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Characterization of products from sawdust biodegradation using selected microbial culture isolated from it

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Characterization of products from sawdust degradation using selected microbial culture isolated from it was carried out. A composite sawdust waste sample was collected from MCC timber market in Calabar. The sample was diluted using serial dilution method and inoculated on Nutrient agar and Sabauraud dextrose agar using pour plate technique and incubated at 370C and 270C respectively. Five bacteria species and 3 species of fungi were identified which included Bacillus sp, Serracia mercescens, Micrococcus sp, Pseudomonas aeruginosa, Streptococcus sp, Aspergillus niger, Rhizopus sp and Penicillumsp. The bacteria and fungi species were individually and in combination inoculated into sterile sawdust containing sterile water. These were kept at 270C for 21 days. Protein, glucose contents and chemical components in the degraded sawdust samples were determined using GC-MS chromatography. It was observed that Aspergillus niger degradation had protein content 7.87g/l, Serracia mercescens and Aspergillus niger 6.56g/l, and Serracia mercescens 5.75g/l. These organisms used lignocellulolytic enzymes and similar enzymes in the degradation process. In the case of glucose, the combined degradation produced 2.24g/l, Aspergillus niger 2.05g/l and Serracia mercescens 1.94g/l. It was also observed that sawdust inoculated with Serratia marcescens produced 14 compounds while the combined species of Aspergillus niger and Serratia marcescens produced only 12 compounds. The following compounds Methylene cyclo propane carboxylic acid, Glycine N-Cyclopropylcarbonyl-methyl ester, acetic acid, propanoic acid, benzaldehyde, thiophene, tetrahydro, furfural and propanoic acid 2,2-Dimethyl-2-ethylhexyl ester were detected from the combination of Serratia marcescens and Aspergillus niger though in different concentrations. The percent concentrations of the compounds from Serratia marcescens range from n-Hexadecanoic acid 3.59 to Methylene cyclo propane carboxylic acid with 11.56 while Serratia marcescens and Aspergillus niger range from Butanoic acid, 4-chloro 3.59 to Benzaldehyde with 11.56. Microbial degradation of sawdust produced economically important products and therefore can contribute to the economy of this country at the same time reduce pollution caused by it which is a novel.

Key words: Sawdust, biodegradation, bioactive, extracts, microbial culture, lignocellulosic.

INTRODUCTION

Microbial biodegradation is the use of bioremediation and biotransformation methods to harness the naturally occurring ability of microbial xenobiotic metabolism to degrade, transform or accumulate environmental

pollutants including hydrocarbons (example oil).

Biodegradation is the process by which organic substances are broken down into smaller compounds by living microbial organisms or their enzymes (Godliving and Yoshitoshi, 2002). These organic substances can either be degraded aerobically with oxygen, or anaerobically without oxygen. Interest in the microbial biodegradation of agricultural wastes has intensified in recent years (Koukkou and Anna-Irini, 2011).

Sawdust is a by-product of woodworking operations such as sawing, milling, planning, routing, drilling and sanding. These operations shatter lignified wood cells and break out whole cells and groups of cells. Shattering of wood cells creates dust known as sawdust. This dust causes a lot of health hazards (Baran and Teul, 2007; Zhang, 2004). It is composed of fine particles of wood made up of 3 major components, cellulose, hemicelluloses and lignin (Green, 2006; Alexander, 1997; Erikson et al., 1990). It is generally used for blotting material, hand cleaner, wood filler, compost, packing and for writing notes (Berendeohu, 2018). The general recalcitrance of cellulose, lignin and hemicelluloses and the importance of their biodegradation in the environment have received much attention for several years (Erikson et al., 1990). Raw sawdust takes an average of 180 days to decompose in the soil due to deficiency of nitrogen (Olayinka and Adebayo, 1989). Microbial degradation of sawdust has been very difficult due to the presence of lignin, a highly recalcitrant component that does not degrade easily (Erkson et al., 1990). Despite this, Lennox et al. (2010) were able to degrade sawdust at certain degrees using microorganisms. Shide et al., (2004) reported that wood sawdust was degraded by *Lentinus squarrosolus* (Mont) singer, a basidiomycete also known as a white rot fungi to form protein, glucose and ethanol. Cellulose fibrils have high tensile strength which is used in the textile industry, paper and miscellaneous materials like vulcanized fibre, plastic filters, filtering media and surgical cotton; other uses include adhesives, explosives, thickening agents, coated paper, cellophane, artificial leather, films and foils (Hitchner and Leatherwood, 1982). This is to say that numerous products can be gotten from biodegradation of sawdust. Fungi of the classes hyphomycetes, zygomycetes, pyrenomycetes, hymenomycetes and the actinomycetes and bacteria of the groups *Cytophage, Erwinia, Pseudomonas, Sporolytophaga, Xanthamonas and Streptomonas* species degrade hemicelluloses (Lennox et al., 2010; Wuyep et al., 2012).

This research is therefore aimed at characterization and identification of products of biodegradation of lignocellulosic material, sawdust using microbial culture isolated from it.

MATERIALS AND METHODS

Samples collection

Fresh and dry undecomposed sawdust composite samples were collected from MCC timber market, Calabar, Cross River State, Nigeria in sterile polythene bags and transported to the laboratory for analysis.

Isolation and identification of indigenous bacteria and fungi involved in sawdust degradation

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 at 37°C for 18 to 24 h. 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 milliter from the dilution above was inoculated on potato dextrose agar and incubated at 27°C for 7 days. The discrete colonies were sub-cultured and the pure cultures were 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°C for 24 to 48 h, respectively. After incubation, the cultural characteristics of the isolates were recorded and compared with descriptive features contained in Bergey's Manual for Determinative Bacteriology (Holt et al., 1994). The isolates were then preserved on nutrient agar slants stored in the refrigerator at 4°C for biochemical characterization and identification.

Biochemical characterization and identification of isolates

The method of Oranusi et al., (2004) as modified was employed for the identification of the bacteria isolates while the fungal isolates were identified based on the taxonomic schemes described by Fawole and Oso (1988). Biochemical and sugar fermentation tests were used for the identification of the bacteria isolates. The biochemical tests carried out were: Catalase, methyl-red, oxidase, citrate utilization, coagulase, indole, coagulase and oxidase tests. The identities of the bacteria were confirmed using the identification aid outlined in Bergey's Manual for Determinative Bacteriology (Holt et al., 1994) as well as that of known taxa as described by Cheesbrough (2003). The fungal isolates were characterized based on colonial morphological features and microscopic examination using the Lactophenol cotton blue stained slide cultures and the results compared with fungal atlas.

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Table 1. Cultural and morphological characterization of fungal isolates.

Isolate code	Colony morphology	Microscopic morphology	Organism
PF_1	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	*A. niger*
PF_2	Black fluffy coloration with powdery appearance.	Non-septate hyphae with sporangiophores	*Rhizopus sp.*
PF_3	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.*
PF_4	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	*A. niger*
PF_5	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.*

Preparation of bacterial and fungal inoculum

The identified bacteria and fungi strains isolated from sawdust sample were transferred from stock agar slant cultures to nutrient agar broths and incubated until a sizable inoculum size of 10^7 cfu/ml was obtained at 35°C.

Sawdust inoculation and degradation

The collected sawdust samples were first autoclaved to kill all indigenous bacteria and fungi present in it. Three sterile conical flasks were obtained and 25 g of sawdust was introduced into each and properly labeled; bacteria, fungi and combined (bacteria + fungi). 150 ml of distilled water was added into each of the conical flasks followed by the addition of the inoculums size of 10% v/v (17.5 ml) into each of the sawdust solutions. The conical flasks were then incubated at room temperature (26 to 28°C) for 21 days. At the end of the 21 days, the supernatants in the conical flasks were carefully filtered out and the residue sawdust was analysed.

Estimation of protein and glucose contents of fermented sawdust

Protein and glucose contents of the fermented sawdust samples were determined using ASTM standard method (1974) as reported by Bakulin et al., (2007).

Gas chromatography mass spectrometry (GC-MS) analysis

The analysis for chemical components present in sawdust degradation extracts 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 Whattman TM 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. 2 µL of sample was injected into the column. The injector temperature was 320°C. The temperature of oven starts at 70°C and hold for 2 min, and then, it was raised at a rate of 7°C per minute up to 320°C, hold 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 to 500 Da 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

Cultural and morphological characterization of fungal isolates

The cultural and morphological characteristics of the fungal isolates results are shown in Table 1. Based on the colony and microscopic morphology, three species of fungi were identified.

Morphological and biochemical characterization and identification of the bacteria isolates

Table 2 shows the results of morphological and biochemical characterization and identification of the

Table 2. 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	Organism
P_1	+	Rods	+	+	-	-	+	-	+	-	+	±	+	+	*Bacillus sp*
P_2	+	Cocci	-	-	-	+	+	-	-	-	+	+	+	+	*Streptococcus sp*
P_3	-	Rods	+	+	+	-	+	-	-	+	-	-	-	-	*P. aeruginosa*
P_4	+	Cocci	+	-	+	±	-	+	-	+	±	-	+	-	*Micrococcus sp*
P_5	-	Rods	+	+	-	-	+	-	+	+	+	-	+	+	*S. marcescens*

Table 3. Estimation of protein and glucose contents of degraded sawdust sample.

S/N	Name of sample		Protein content (g/l)	Glucose content **(g/l)**
1	*S. marcescens*	Initial	5.25	3.74
		Degraded	5.75	1.94
2	*A. niger*	Initial	5.25	3.74
		Degraded	7.87	2.05
3	*S. marcescens* *A. niger*	Initial	5.25	3.74
		Degraded	6.56	2.24

isolates from the sawdust sample analyzed. Five bacteria species were identified.

Estimation of protein and glucose contents

The available protein and glucose contents in the degraded sawdust sample were estimated as presented in Table 3. From the result, it was observed that the fungus degradation had higher protein content available followed by the combined (bacteria and fungi) with the bacterium degradation having the least available protein. However, in the case of glucose, the combined degradation recorded the highest glucose content while the least was seen in the bacterium degradation.

Sawdust degradation (bacteria) components identified by GC-MS analysis

The results of GC-MS analysis of sawdust sample degraded by inoculated bacteria isolate identified fourteen bioactive chemical constituents present in the extracts. The gas chromatogram shows the relative concentrations of various compounds getting eluted as a function of retention time as presented in Table 4 and Figure 1 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 (Table 5). 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 (Figure 2).

DISCUSSION

A total of 8 microbial species were isolated from the sawdust; 5 bacteria and 3 fungi. Microbial degradability of sawdust through the isolation of autochthonous bacterial and fungal species utilizing sawdust as their carbon and energy sources and the observation of differences in sawdust before and after degradation have been demonstrated by Lennox et al. (2010). This corroborates the findings of Godliving and Yoshitoshi (2002) and Zhang et al., (2018) that bacteria and fungi degrade wood sawdust by producing enzymes such as carboxymethyl cellulose, lignin peroxidase and xylanase. Beguin and Aubert (1994) also reported the biodegradability of cellulose. This agrees with the finding of Dosoretz et al*.,* (1990), when they reported the reduction in carbon content of sawdust when subjected to microbial degradation. Hitchner and Leatherwood (1982) reported the ability of cellulase enzyme in the degradation of

Table 4. Sawdust degradation components identified by GC-MS analysis using *S. marcescens.*

Temperature	RT (min)	Name of compound	Concentration (%)
320°C	24.57	Methylene cyclo propane carboxylic acid	11.56
	30.46	Glycine, N-cyclopropylcarbonyl-methyl ester	7.58
	32.12	Acetic acid	4.64
	16.53	Propanoic acid	3.59
	16.76	2(5H)-furanone	5.84
	24.15	Benzaldehyde	5.91
	36.06	1H-pyrazole, 3,4-dimethyl(C)	4.83
	12.56	Pentanoic acid 2-ethylhexyl ester	10.6
	28.97	Thiophene, tetrahydro	9.38
	28.98	Furfural	4.63
	44.2	Thiophene, tetrahydro	3.98
	37.84	Propanoic acid, 2,2-dimethyl-,2-ethylhexyl ester	4.2
	33.86	4H-Pyran-4-one	6.09
	44.19	n-Hexadecanoic acid	3.59

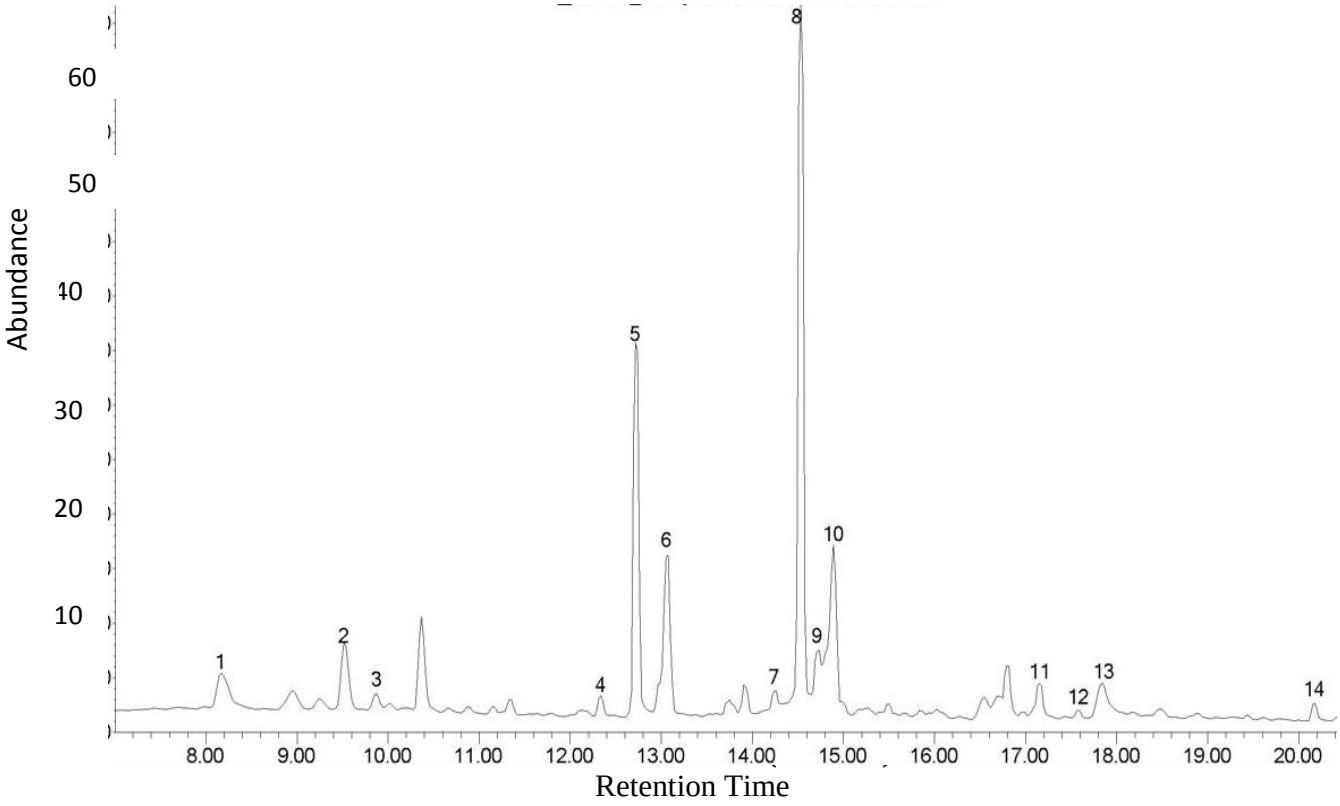

Figure 1. GC-MS chromatogram obtained from *sawdust* degraded extract by *Serratia marcescens.*

cellulose. Microbial species have different capabilities of degradation of sawdust (Lennox et al., 2010). This indicates that only few of the isolates are capable of utilizing sawdust as its source of carbon and energy for growth. The microbial isolates, *Bacillus* sp*, Streptococcus* sp.*, P. aeruginosa, Micrococcus* sp., *S. marcescens, A. niger, Rhizopus* sp. and *Penicillium* sp. in this study are similar to those isolated by *Lennox* et al. (2010) except *Cellulomonas* sp., *Cytophaga* sp., *Flavobacterium* sp., *Xanthomonas* sp., for bacteria, but only 2 fungi species, *Rhizopus* sp. and *Penicillium* sp. agree with the findings of Lennox et al. (2010). Two out of the eight microbial

Table 5. Sawdust degradation components identified by GC-MS analysis using **S**. *marcescens* and *A. niger*.

Temperature	RT (min)	Name of compound	Concentration (%)
320°C	24.57	Benzaldehyde	11.56
	30.46	Acetic acid	7.58
	32.12	Furfural	4.64
	16.53	Butanoic acid, 4-chloro	3.59
	16.76	Propanoic acid, 2,2-dimethyl-,2-ethylhexyl ester	5.84
	24.15	4H-Pyran-4-one	5.91
	36.06	n-Hexadecanoic acid	4.83
	12.56	thiocyanic acid, methylene ester	10.6
	28.97	Methylene cyclo propane carboxylic acid	9.38
	28.98	Propanoic acid	4.63
	44.2	Thiophene, tetrahydro	3.98
	37.84	Glycine, N-cyclopropylcarbonyl-methyl ester	4.2

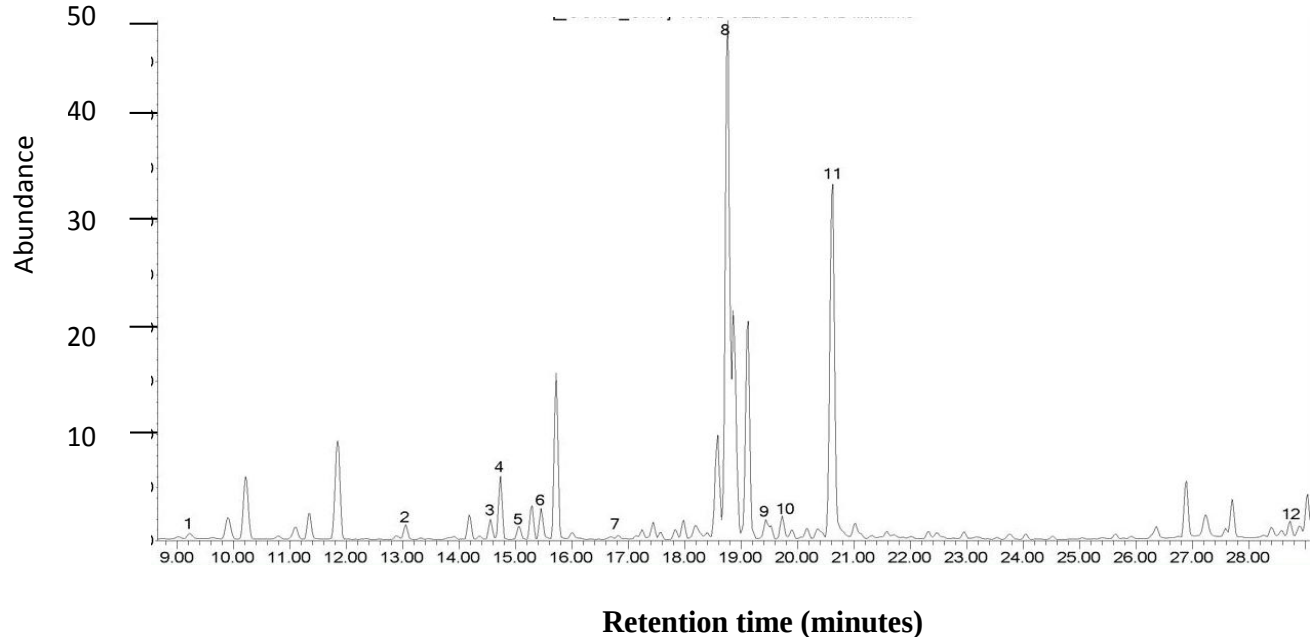

Figure 2. GC-MS chromatogram obtained from *sawdust* degraded extract by *S. marcescens* and *A. niger*.

species isolated from the sawdust were used for this research. *S. marcescens* and *A. niger* used in the biodegradation showed their capability to utilize saw dust to produce important products. 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 (Lennox et al., 2010). The saw dust inoculated with *S. marcescens* produced 14 compounds while the combined species of *A. niger* and *S. marcescens* produced only 12 compounds. The following compounds, Methylene cyclo propane carboxylic acid, Glycine N-Cyclopropylcarbonyl-methyl ester, acetic acid, propanoic acid, benzaldehyde, thiophene tetrahydro, furfural and propanoic acid 2,2-Dimethyl-2-ethylhexyl ester were detected in both the *S. marcescens and A.niger/S. marcescens* inoculated saw dust samples though in different concentrations. Methylene cyclo propane carboxylic acid with 11.56% was the highest produced by *S. marcescens* but only 9.38% from the combined. This could be as a result of some antagonistic activities of the organisms by producing several hydrolytic enzymes like chitinase, protease, lipase and cellulase (Purkayastha et al., 2018).

S. marcescens produced 10.6% of pentanoic acid 2-ethylhexyl ester which was absent in the combined. This also could be some antagonistic or use of the compound by *A. niger.* Benzaldehyde concentration was 11.56% from the combined but only 5.91% from *S. marcescens*. Thiocyanic acid, methylene ester 10.6% produced by the combination was not produced by *S. marcescens*. The results here show that selective utilization of these organisms in the degradation of saw dust can lead to the production of economically important products. These compounds produced by these microorganisms are the first of its kind in scientific world and therefore a major contribution to knowledge.

The protein and glucose analysis revealed an increase in protein content by 9.5, 49.9 and 25% using *Sarratia marcescens, Aspergillus niger* and combination of *S. marcescens* and *A. niger* respectively. For the glucose, there were decreases of 48.6, 45.2 and 40% using *S. marcescens, A. niger* and combination of *S. marcescens* and *A. niger* respectively. This is to say that these microorganisms were able to utilize saw dust to increase the protein content while at the same time utilized the glucose contained in the saw dust. This is contrary to the findings of Bohdan and Yaser (2011), Reddy and Yang (2009), Van Wyk (2001) and Ndukwe et al., (2012) who detected an increase in glucose levels, though the saw dust samples were pretreated. This is pretreatment of the lignocelluloses wastes to a greater extent must have removed lignin from the different saw dust materials. This research has shown that saw dust waste can be bio converted to useful products without pretreatment as has always been believed. Most of the products produced by the fermentation of the sawdust are very useful. Glycine, N-Cyclopropylcarbonyl-methyl ester produced by *S.marcescens* is used as additive in pet food and animal feed, in foods and pharmaceuticals as a sweetener/taste enhancer, or as a component of food supplements and protein drinks. It is also used in cosmetics. Benzaldehyde, 11.56% produced by *S. marcescens* and *A. niger* is also an important compound used chiefly in the organic synthesis of dyes, perfumes, and flavors, and as a solvent. Methylene cyclo propane carboxylic acid (11.56%) and Pentanoic acid 2-Ethylhexyl ester (10.6%) also produced from the degradation of sawdust by *S. marcescens* have been found to be very useful as an intermediates in the production of pesticides, pharmaceuticals and other agrochemicals. Acetic acid (7.58%) produced by *S. marcescens* and *A. niger* has several health benefits which include reducing inflamation, lowering blood pressure, and keeping blood sugars spikes at bay. Acetic acid is also used as a condiment and in the pickling of raw vegetables and other foods

The direct fermentation of sawdust without pretreatment with selected indigenous microbial flora is a novel and the amounts of economically important products resulting from the fermentation are also novels. The use of sawdust for the production of these compounds will lead to reduction of pollution caused by sawdust.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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