g), $ZnCl.7H_2O$ (0.0003 g), yeast extract (0.5 g), deionised water (1000 ml)] as the sole carbon source. Autoclaving was achieved at 121 °C for 15 min while 1.0 ml of 48 h-old cell culture of selected bacterial isolates were introduced into each of the flasks while the flasks were plugged appropriately with sterile non-absorbent cotton wool and agitated continuously (150 rpm, 30 °C). Harvesting was done every 2 weeks to determine the weight, lignin and carbohydrate losses.

2.13. Measurement of sawdust degradation

2.13.1. Weight loss determination

Harvesting was done by withdrawing a bottle from the *in situ* floating raft microcosm set-up and a flask from laboratory set-up. The contents were filtered using pre-weighed filter paper Whatman No 42, oven-dried to constant weight (65 °C, 48 h). Final weights were obtained by subtracting the weight of the filter-paper (Deschamps et al., 1981).

2.13.2. Lignin evaluation in degraded wood residues

Lignin was extracted from wood residues (5 mg) using acetylbromide (25%) in glacial acetic acid (24 ml), hydroxylamine hydrochloride (7.5 ml) and NaOH (4.5 ml, 2 M) as described previously

(Van Zyl, 1978). Lignin was monitored at 280 nm using a Spectrophotometer (Spectra Max PLUS 384, molecular device Corp. Sunnyvale, California USA). Graded concentrations of kraft lignin (Indulin AT, Mead Westvaco, Glen Allen, VA,USA) were obtained through dilution in series. Standard curves were established with a stock solution (1.0 g L^{-1}) of kraft lignin using 20% NaOH (0.1 N) in deionised water as diluent. Further dilutions were made as appropriate. A straight line curve with correlation coefficient of 0.998 was obtained. Lignin values were extrapolated from the standard curve.

2.13.3. Estimation of carbohydrate contents

The Anthrone reagent used was prepared as described previously (Trevelyan et al., 1952). Anthrone reagent (BDH chemicals, 0.2 g) was dissolved in 100 ml of H_2SO_4 made by adding 500 ml of concentrated acid to 200 ml of water. The reagent was allowed to stand for 40 min with occasional shaking until it was clear. The reagent was freshly prepared each day and used within 12 h.

Anthrone reagent (4.0 ml) was pipetted into thick-walled Pyrex tube (150 × 25 mm) and chilled in iced water. One millilitre (1.0 ml) of the supernatant from the sawdust under test was layered on the acid, cooled for a further 5 min and then mixed thoroughly while still immersed in ice. The tubes were loosely covered with corks, heated in a vigorously boiling constant level water bath for 10 min and then cooled in water for 5 min. The measurements of test solutions and of reagent blanks were made against water as a reference. Absorption spectra were determined using a spectrophotometer at 620 nm. The amount of total sugars released was extrapolated from the standard curve.

3. Results and discussion

3.1. Cultural, morphological, biochemical and genotypic characterization of bacterial isolates

Twenty-five bacterial strains were isolated from decomposing sawdust in the Lagos lagoon. All selected strains were Gram positive. Strains AOB, BOB, COB, DOB, EOB and FOB appeared woolly on the light microscope while strains NOB, OOB and ROB were Gram positive rods. Strains AOB, BOB and COB appeared tough, leathery, greyish with powdery whitish surface, fruity/earthy smell and produced black melanin-like pigments.

Scanning electron micrographs showed that strains AOB, BOB, COB, DOB, EOB and FOB on the Starch-Casein agar appeared like filamentous-bacteria (Fig. 1). All isolates were catalase and oxidase positive with the exception of strain NOB (Table 1). All isolates were able to hydrolyse starch, gelatin and casein except strains OOB and ROB which could not hydrolyse casein. All isolates were lactose fermenters except strain ROB. The isolates were able to produce gas from glucose except strain OOB.

The 16S rDNA genes of strains AOB, BOB, COB, DOB, EOB, FOB, NOB OOB and ROB were sequenced (1519, 1492, 1522, 1514, 1492, 1388, 1508, 1540 and 1503 bp respectively) and had been assigned the following GenBank accession numbers; KF977548, KF977549, KF977550, KF977551 KF977552, KF977553, KF97754, KF977555 and KF977556 respectively. Evidences from 16S rDNA gene sequence analyses in the GenBank database (using BLAST) showed that the 16S rDNA genes of strains AOB and COB had 100% similarities to *Streptomyces albogriseolus* GQ925802 and *Streptomyces coelicolor* HQ848084 respectively, while the 16S rDNA genes of strains NOB and ROB are similar to those of *Bacillus megaterium* L2S3 EU221414 and *Paenibacillus* sp. HQ-5 by 98 and 99.8% respectively (Table 2).

Several authors have previously isolated lignocellulolytic bacterial species from various ecosystems. Njoku and Antai (1987) isolated six *Streptomyces* strains from decaying plant materials that were able to degrade guinea grass over a period of 12 weeks. Also, Padmavathi et al. (2013) isolated *Bacillus pumilus* from coastal area of Tamil Nadu, India, which exhibited maximal degradation of various lignocellulosic biomass for a period of 60 days, while *Paenibacillus* species were previously isolated by Maki et al. (2011) from paper sludge which completely broke down filter paper within 72 and 96 h of incubation.

3.2. Physicochemical characteristics of lagoon water

The physicochemical properties of the lagoon water at the *in situ* experimental site showed that the mean values for some of the key parameters were within the locally and internationally acceptable regulatory limits. However, the mean values for electrical conductivity (11737.75 mg L^{-1}), total dissolved solids (4907.083 mg L^{-1}), copper (22.64 μg L^{-1}) and lead (1.39 μg L^{-1}) were higher than the Nigerian local (Federal Environmental Protection Agency) and international (World Health Organization) standards. This may not be out of place since the Lagos lagoon is a repository for other wastes including sewage, industrial and domestic effluent and hydrocarbons. The higher values are most likely to be an indication of a polluted water body. However, the salinity level of the lagoon indicated a typical estuarine environment (Table 3).

3.3. In situ and laboratory studies of lignocellulose degradation

In situ degradation studies showed that *S. albogriseolus* AOB and *Bacillus bataviensis* FOB brought about the highest total weight loss of 59 and 59.2% respectively in sawdust. Up to 82.52% of lignin content was degraded at the rate of 1.80 × 10^{-5} g d^{-1} cm^{-3} by *Bacillus megaterium* NOB (Table 4), whilst 58–85 % of carbohydrate contents were utilized by the bacterial isolates at the rates of 1.496 × 10^{-7} to 2.192 × 10^{-7} g d^{-1} cm^{-3}. In Table 5, laboratory studies revealed that the highest total weight loss of sawdust residues was by *Bacillus megaterium* strain NOB. Caracciolo et al. (2013) evaluated the natural bioremediation of contaminated ecosystems using microcosm experiments. The authors stressed the importance of experimental conditions in the natural attenuation capabilities of microbial communities on contaminants. Several physico chemical factors such as species of litter, varying composition and proportion of lignin and polysaccharides and other plant matters, temperature, pH, salinity, nitrogen content, submersion and agitation have been reported to limit lignin and cellulose degradation by microorganisms (Benner et al., 1985; Valiela et al., 1985; Pometto and Crawford, 1986).

Heald (1971) observed a higher rate of weight loss from mangrove leaves contained in litter bags suspended in sea water than litter bags suspended in low salinity water. Benner and Hodson (1985), reported that biodegradation of mangrove lignocellulose was higher in samples from stations with salinities ranging from 15 to 24% compared to the low salinity (4%) station. White and Trapani (1982) suggested that submersion time may be a determining factor in microbial decomposition of lignocelluloses, whereas Valiela et al. (1985) are of the opinion that rates of degradation are not increased by submersion time.

According to Newell (2002), bacteria may be favoured over fungal decomposition in environments where physical agitation occurs such as the water bodies, a scenario relevant to the Lagos lagoon. The author also reported that the differences in the mineralization rates could be a reflection of the differences in the variation in size and diversity of the lignocellulolytic microbial population, inorganic and organic nutrients concentration and lignin-nitrogen ratio. They reported a higher lignin-nitrogen ratio which led to more resistance in *Spartina alterniflora* lignocelluloses.

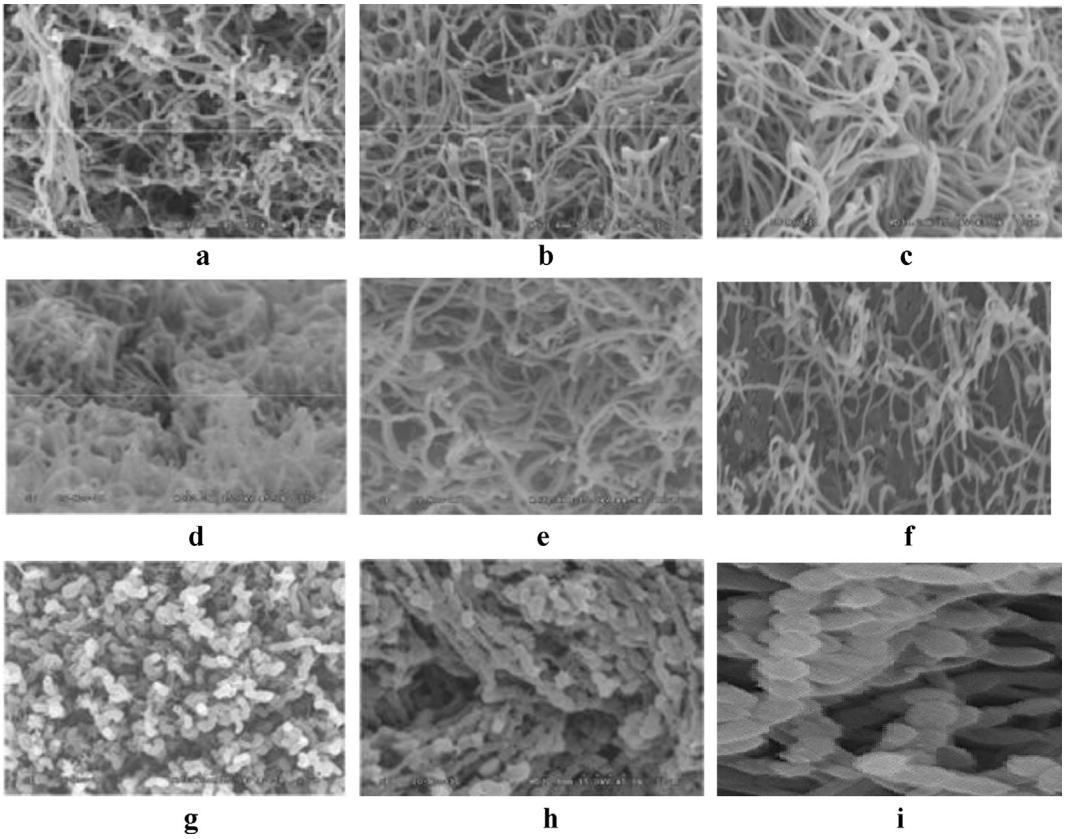

Fig. 1. Scanning electron micrographs of: (a) *Streptomyces albogriseolus* AOB KF977548 (×3.5K) (b) *Streptomyces aureus BOB* KF977549(×3.5K) (c) *Streptomyces ceolicolor* COB KF977550 (×5.0K) (d) *Streptomyces albus* DOB KF977551 (×5.0K) (e) *Streptomyces pseudogriseolus* EOB KF977552 (×4.5K) (f) *Bacillus bataviensis* FOB KF977553 (×4.5K) (g) *Bacillus megaterium* NOB KF977554 (×2.5K) (h) *Bacillus* sp. OO BKF977555 (× 2.5K) (i) *Paenibacillus* sp. ROB KF977556 (×10.0K).

Gallagher et al. (1976) reported that the variation in dissolved organic carbon released from plant biomass was due to temperature fluctuations, rainfall, and frequency of tidal inundation, age of leaf/plant and photosynthetic rates of leaves. In their experiment using litter bags of submerged plants, higher rates of plant wastes weight loss have also been reported by White and Trapani (1982) at higher temperatures.

3.4. Statistical data analysis

The statistical analysis using chi-square tests showed that there were significant differences between the total weight losses, lignin and carbohydrate degraded under *in situ* and laboratory conditions. For weight losses, the data obtained (*in situ)* differed significantly from laboratory data at 0.01 level of significance (41.311 > 21.7). The lignin and carbohydrate *in situ* degradation data also differed significantly from laboratory data at 0.05 level of significance (276 > 15.5 and 142.4 > 15.5 respectively). This validates the observed higher rate of degradation of the lignocellulosic residues under *in situ* conditions.

The higher rate of wood degradation under *in situ* environmental conditions as compared to the laboratory situation could be attributed to a couple of reasons. The *in situ* set up operated like a continuous culture system which allowed the replacement of spent

Table 1
Physiological and biochemical properties of bacterial isolates.

Properties	AOB	BOB	COB	DOB	EOB	FOB	NOB	OOB	ROB
Gram reaction	+	+	+	+	+	+	+	+	+
Pigmentation (melanin-like)	+	+	+	−	+	−	−	−	−
Earthly smell	+	+	+	+	+	+	−	−	+
Starch hydrolysis	+	+	+	+	+	+	+	−	+
Cellulose hydrolysis	+	+	+	+	+	+	+	−	+
Gelatin liquefaction	+	+	+	+	+	+	+	−	−
Catalase	+	+	+	+	+	+	+	+	W+
Nitrate Reduction	+	+	+	+	+	+	−	−	W+
Casein hydrolysis	+	+	+	+	+	+	+	−	−
Appearance on starch casein −agar	Grey	Grey	GWPS	Cream	GWS	WS	GCYS	WSS	WRS
Lactose fermentation	+	+	+	+	+	+	+	+	+
Oxidase	+	+	+	+	+	+	−	+	+
Arabinose	+	−	+	+	+	+	+	−	+
Xylose	+	−	+	+	+	+	+	−	+
Gas production from glucose	+	−	+	+	+	+	+	−	+

+ = positive; = negative; W+ = weakly+; GWPS = greyish white powdery surface; GCY = glossy cream -yellow surface; W = whitish surface; WS = white smooth surface; WRS = white rough surface.

Table 2
Genotypic identities of lignocellulose-degrading bacterial isolates from amplified sequences of 16S rDNA fragment of genomic DNA.

Bacterial strain	Closest relative	% Identity
AOB (KF977548)	*Streptomyces albogriseolus* ABRIIW EA1145 (GQ925802.1)	100
BOB (KF977549)	*Streptomyces aureus* 3184 (EF371429)	99
COB (KF977550)	*Streptomyces coelicolor* WBF-16 (HQ848084)	100
DOB (KF977551)	*Streptomyces albus* NBRC 13689 (AB249915)	95
EOB (KF977552)	*Streptomyces pseudogriseolus* S41 (HQ850412)	98
FOB (KF977553)	*Bacillus bataviensis* LMG 21832 (AJ542507)	98
NOB (KF977554)	*Bacillus megaterium* L2S3 (EU221414)	98
OOB (KF977555)	*Bacillus* sp. LS29 (FJ937891)	97
ROB (KF977556)	*Paenibacillus* sp. HQ-5 (AM162315)	99.8

Parenthesis – GenBank Accession number.

Table 3
Physico-chemical properties of Lagos lagoon water at the *in situ* experimental site. (Jan–Dec 2011).

Parameter	Range	Mean values	Nigerian local standard	W.H.O standard
Turbidity (FTU)	2.0–35.0	9.56	N/A[a]	–[b]
Temperature (°C)	25–30	26.7500	<40	<40
pH	6.53–7.7	7.1325	6.0–9.0	6.0–9.0
Electrical conductivity ($\mu S\ cm^{-1}$)	436.0–35600.0	11737.75	2000	2000
TDS ($mg\ l^{-1}$)	162–17,700	4907.083	2000	2500
TSS ($mg\ l^{-1}$)	5.0–18.0	11.167	30	<100
Acidity ($mg\ l^{-1}$)	8.0–48.0	21.50	N/A	–
Alkalinity ($mg\ l^{-1}$)	100–1090	216.67	N/A	>20
Total Hardness ($mg\ l^{-1}$)	98–4460	1923.33	N/A	–
Calcium Hardness ($mg\ l^{-1}$)	52–2400	621.5	N/A	–
Chloride (mgl^{-1})	204–1600	763.5	600	<1000
Dissolved Oxygen ($mg\ l^{-1}$)	2.7–6.2	7.0333	8–10	8–10
Nitrate ($mg\ l^{-1}$)	1.25–100.2	17.331	20	<50
Phosphate ($mg\ l^{-1}$)	00.5–6.81	2.6716	5.0	–
Nitrite ($mg\ l^{-1}$)	1.25–100.2	28.885	N/A	–
Salinity (‰)	0.36–24.60	8.5083	600	600
BOD_5 ($mg\ l^{-1}$)	3.0–15.0	7.47	50	30–300
COD ($mg\ l^{-1}$)	5.0–25.0	12.49	40	<200
Copper ($\mu g\ l^{-1}$)	0.27–86.20	22.64	<1.0	<1.0
Lead ($\mu g\ l^{-1}$)	0.06–3.18	1.39	0.05	<1.0
Zinc ($\mu g\ l^{-1}$)	3.84–136.50	59.86	1.00	–

N/A [a] = Not available; – [b] = Varied between countries, WHO–World Health Organization.

nutrients and provided continuous aeration due to tidal wave activities. The system prevented the accumulation of toxic products of metabolism while the microbial cells were probably maintained in a steady state at the exponential phase.

The Laboratory setup in this study on the other hand operated as a batch system, which allowed depletion of nutrients and oxygen, leading to cell death arising from accumulation of toxic products of metabolism. Amer and Drew (1980) alluded that aeration is an important limiting factor in the flask cultures as lignin degradation is an aerobic process.

Other factors such as temperature fluctuation, rainfall, changes in tidal movement, activities of other living components within the ecosystem and nature of human and industrial discharges and activities probably did influence on the rate of degradation as observed in the *in situ* microcosm study. According to Turner (1993), some organic and inorganic forms of carbon, phosphorus, sodium, manganese, iron, and zinc are released into water by water plants and leaves, some of which may be able to penetrate the micron filters and may have acted as inducers in the production of lignocellulolytic enzymes by the bacterial species in the *in situ* experiment. Since the Lagos lagoon is a repository for industrial, agricultural, and other discharges, the nature of the discharges may have had an impact on the degradation rates of lignocellulose. The *in situ* study was carried out between October 2010 and March

Table 4
Weight losses and bioremoval of lignin and carbohydrates components of wood residues under *in situ* conditions.

Organism	Weight loss		Lignin degraded		(%) carbohydrate utilization	
	%	$g\ d^{-1}\ cm^{-3}$	%	$g\ d^{-1}\ cm^{-3}$	%	$g\ d^{-1}\ cm^{-3}$
Streptomyces albogriseolus AOB KF977548	59	1.171×10^{-4}	58.7	1.28×10^{-5}	81	2.089×10^{-7}
Streptomyces aureus BOB KF977549	42.4	8.41×10^{-5}	77.48	1.69×10^{-5}	79	2.038×10^{-7}
Streptomyces coelicolor COB KF977550	55.2	1.095×10^{-4}	79.9	1.74×10^{-5}	80	2.063×10^{-7}
Streptomyces albus DOB KF977551	50.2	9.96×10^{-5}	81.7	1.78×10^{-5}	80	2.063×10^{-7}
Streptomyces pseudogriseolus EOB KF977552	28.3	5.62×10^{-5}	77.4	1.69×10^{-5}	69	1.780×10^{-7}
Bacillus bataviensis FOB KF977553	59.2	1.175×10^{-4}	75.02	1.64×10^{-5}	85	2.192×10^{-7}
Bacillus megaterium NOB KF977554	52.2	1.036×10^{-4}	82.52	1.80×10^{-5}	81	2.089×10^{-7}
Bacillus sp. OOB KF977555	46.3	9.19×10^{-5}	52.49	1.15×10^{-5}	58	1.496×10^{-7}
Paenibacillus sp. ROB KF977556	46.9	9.31×10^{-5}	52.33	1.14×10^{-5}	67	1.728×10^{-7}

Table 5
Summary of laboratory degradation studies of isolates on wood residues.

Organism	Weight loss		Lignin degraded		(%) carbohydrate utilization	
	%	g d^{-1} cm^{-3}	%	g d^{-1} cm^{-3}	%	g d^{-1} cm^{-3}
Streptomyces albogriseolus AOB KF977548	49.6	5.9×10^{-5}	47.51	1.037 x 10^{-6}	58	4.488×10^{-8}
Streptomyces aureus BOB KF977549	45.4	5.4×10^{-5}	44.98	9.817×10^{-7}	50	3.869×10^{-8}
Streptomyces coelicolor COB KF977550	46	5.48×10^{-5}	44.99	9.821×10^{-7}	48	3.714×10^{-8}
Streptomyces albus DOB KF977551	50.5	6.01×10^{-5}	44.99	9.821×10^{-7}	59	4.565×10^{-8}
Streptomyces pseudogriseolus EOB KF977552	49.7	5.92×10^{-5}	47.51	1.037×10^{-4}	50	3.869×10^{-8}
Bacillus bataviensis FOB KF977553	50.3	5.99×10^{-5}	50	1.091×10^{-6}	52	4.024×10^{-8}
Bacillus megaterium NOB KF977554	51.3	6.11×10^{-5}	52.49	1.146×10^{-6}	58	4.488×10^{-8}
Bacillus sp. OOB KF977555	25.95	5.19×10^{-3}	22.49	4.909×10^{-7}	28	2.167×10^{-8}
Paenibacillus sp. ROB KF977556	31.9	3.8×10^{-5}	22.49	4.909×10^{-7}	40	3.095×10^{-8}

2011, a period of low rainfall, when there is the likelihood of increased pollution and higher concentration of nitrates, and other ions in the water. Increased nitrate in the water during this period may encourage algal bloom and rapid growth of other water plants. Consequently, the nitrogen content of water may increase; hence, the degradation rates of lignocelluloses may be enhanced.

Sherr et al. (1982) reported that the presence of detritus-feeders, such as *Littorina* snails and other animals in the aquatic ecosystem negatively impact microbial decomposition of lignocelluloses. In this *in situ* study however, it is very unlikely that the rate of decomposition was influenced by detritus feeders since the set up used were sealed with micron filters which were still intact at the time of harvest.

4. Conclusions

This study clearly demonstrated the biodegradation of lignocellulosic wastes by bacteria in an estuarine setting. The outcome is an indication that an *in situ* microcosm approach improved the conversion of the sawdust substrate. The rate of wood component degradation by the autochthonous bacterial species was also established.

Acknowledgements

We appreciate the molecular and cellular imaging centre, Ohio Agricultural Research Development Centre (OARDC), The Ohio state University, (Wooster Campus), USA, for the sequencing of DNA. We thank Andrea Kaszas of the microscopy unit for help with the scanning electron microscopy. Thanks to Dr. Thaddeus Ezeji of the same University, for a critique of the manuscript.

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