Products of spontaneous biodegradation of saw dust.

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Abstract

The aim of this research was to determine if natural microorganisms indigenous to sawdust can degrade the sawdust waste and also to determine the products of the degradation. Two types of sawdust, fresh dry and wet decomposing samples were collected from timber market in Calabar. Serial dilutions of the wet sawdust were prepared and inoculated onto the following media, nutrient agar, MacConkey agar, Sabouraud dextrose agar and CLED agar using pour plate and spread plate techniques and incubated at 37^0C for 24 hours. The sawdust biodegradation was carried out using two grams each of the sawdust placed in 3 test tubes and incubated at 37^0 C for 21 days. The products of the sawdust fermentation were analyzed using wet chemistry and GC-MS methods. Several bacteria and fungi were isolated from the wet sawdust such as *Bacillus* sp, *Serratia Marcescens, Micrococcus* sp, *Pseudomonas aeruginosa, Streptococcus* sp, *Asperigillus niger, Rhizopus* sp and *Penicillium* sp. It was observed that products of the degradation of the sawdust were ethanol 1.98%, protein 4.37% and glucose 3.16% while the GC-Ms analysis showed the presence of 1H-pyrazole, 3,4-dimethyly (c) 36.65%, Glycine, N Cyclopropylcarbonyl-methyl ester 21.9%, propanoic acid, 2,2-Dimethyl-,2- Ethylhexyl ester 11.7%, Thiophene Tetrahydro 5.98%, Benzaldehyde 5.04% pentanoic acid 2-Ethylhexyl ester 4.49%, 2(5H)-furanone 4.48% and Tetrahydro 4.22%. It is therefore important to know that microorganisms indigenous to sawdust have been demonstrated in this research to be effective in its degradation and production of compounds of economic importance. This natural sawdust fermentation can be enhanced to serve as a remediation for sawdust waste that has been an environmental problem and also produce useful products from it.

Key words: biodegradation, fermentation, sawdust, microorganisms, decomposing.

Introduction

Sawdust is composed of fine particles of wood produced from cutting with a saw, hence its name. It is the main by-product of wood processing in sawmills and is generally regarded as a

waste (Eze *et al.,* 2011). Wood as an essential material for man is a material source for energy and construction works, wood debris (sawdust) by-product of wood processing (Stride *et al.,* 2004). It pollutes the environment even though these debris are materials suited to degradation (Williams, 2001). Sawdust if not degraded or reused may cause damage to the environment such as leachates into water systems and block the water ways thereby creating an environmental hazard, water-borne disease and depletion of oxygen in aquatic environments (Pe'rez *et al*., 2012). This high biological oxygen demand can suffocate fish and other organisms. It is not advisable to use sawdust within home aquariums as was once done to save some expense on activated charcbal (Liu *et al.,* 1998).

Wood is made up of cellulose, hemicellulose and lignin (Erikson *et al*., 1990). Hemicellulose and lignin make up about a third of the mass of a typical wood and also add great strength to the wood. It is a complex set of aromatic molecules and a polymer of sugars (Anderson *et al*., 2007). Cellulose fibrils have high tensile strength which is used in the textile industry, paper and miscellaneous materials like Vulcanized fibre, plastic filters, etc. Other uses include adhesive exposures thickening agents, coated paper cellophane, artificial leather, films and foils (Hitchner and Leatherwood, 1982). Cellulose constitutes one-third to one half of the approximately 150 billion tones of organic matter synthesized annually. Hemicellulose is an ill-defined group of carbohydrate and is of the major plants constituents second in quality to cellulose. The general recalcitrance of cellulose lignin and hemicelluloses and the importance of their biodegradation in the environment have received much attention for several years (Lennox *et al.,* 2010). Most of the cellulolytic microorganisms belong to eubacteria and fungi even through some anaerobic protozon and slime molds able to degrade cellulose have also been described. Cellulolytic microorganism can establish synergistic relationship with non-cellulolytic

species in cellulose wastes. The interactions between both population lead to complete degradation of cellulose releasing carbondioxide and water under aerobic condition, and carbondioxide, methane and water under anaerobic condition.(Cohen, *et al.,* 2006). Biodegradation is the natural process of breaking down organic pollutants by microorganisms to harmless economic compounds or recycling wastes to nutrients which can be used by other organisms. Degradation is carried out by huge assortment of bacteria, fungi insects worms and other organisms that eat materials and recycle them into new forms (Singleton and Samburg 1998). Wood sawdust have been reported by Stride *et al.*, (2004) to be degradable by Letinus sqarrosolus (mont) Singer, a basidiomycetes also know as a white rot fungi to form protein, glucose and ethanol (Eze and Ikeri 2011). Only protein and glucose have been so far reported as products of biodegradation of sawdust. The end products of effective biodegradation are non-toxic such as carbon dioxide and water and can be accommodated without harm to the environment and living organisms. The organism multiplies in number in the process (Okpokwasili, 1994). Sawdust can be used in manufacturing, energy and agricultural processes. Cellulose, a component of sawdust can be used to produce cotton (Bian *et al*., 2005), cellophane (Korpinen and Fardum, 2006), Glucuronolaction, vitamin, carbohydrate, industrial sugar, biofuel production, oil production, ester production paper, movie film frames, toy cellulose, ethanol (Javis, 2003) and Waste to Wealth (Rominiyi *et al.,* 2017). This research will determine other useful products of sawdust biodegradation if any.

Materials and methods

Samples collection

Fresh and dry un-decomposed sawdust samples were collected from MCC timber market, Calabar, Cross River State, Nigeria in sterile polythene bag and transported to the laboratory for analysis.

Isolation and identification of indigenous bacteria and fungi in sawdust

Bacteria were isolated and identified by carrying out a ten-fold dilution of the wet decomposing sawdust as described by Lennox *et al*. (2010). Antifungal agent, nystatin was incorporated into nutrient agar. One milliliter of the diluted solution of the sawdust was plated and incubated. The discrete colonies were sub-cultured and stock cultures were prepared from the pure cultures and stored at 4°C until needed.

The method of Anderson *et al*. (1973) was used in the isolation of fungi. One milliliter from the dilution above was plate on Sabouraud dextrose agar and incubated at 27°C for 3 days. The discrete colonies were sub cultured and the pure cultures stocked and stored until needed. The isolated colonies were characterized and identified.

Purification and maintenance of isolates

Each discrete colony on a Petri dish was transferred using a sterile inoculating loop into plates containing freshly prepared Nutrient agar and were incubated at 37^0C for 24-48hrs respectively. After incubation, the cultural characteristics of the isolates were recorded and compared with descriptive features contained in Holt and Bergey, (1994). The isolates were then preserved on nutrient agar slants stored in the refrigerator at 4^oC for biochemical characterization and identification.

Biochemical characterization and identification of isolates

Standard microbiological methods were employed for the identification of the bacteria and fungi isolates using the methods of Holt and Bergey (1994) and Cheesebrough (2000).

Glucose determination

Glucose was determined using dinitrosalicylic (DNS) reagent standard glucose solution was prepared by dissolving 10g 3.5-dinitrosalicylate in 200ml 2M- NaOH and mixed with solution of 300g Rochele salt in 500ml water. This mixture was then diluted to 1 liter to contain 2g crude sugar per liter (5mM glucose solution).

Different dilutions of the standard DNS reagent were made and absorbance recorded. A standard curve for the determination of glucose was constructed using the results. Two mls of test solution were measured into a test tube, 0.5ml distilled water and 2 mls DNS were added mixed and placed in water bath at 90^0C for 5 min. The content was then diluted to 20 mlsl with water and cooled. The absorbance determined this procedure was repeated twice and the average value of the three absorbance, was determined. Using the average value of the absorbance the concentration of the test sample was determined from the standard curve. Shide *et al.,* (2004).

Protein determination

Protein was determined using Biuret reagent. Standard albmin solution containing 10ml standard albumin per ml solution was prepared and fractions of 0.2, 6.4, 0.7 and 1.0ml were measured out into four different test tubes. Distilled water was added to the first three test tubes to make up 0.1ml solution. Four mls of Biuret reagent were added to each of the content of the test tubes and cautiously shaken to homogenize and left for 30min at 29^0C. The averages of three absorbance measured using a spectrophotometer (pyeunican, model sp192) at 540nm, each of the test tubes, were recorded from which the standard curve was obtained. The procedure was repeated using 1.0ml of the solution and corresponding concentration of protein determined from standard curve.

Alcohol determination

Concentration alcohol (92% v/v) was diluted to 2.0, 4.0, 6.0 and 8.0 and the absorbance measured using a spectrophotometer (pyeunican, model sp192) at of 540 nm. The standard curve was obtained from the plot of these values. The absorbance of aliquot sample were determined and concentration found from the standard curve and appropriate conversion made and recorded Shide *et al*., (2004).

Degradation of sawdust

Three test tubes were obtained and 2 g of sawdust was introduced into each. Eighteen mls of distilled water was also added into each of the test tubes. The tubes were then incubated at 37°C for 21 days. At the end of the 21 days, the sawdust mixture was filtered and the liquid portion was obtained and the residue was discarded.

Gas chromatography - mass spectrometry (GC-MS) analysis

The analysis for chemical components present in the degraded sawdust was carried out using GC-MS analyzer (BRUKER SCION 436-GC SQ). Extracts were dissolved in methanol (high-performance liquid chromatography grade) and filtered through Whatman™ filter device (0.2 µm). Helium (99.99%) was used as carrier gas, at a flow rate of 1 ml/min in split mode. RESTEK Rtx®-5 (Crossbond® 5% diphenyl/95% dimethyl polysiloxane) with 30 m length, 0.25 µmdf, and 0.25 mm ID column was used for separation of chemical components. Two microliter of sample was injected into the column. The injector temperature was 320°C. The temperature of oven starts at 70°C and holds for 2 min, and then, it was raised at a rate of 7°C per minute up to 320°C, holding for 1 min. Temperature of ion sources was maintained at 250°C. The mass spectrum was obtained by electron ionization at 70eV and detector operates in scan mode 30–500Da atomic units. Total running time was 38.71 min including 3 min solvent delay. The spectrum of the unknown component was compared with the spectrum of the known components

using computer searches on a NARICT Ver.2.1 MS data library. The name, molecular weight, retention time and structure of the components of the test materials were ascertained and results were recorded.

Results

Biochemical characterization and identification of the isolates

Table 1 shows the biochemical characterization and identification of the isolates from the sawdust sample analyzed. Five bacteria species were identified and included *Bacillus* sp, *Serracia mercescens*, *Micrococcus* sp, *Pseudomonas aeruginosa* and *Streptococcus* sp.

The fungal isolates are shown in Table 2. Based on the colony and microscopic morphology, three species of fungi were identified namely; *Aspergillus niger*, *Rhizopus* spp and *Penicillium* sp. Table 3 shows the results of the protein, glucose and alcohol contents of the fermented sawdust.

GC-MS analysis results

The results of GC-MS analysis of sawdust sample degraded by its indigenous microorganisms identified twelve bioactive chemical constituents. The gas chromatogram showed the relative concentrations of various compounds eluted as a function of retention time as presented in Figure 1 and Table 4 respectively. The heights of the peak indicate the relative concentrations of the compounds of the components present in the plant extracts. The mass spectrometer analyzed the compounds eluted different times to identify the natures – and structure of the compounds. The compounds which were identified by GC-MS analysis were 12 in total. Identification of the bioactive compounds was carried out by comparison of their mass spectra and retention time with those of reference standard and published data in NIST MS library.

Table 1. Biochemical characterization and identification of the isolates from sawdust sample

Isolate code	Gram reaction	Shape	Catalase	Motility	Oxidase	Indole	Citrate	MR	VP	Pigment	Glucose	Lactose	Mannose	Sucrose	Probable organism
P1	+	Rods	+	+	-	-	+	-	+	-	+	±	+	+	*Bacillus* sp
P2	+	Cocci	-	-	-	+	+	-	-	-	+	+	+	+	*Streptococcus* sp
P3	-	Rods	+	+	+	-	+	-	-	+	-	-	-	-	*Pseudomonas aeruginosa*
P4	+	Cocci	+	-	+	±	-	+	-	+	±	-	+	-	*Micrococcus* sp
P5	-	Rods	+	+	-	-	+	-	+	+	+	-	+	+	*Serratia marcescens*

Key:

+ positive
- negative

Table 2. Cultural and morphological characterization of fungal isolates

Isolate code	Colony morphology	Microscopic morphology	Probable organism
PF1	Growth begins as yellow colonies that soon develop a black, dotted surface as conidia are produce within 26 days the colony becomes jet black and powdery and the reverse remains cream color	Exhibits septate hyphae long conidiophores that support spherical vesicle that give rise to mutulae and phalides from which conidia are produce.	*Aspergillus niger*
PF2	Black fluffy coloration with powdery appearance	Non-septate hyphae with sporabgiophores	*Rhizopus* sp
PF3	Green colonies, surface of colonies becomes powdery due to presence of conidia	Hyphae are septate and produce brush like conidiophores, conidiophores produce metulae from which phalides producing chains of conidia arise	*Penicillium* sp
PF4	Growth begins as yellow colonies that soon develop a black, dotted surface as conidia are produce within 26 days the colony becomes jet black and powdery and the reverse remains cream color	Exhibits septate hyphae long conidiophores that support spherical vesicle that give rise to mutulae and phalides from which conidia are produce.	*Aspergillus niger*
PF5	Green colonies, surface of colonies becomes powdery due to presence of conidia	Hyphae are septate and produce brush like conidiophores, conidiophores produce metulae from which phalides producing chains of conidia arise	*Penicillium* sp

Table 3. Estimation of protein, glucose and alcohol contents of the raw sawdust and degraded sawdust sample

Name of sample	Protein%	Glucose (g/L glucose)	Alcoholic content %
Raw sample	5.25	3.74	-
After degradation	4.37	3.16	1.98

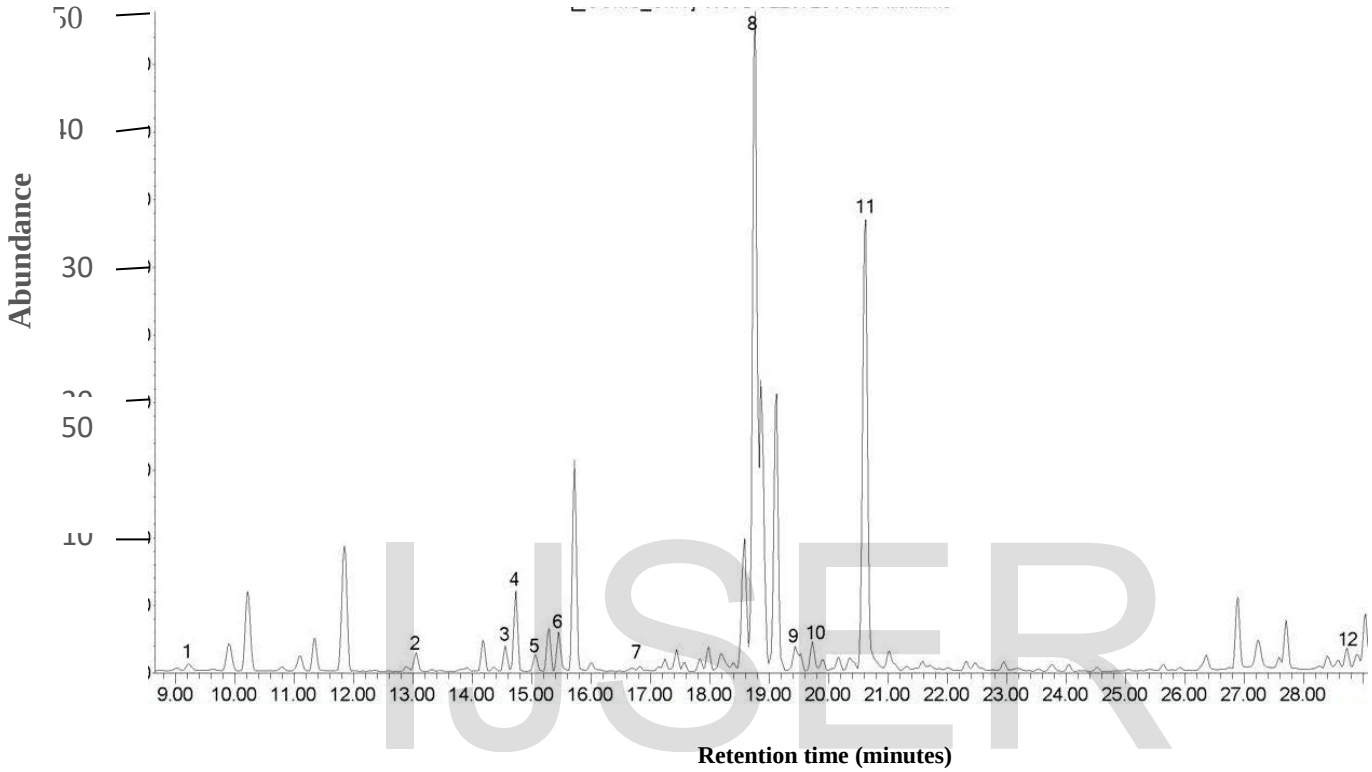

FIG. 1: GC-MS Chromatogram obtained from *sawdust* degraded extract (spontaneous)

Table 4. Sawdust degradation (spontaneous) components identified by GC-MS analysis

Temperature	RT (min)	Name of compound	Peak area (%)
	14.99	thiocyanic acid, methylene ester	1.41
	17.46	Butanoic acid, 4-chloro	0.26
	19.20	Furfural	0.53
	20.63	Furanmethano	1.45
	22.43	2(5H)-furanone	4.48
320◦C	23.48	Benzaldehyde	5.04
	26.78	1H-pyrazole, 3,4-dimethyl(C)	36.65
	26.86	pentanoic acid 2-Ethylhexyl ester	4.49

27.42	Thiophene, Tetrahydro	4.22
28.83	Glycine, N-Cyclopropylcarbonyl-methyl ester	21.9
29.04	Thiophene, Tetrahydro	5.98
29.89	propanoic acid, 2,2-Dimethyl-,2-Ethylhexyl ester	11.7

Discussion

A total of 8 microbial species were isolated from the decaying sawdust; 5 bacteria and 3 fungi. This is against the 16 species of microorganisms isolated by Lennox *et al*., (2010). These indigenous microbial isolates could have used part of the sawdust for their growth and energy and also could have been transit organisms. Though Lennox *et al.,* (2010) also isolated some of these microbes, *Bacillus* sp, *Pseudomonas* sp, *Streptococcus* sp, *Micrococcus* sp, *Aspergillus* sp, *Rhizopus* sp and *Penicillium* sp, it therefore means that these species are resident flora in sawdust. Microbial degradability of sawdust through the isolation of autochthonous bacterial and fungal utilizing sawdust and the observation of differences in cellulose content of sawdust before and after treatment have been demonstrated by Ochonogor and Onilude (1985).

. Eight microbial species isolated from the decaying sawdust had different capabilities of degradation of the cellulose component of the sawdust (Cohen *et al*., 2006). This indicates that only few of the isolates are capable of utilizing sawdust as its source of carbon and energy for growth. This result is in agreement with the work of Lennox *et al.,* (2010) who also isolated different bacteria and fungi responsible for the biodegradation of sawdust. This corroborates the findings of Godliving and Yoshitoshi (2002) that bacteria and fungi degrade wood sawdust. Focher *et al.,* (1991) also reported the biodegradability of cellulose. This also agrees with the findings of Dosoretz *et al.,* (1990), where they reported the reduction in carbon content of sawdust when subjected to microbial degradation. (Hitchner and Leathrewood 1982) reported the ability of cellulase enzyme in the degradation of cellulose. The efficacy of fungi in cellulose

degradation has also been reported (Deeble and Lee, 1985; Kelsey and Shafizadeh, 1980). These reports have provided insight into the possibility of degradation of sawdust using indigenous microorganisms, thereby paving way for enhanced natural attenuation of sawdust polluted sites.

The degradation of sawdust by the indigenous microorganisms isolated and their differences in utilization of the cellulose content of the sawdust after 26days incubation period has been demonstrated as shown in Table 4. This indicates that the isolates are capable of utilizing sawdust as its source of carbon and energy for growth. This finding buttresses the argument by Shide *et al.*, (2004), Shewale and Sadana, (1978), that bacteria degrade organic wastes such as sawdust. Focher *et al.*, (1991) also reported the biodegradability of cellulose. This agrees with the findings of Erickson *et al.*, (1990) when they reported the reduction of carbon content of sawdust and other agro wastes when subjected to microbial degradation. Lennox *et al.*, (2010) also showed that indigenous bacteria and fungi are capable of degrading sawdust thereby using the cellulose content as their main source of carbon.

The protein and glucose contents decreased from 5.25 to 4.37% and 3.74 to 3.16g/L respectively while the alcohol content was 1.98%. These values are higher than what Chide *et al.*, (2004) reported for both treated and untreated sawdust.

Spontaneous fermentation of the sawdust also resulted in the production of various chemical compounds many of which are economically useful. This is novel because no other works have been carried out on this. Benzaldehyde, a product of the fermentation is commonly employed to confer almond flavor to foods and scented products. It is sometimes used in cosmetics products. In industrial settings, benzaldehyde is used chiefly as a precursor to other organic compounds, ranging from pharmaceuticals to plastic additives. Also, 2(5H)-Furanone, one of the products of the fermentation is a food additive.

Conclusion

It has been determined by this research that the isolated normal flora such as *Bacillus* spp, *Serracia mercescens Micrococcus* spp, *Pseudomonas aerugnosa*, *Streptococcus* spp, play important role in degrading sawdust. All organisms known to degrade cellulose efficiently produce a battery of enzymes with different specificities which act together in synergism. The products of effective degradation are non toxic and they include carbon dioxide, water, alcohol, glucose and protein and organic acids. These products can be accommodated without harm to the environment and living organisms. ,Normal flora tend to utilize it as a nutrient and multiply in the process. This research has provided insight into the possibility of degradation of sawdust using normal microbial flora, thereby paving the way for enhanced natural attenuation of sawdust polluted sites. It also leads to new findings and development on how degradation could be done fast, efficient and effectively concerning their mechanism of action.

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