



MICROBIAL ANALYSES OF BIODEGRADING LIGNOCELLULOSIC TISSUES FROM LEFT-OVER *BAMBUSA* *VULGARIS* STUMPS IN SOME LOCATIONS WITHIN SELECTED CONTIGUOUS STATES IN NIGERIA

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ABSTRACT: After harvesting and removal of bamboo stems, their stumps are left behind on site and subsequently exposed to several agents of biodegradation. Thus, it is considered important to be constantly acquainted with these agents in order to be prepared for certain eventualities whenever there may be the need for necessary interventions. This research was conducted by sampling biodegrading bamboo lignocellulosic tissues from left-over stumps in five locations respectively for Anambra, Delta and Edo States, Nigeria. The microbial organisms in these tissues were purely cultured and isolated using Sabouraud Dextrose, Potato Dextrose and Nutrient Agars in line with standard procedures at room temperature of $35\pm2^{\circ}\text{C}$. The pure isolates of the prevailing microbes were identified using biochemical, cultural and microscopic characteristics. The colony-forming unit (CFU) were also estimated for all the micro-organisms cultured at the same room temperature of $35\pm2^{\circ}\text{C}$. The identified purely isolated microbes in the biodegrading bamboo samples were collectively identified as fungi (*Alternaria spp.*, *Aspergillus spp.*, *Fusarium spp.*, *Penicillium spp.* and *Trichoderma spp.*) and bacteria (*Acidobacteria spp.*). Cumulatively in the three states, the microbial count, based on CFU, for *Alternaria spp.* ranged from 0.1×10^5 to 1.5×10^5 CFUml⁻¹ while it ranged from 1.5×10^5 to 8.7×10^5 CFUml⁻¹ for *Aspergillus spp.*. It ranged from 3.0×10^5 to 3.8×10^5 CFUml⁻¹ for *Fusarium spp.* while it was between 0.1×10^5 and 4.0×10^5 CFUml⁻¹ for *Penicillium spp.*. In addition, the only value for *Trichoderma spp.* in a location within Sapele Local Government Area was 22.2×10^5 CFUml⁻¹ while that for *Acidobacteria spp.* in the three states ranged from 0.2×10^5 to 10.8×10^5 CFUml⁻¹. These were the microbial organisms present in samples of biodegrading lignocellulosic tissues sourced from the bamboo stumps within the study area as at the time the experiments being reported were conducted. Consequently, it was found necessary that they should be documented while more advanced studies are recommended to improve knowledge.

Keywords: Biodegradation, Bamboo stumps, Micro-organisms, Lignocellulose, Microbial count

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1. INTRODUCTION

The literature has it that the stems of many woody bamboo species are valuable lignocellulosic material that have found usefulness in many applications ranging from simple traditional to high value-added industrial uses (Sekar *et al.* 1998; Gielis 2002; Nordin *et al.* 2007; He *et al.* 2013). This multiplicity of bamboo usefulness has necessitated the need for their sustainable sourcing from natural and planted stands. Whatever the source (from the wild or planted), there are stumps, almost always left behind on site, after harvesting the stems (except in rare case of complete extraction). In view of

the fact that bamboo culms possess poor natural durability against agents of biodegradation such as certain insects and micro-organisms, it should not be unexpected that the stumps left behind after bamboo harvest will be unprotected against the agents of biodegradation present in these sites.

It is implicit in the foregoing that these left-over stumps may be invaded by some micro-organisms, at different stages, for different purposes. Nevertheless, the assumption is that these stumps are not useful after the extraction of appreciable length of bamboo culm, thus, the left-over stumps can be allowed to rot away on site.

This position may appear reasonable on one hand but on the other, for instance, when considering setting up new bamboo plantation or replanting harvested sites, in order to ensure success, part of the information that will be needed must have to do with potential and prevalent pests and diseases in that locality. The means by which this can be ascertained is through properly conceptualised and executed research. This is part of what led to earlier similar researches such as Erakhrumen (2012) and Erakhrumen (2014) on bamboo culms.

The published studies cited earlier had been on harvested bamboo culms, however, it is also necessary that researches, particularly those that have to do with microbial colonisation, be conducted on left-over stumps. This is not only because these stumps might serve as reservoir for certain micro-organisms that may threaten the survival of new bamboo plantations but also that this kind of studies will assist in cataloguing these microbes in such ways that will serve as source of first-hand information for identifying and managing them. In line with this thinking, this research, aimed at identifying and documenting the prevalent micro-organisms in some bamboo stumps, was conducted in locations within Anambra, Delta and Edo States, Nigeria. Lignocellulosic tissues were obtained from biodegrading stumps in five different Local Government Areas in each of the states.

2. MATERIALS AND METHODS

2.1. Brief description of the locations where samples were sourced

The samples of biodegrading tissues from left-over stumps were obtained from locations in each of the selected five Local Government Areas (LGAs) in Anambra, Delta and Edo States, Nigeria. The three states share some forms of contiguity in terms of boundary delineations. In specific details, Delta State has land and other boundaries with both Anambra and Edo States. Variously published records have it that Anambra State has an annual rainfall and temperature that vary from 1400mm to 2500mm and 20°C to 35°C respectively and falls within the tropical rainforest vegetation zone with relative humidity that can be up to 81%. Delta State, which falls mainly within the mangrove forest vegetation zone, has an annual rainfall that varies between 2400mm and over 4000mm with temperature varying from 26°C to 32°C and relative humidity of up to 95%.

Furthermore, Edo State that falls within the moist rainforest vegetation zone has an annual rainfall that vary from 1500mm to 2500mm with a temperature

range of 24°C to 32°C and relative humidity that can also be up to 95%. The names of the specific locations having the left-over bamboo stumps from which samples of biodegrading lignocellulosic tissues were sourced are:

(a). Anambra State: Idemili North, Idemili South, Nnewi North, Nnewi South and Oyi LGAs.

(b). Delta State: Ethiope West, Okpe, Oshimili South, Sapele and Uvwie LGAs.

(c). Edo State: Egor, Ikpoba-Okha, Oredo, Ovia North and Uhunmwonde LGAs.

2.2 Collection of biodegrading bamboo samples from left-over stumps

Biodegrading bamboo's lignocellulosic tissues, from left-over stumps, were obtained from the different locations between the 3rd and 18th of March, 2017. This was done by aseptically obtaining samples of the tissues from the left-over stumps that show physical outward manifestations of biodegradation to the naked eye. These samples were immediately transferred differently into properly labelled chemically clean sealed polythene bags ensuring their protection from external contamination. This procedure was adopted in sourcing the samples in all the fifteen locations. Subsequently, all the samples were moved to the laboratory for further processing and analyses.

2.3. Laboratory analyses

2.3.1. Preparation of culture media

The culture media used for the experiments were Sabouraud Dextrose Agar (SDA), Potato Dextrose Agar (PDA) and Nutrient Agar (NA). This is to ensure that most of the micro-organisms present in the biodegrading tissues, as at the time of the study, were cultured, isolated and properly identified. These culture media were carefully prepared at ambient room temperature of 35±2°C in line with adapted laboratory procedures earlier described and adopted in studies by Erakhrumen (2012), Erakhrumen (2014), Erakhrumen and Okon (2015) and Erakhrumen and Boyi (2016).

Each culture medium was subsequently poured into aseptically clean culture plates using a lamina flow hood with its surface cleaned with cotton wool dipped in methylated spirit. The mouths of the conical flasks in which the culture media were prepared were flamed to free them from contaminants. Twenty (20) ml of each the culture media were poured into fifteen (15) different sterile plates making a total of forty-five (45) plates for

the three types of culture media. They were immediately covered with cling film and sealed with a masking tape to prevent contamination.

2.3.2. Culturing of micro-organisms from biodegrading bamboo samples

Samples from all the biodegrading bamboo tissues from the different sites were obtained using sterile forceps and placed into the three-culture media in the different culturing plates in such a way that each sample was placed differently in the three media (SDA, PDA and NA). The plates were again covered with cling film to protect them from being contaminated and kept in a laboratory incubator at a temperature of $35\pm2^{\circ}\text{C}$. The culturing plates were monitored for microbial growth with records kept daily for seven days. The processes of culturing the micro-organisms were carried out in the laboratory within the Faculty of Agriculture, University of Benin, Benin City, Nigeria.

2.3.3. Isolation, purification, identification and microbial count of cultured micro-organisms

In order to obtain a pure culture of the differently grown micro-organisms for proper and reliable identification, the microbial organisms cultured and grown in the first set of Petri dishes were sub-cultured by aseptically inoculating the different corresponding growth medium with the micro-organisms in another new set of sterilised Petri dishes. The isolated and pure cultured micro-organisms were subsequently identified based on characteristics obtained from biochemical tests such as gram staining, citrate, oxidase, indole production, urea, colonial and morphological characteristics for bacterial isolates in line with Barnet and Hunter (1998).

Identifications for fungal isolates was based on the colour of aerial hyphae and substrate mycelium, arrangement of hyphae, conidial arrangement as well as morphology in line with Erakhrumen (2012), Erakhrumen (2014), Erakhrumen and Okon (2015) and Erakhrumen and Boyi (2016) based on the classical methods of identifying these organisms. It is worth to note that efforts were made to prevent false identification, even as the differences in the morphological features among related species were difficult to distinguish due to the level of sophistication of the methodologies adopted. The colony-forming unit (CFU) used in estimating the number of viable bacterial/fungal cells in a cultured sample were estimated by serial dilutions, plating and counting of live organisms to determine their number in a given population using equation 1 below:

$$\text{Number of CFU} \times [\text{Volume plated (ml)} \times \text{total dilution used}]^{-1} = \text{Number of CFU ml}^{-1} \quad \text{----- (1)}$$

3. RESULTS

The results from the experiments aimed at identifying all the purely cultured micro-organisms from all the stumps sourced from the study area are in Table 1. The table showed that *Alternaria spp.* was identified in all the samples from stumps in the three states using SDA. In addition, the outcome of the microbial identification for organisms grown using PDA had it that *Aspergillus spp.* and *Penicillium spp.* were common in the three states with *Trichoderma spp.* and *Fusarium spp.* present in some of the locations within Delta and Edo State respectively while the only micro-organism, a bacterium, isolated and identified in all the locations using NA was *Acidobacteria spp.*

Table 1 also have the CFU counts for all the isolated and identified micro-organisms from all the tissues from stumps in the three states. These values, cumulatively in the three states, ranged from 0.1×10^5 to 1.5×10^5 CFU ml $^{-1}$ for *Alternaria spp.* In addition, those for *Penicillium spp.* ranged from 0.1×10^5 to 4.0×10^5 CFU ml $^{-1}$ while those for *Aspergillus spp.* ranged from 1.5×10^5 to 8.7×10^5 CFU ml $^{-1}$. *Fusarium spp.* had values ranging from 3.0×10^5 to 3.8×10^5 CFU ml $^{-1}$ with the only value for *Trichoderma spp.* in a location within Sapele LGA being 22.2×10^5 CFU ml $^{-1}$. Furthermore, the values obtained for *Acidobacteria spp.* in all the states ranged from 0.2×10^5 to 10.8×10^5 CFU ml $^{-1}$.

4. DISCUSSION

It was observed that all the microbial organisms isolated and identified within the limit of this study were either fungal or bacterial species. These outcomes appeared to be in alignment with the almost customary view that biodegradation of lignocellulosic tissues occurs mainly as a result of the activities of the species belonging to these groups (fungi and bacteria) perhaps because only a few of them – particularly fungi – have the natural ability to perform this specific function (Shigo 1984; Mohanan 1994; Leininger *et al.* 2001; Schwarze 2007; Selvam *et al.* 2012; Erakhrumen and Okon 2015; Erakhrumen and Boyi 2016). Similar to this is that there appeared to be a sort of similarity in the type of bacterial species present in stumps within the sampled locations within the three states. The reason(s) for this observation may not be apparent from this study; however, this bacterial species appeared to be the one prevalent during this stage of stump biodegradation in these sites.

Availability of microbial flora in the form of fungal and bacterial species and their growth variations with time during the different decomposition stages of bamboo stumps has been documented in some studies such as Li *et al.* (2015). There are assumptions of microbial presence in decomposing biological matter but what cannot be assumed are the microbial types, conditions favouring them and their activities including their sources. For instance, in this study, the source(s) of these microbial species is/are not known owing to the limit of the study. Nevertheless, many of them have been found to be air-borne, soil inhabiting and/or endophytic and might have been involved in triggering the development of early stages of stump decay sometimes in combination with other, mostly unknown, triggering factors (Schwarze *et al.* 2000; Baum *et al.* 2003; Eichorst *et al.* 2007; Saikonen 2007; Sieber 2007; Oses *et al.* 2008; Ruangsanka 2014; Yuan *et al.* 2015).

Since bamboo stumps are assumed to be useless after harvesting, they are left to rot away on site thereby contributing to the balance in the dynamics of biomass accumulation and its breakdown resulting in maintaining the recycling of carbon and minerals (Schwarze 2007; Siripong *et al.* 2009). This natural phenomenon, of course, has its role in biodiversity maintenance but there is the need to keep updating the database concerning the various microbes and other organisms contributing to this natural occurrence in different localities so that those that may pose danger in certain instances are identified for necessary intervention efforts. For example, the micro-organisms identified in this study are mostly different from those in bamboo and other lignocellulosic tissues in this and other parts of the country in earlier similar studies reported by Erakhrumen (2012), Erakhrumen (2014), Erakhrumen and Okon (2015) and Erakhrumen and Boyi (2016).

Irrespective of the differences in the types of microbial organisms isolated from the different samples of lignocellulosic materials in the published studies cited earlier, the outcome of this research being reported, regarding fungi, appeared to be in line with those of Wu *et al.* (1994), Zhao *et al.* (1994), Wu and Wen (2000) and Ma and Jiang (2009) where the dominant fungal genera they identified from samples of bamboo culms and bamboo-products from some locations within China were *Trichoderma*, *Aspergillus*, *Penicillium*, *Fusarium*,

Alternaria and *Botryodiplodia*. These similarities do not foreclose the possibility for differences in fungal species owing to difference in climate, geographical area, and bamboo species. It is noteworthy that these fungi are classified as either mould, stain or rot fungi. Freshly cut bamboo culms are susceptible to moulds and stain fungi due to the relative high content of carbohydrates in the tissue and the high moisture content.

The various microbial counts obtained for the differently cultured fungal and bacterial species in this research indicate that they possess the ability to vigorously multiply within a short period under suitable environmental and other necessary conditions for their activities as also observed for some other fungal and bacterial species in another reported study by Erakhrumen and Boyi (2016). This observation, being that for experimental condition, is likely to also be that for natural situation. This natural phenomenon may be overlooked when the lignocellulosic tissues being invaded and biodegraded are those within the left-over stump not intended for any practical use. However, this information becomes one to be taken note of, and necessary corrective measure put in place, if this invasion might be deleterious to other nearby standing bamboo culms and future plantations including harvested culms for applications requiring good strength/mechanical properties as these microbial activities are known to impact negatively on bamboo's strength/mechanical properties.

5. CONCLUSION

1. As at the time this study was conducted it was observed that the microbial organisms within samples of biodegrading left-over stumps in the study area were mainly five fungal groups identified to genus level as *Alternaria spp.*, *Aspergillus spp.*, *Fusarium spp.*, *Penicillium spp.* and *Trichoderma spp.* and one bacterial group as *Acidobacteria spp.*
2. These identified microbes appeared to be similar in terms of their presence in all the fifteen Local Government Areas from which biodegrading lignocellulosic tissue samples were obtained.
3. Owing to possibilities of emerging trends in the future it was therefore recommended that more similar studies, with advanced techniques, should be carried out to improve knowledge in this area.

Table 1: Isolated and identified micro-organisms obtained from tissues of biodegrading bamboo stumps within the study site

Source of biodegrading bamboo tissues		Culture medium	Cultured Micro-organism	Microbial count (CFU ml ⁻¹)	Family	Kingdom
State	Location					
Anambra	Idemili North	SDA	<i>Alternaria spp.</i>	0.9 x 10 ⁵	Pleosporaceae	Fungi
	LGA	PDA	<i>Aspergillus spp.</i>	1.5 x 10 ⁵	Trichocomaceae	Fungi
		NA	<i>Acidobacteria spp.</i>	0.3 x 10 ⁵	Acidobacteriaceae	Bacteria
Anambra	Idemili South	SDA	<i>Alternaria spp.</i>	0.9 x 10 ⁵	Pleosporaceae	Fungi
	LGA	PDA	<i>Penicillium spp.</i>	2.0 x 10 ⁵	Trichocomaceae	Fungi
		NA	<i>Acidobacteria spp.</i>	0.6 x 10 ⁵	Acidobacteriaceae	Bacteria
Anambra	Nnewi North	SDA	<i>Alternaria spp.</i>	0.1 x 10 ⁵	Pleosporaceae	Fungi
	LGA	PDA	<i>Penicillium spp.</i>	4.0 x 10 ⁵	Trichocomaceae	Fungi
		NA	<i>Acidobacteria spp.</i>	0.3 x 10 ⁵	Acidobacteriaceae	Bacteria
Anambra	Nnewi South	SDA	<i>Alternaria spp.</i>	0.11 x 10 ⁵	Pleosporaceae	Fungi
	LGA	PDA	<i>Penicillium spp.</i>	2.5 x 10 ⁵	Trichocomaceae	Fungi
		NA	<i>Acidobacteria spp.</i>	0.8 x 10 ⁵	Acidobacteriaceae	Bacteria
Anambra	Oyi LGA	SDA	<i>Alternaria spp.</i>	1.5 x 10 ⁵	Pleosporaceae	Fungi
		PDA	<i>Penicillium spp.</i>	1.3 x 10 ⁵	Trichocomaceae	Fungi
		NA	<i>Acidobacteria spp.</i>	0.2 x 10 ⁵	Acidobacteriaceae	Bacteria
Delta	Ethiope West	SDA	<i>Alternaria spp.</i>	0.3 x 10 ⁵	Pleosporaceae	Fungi
	LGA	PDA	<i>Aspergillus flavus</i>	2.3 x 10 ⁵	Trichocomaceae	Fungi
		NA	<i>Acidobacteria spp.</i>	1.7 x 10 ⁵	Acidobacteriaceae	Bacteria
Delta	Okpe LGA	SDA	<i>Alternaria spp.</i>	0.9 x 10 ⁵	Pleosporaceae	Fungi
		PDA	<i>Penicillium spp.</i>	0.1 x 10 ⁵	Trichocomaceae	Fungi
		NA	<i>Acidobacteria spp.</i>	0.2 x 10 ⁵	Acidobacteriaceae	Bacteria
Delta	Oshimili South LGA	SDA	<i>Alternaria spp.</i>	0.3 x 10 ⁵	Pleosporaceae	Fungi
		PDA	<i>Penicillium spp.</i>	0.5 x 10 ⁵	Trichocomaceae	Fungi
		NA	<i>Acidobacteria spp.</i>	1.2 x 10 ⁵	Acidobacteriaceae	Bacteria
Delta	Sapele LGA	SDA	<i>Alternaria spp.</i>	0.4 x 10 ⁵	Pleosporaceae	Fungi
		PDA	<i>Trichoderma spp.</i>	22.2 x 10 ⁵	Hypocreaceae	Fungi
		NA	<i>Acidobacteria spp.</i>	0.4 x 10 ⁵	Acidobacteriaceae	Bacteria
Delta	Uvwie LGA	SDA	<i>Alternaria spp.</i>	0.9 x 10 ⁵	Pleosporaceae	Fungi
		PDA	<i>Penicillium spp.</i>	1.3 x 10 ⁵	Trichocomaceae	Fungi
		NA	<i>Acidobacteria spp.</i>	0.6 x 10 ⁵	Acidobacteriaceae	Bacteria
Edo	Egor LGA	SDA	<i>Alternaria spp.</i>	0.4 x 10 ⁵	Pleosporaceae	Fungi
		PDA	<i>Aspergillus spp.</i>	8.7 x 10 ⁵	Trichocomaceae	Fungi
		NA	<i>Acidobacteria spp.</i>	10.8 x 10 ⁵	Acidobacteriaceae	Bacteria
Edo	Ikpoba-Okha	SDA	<i>Alternaria spp.</i>	0.3 x 10 ⁵	Pleosporaceae	Fungi
		PDA	<i>Fusarium spp.</i>	3.0 x 10 ⁵	Nectriaceae	Fungi
		NA	<i>Acidobacteria spp.</i>	0.6 x 10 ⁵	Acidobacteriaceae	Bacteria
Edo	Oredo LGA	SDA	<i>Alternaria spp.</i>	0.3 x 10 ⁵	Pleosporaceae	Fungi
		PDA	<i>Penicillium spp.</i>	2.5 x 10 ⁵	Trichocomaceae	Fungi
		NA	<i>Acidobacteria spp.</i>	3.1 x 10 ⁵	Acidobacteriaceae	Bacteria
Edo	Ovia North	SDA	<i>Alternaria spp.</i>	0.3 x 10 ⁵	Pleosporaceae	Fungi
		PDA	-----	-----	-----	-----
		NA	<i>Acidobacteria spp.</i>	0.3 x 10 ⁵	Acidobacteriaceae	Bacteria
Edo	Uhunmwonde LGA	SDA	<i>Alternaria spp.</i>	0.2 x 10 ⁵	Pleosporaceae	Fungi
		PDA	<i>Fusarium spp.</i>	3.8 x 10 ⁵	Nectriaceae	Fungi
		NA	<i>Acidobacteria spp.</i>	0.9 x 10 ⁵	Acidobacteriaceae	Bacteria

SDA = Sabouraud Dextrose Agar NA = Nutrient Agar

PDA = Potato Dextrose Agar

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