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

Olanike M. Buraimoh [a, b, *], Matthew O. Ilori [a], Olukayode O. Amund [a], Frederick C. Michel Jr. [b], Sukhbir K. Grewal [b]

[a] Department of Microbiology, University of Lagos, Akoka, Yaba, Lagos, Nigeria
[b] Department of Food, Agricultural and Biological Engineering, The Ohio State University, Wooster, OH 44691, USA

ARTICLE INFO

Article history:
Received 15 October 2014
Received in revised form
10 June 2015
Accepted 10 June 2015
Available online 20 June 2015

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

* Corresponding author. Department of Microbiology, University of Lagos, Akoka, Yaba, Lagos, Nigeria.
E-mail addresses: marianiks@yahoo.com (O.M. Buraimoh), olusojiilori@yahoo.com (M.O. Ilori), kay_amund@yahoo.com (O.O. Amund), michel.36@osu.edu (F.C. Michel), sukhbirgrewal.9@gmail.com (S.K. Grewal).

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.