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Phenotypic characterization of Mesophilic Bacteria Species involved in the Biodegradation of Rice Husk from Abakaliki Rice Mill Ebonyi State South East Nigeria.

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ABSTRACT

The research was carried out to isolate and characterize mesophilic bacteria involved in the biodegradation of rice husk from Abakaliki rice mill Abakaliki. Ebonyi State. Eighteen (18) mesophilic species were isolated from an old rice husk dump at the rice mill. The isolated species were used to hydrolyze fresh rice husk and their ability to degrade rice husk were determined by their ability to release reducing sugars from the rice husk. In all, eight (8) isolates were found to release substantial amounts of reducing sugars. They were identified as *Bacillus brevis. Bacillus coagulans. Bacillus laterosporus. Bacillus firmus, Pseudomonas mallei, Pseudomonas putida, Cellulomonas fimi* and *Cellulomonoas flavigena.* The physic-chemical analysis of the soil from the old rice husk dump showed that the rice husk impacted positively on the soil quality. Daily analysis of reducing sugar showed that the highest value of reducing sugar for most of the organisms was on the 3rd day of hydrolysis while the highest reducing sugar value of 37.1mg/g was produced by *Pseudomonas mallei.* Results of cellulose enzyme assay showed that the organisms produced cellulose but cellulose activity for all the organisms were found to be highest on the 1st day of hydrolysis. Statistical analysis revealed that there were significant differences in the reducing sugar and cellulose produced by the organisms daily. The ability of these organisms to degrade the rice husk was based on their ability to produce cellulose and thus these organisms can be used in the bioconversion of rice husk to useful products.

Keywords: Rice husk, Biodegradation, Bacterial species, Reducing sugar, Cellulose.

INTRODUCTION

Rice husk is the coating of the seed of grains of the rice plant. It is the hard protecting covering of grains of rice. The husk is removed during the milling of the rice grains. It is mostly indigestible to humans and accounts for 20-25% of the weight of the rice paddy [1]

Rice husk is made up of cellulose 32.1%, hemicelluloses 24.1% and lignin 12.5%, [2]. The polymers lignin, hemicelluloses and cellulose combine in various proportions to form a “lignocellulosic” structural support system for nearly all plants. This constitutes a vast biomass that is often a waste product of agriculture, timber processing and other human activities ad needs to be disposed off in a safe and efficient manner or used as a resource [3]. Lignocellulosic materials are grouped into three classes namely: Primary cellulosics: This includes plants that are harvested specifically for cellulosics content, structural use or feed value e.g. cotton, timber and hay. Agricultural waste cellulosics: This are the plant materials that remain after harvesting and processing e.g. straws, corn stover’s rice husks (hulls), sugar cane bagasse, animal manures and timber residues. Municipal waste cellulosics: This encompasses waste papers and other discarded paper products.

Rice is a general food which forms an important part of the diet of many people worldwide and as such is a staple food to many especially to the developing and underdeveloped nations [4]. Domesticated rice comprises two species of food crops

in the *Oryza* genus of the poaceae ("true grass") family. Asian rice, *Oryza sativa,* is native to tropical and subtropical Southern Asia: African rice, *Oryza glaberrima,* is native to west Africa [5]. Rice is grown as a monocarpic annual plant although in tropical areas, it can survive as a perennial. Rice can grow to 1.0-1.8m tall depending on the variety and soil fertility. The grass has long slender leaves 50-1000cm long and 2-2.25cm broad. The small wind pollinated flowers are produced in a branch arching to pendulous inflorescence 30-50cm long. The edible seed is a grain (caryopsis) 5-12cm long and 2-3mm thick [6]

Rice cultivation is well suited to countries and regions with low labour costs and high rainfall as it is very labour intensive to cultivate and require plenty of water for cultivation [7]. Rice cultivation began in China [8]. In Africa, rice has been cultivated for 3,500 years between 1,500 and 800 BC [9]. The African species *Oryza glabberima* propagated from its origin, the Niger River Delta was extended to Senegal.

Lignocelluloses, the major component of plant biomass, make up about half of the matter produced by photosynthesis. It consists of three types of polymers: cellulose, hemicelluloses and lignin that are strongly intermeshed and chemically bonded by non-covalent forces and covalent cross-linkages. A great variety of fungi and bacteria can breakdown these macromolecules by using a battery of hydrolytic or oxidative enzymes. In native substrates, binding of the polymers hinder their biodegradation [10]. Molecular genetics of cellulose, hemicelluloses and lignin-degrading systems advanced considerably during the 1990s [11]. Most of the enzymes have been cloned, sequenced and expressed both in homologous and in heterologous hosts. Much is known about the structure, genetic organization and regulation of the genes encoding these proteins [12]

Lignocelluloses in nature are found in wood, grass, agricultural residues, forestry wastes and municipal solid wastes. Several methods for lignocelluloses recycling based on the enzymology of cellulose, hemicelluloses and lignin degradation have been suggested [13]. Among them, composting and their use as raw material for the production of bioethanol as an alternative source of energy seem to be the most economically feasible [14]. Cellulose and hemicelluloses are macromolecules from different sugars whereas lignin is an aromatic polymers synthesized from phenyl propaniod precursors [15]. The composition and percentage of these polymers vary from one plant species to another. Moreover, the composition within a single plant varies with age, stage of growth and other condition [16].

Fungi and bacteria are the main natural agents of cellulose degradation [17]. However, protozoa living in the gut of lower termites have long been known to digests cellulose and more recent work suggests that in higher termites, cellulose activity is produced by the midget tissue and the salivary glands of the termite itself. In addition, plants synthesize cellulose, which play a role in morphogenesis and developmental processes (e.g. in the ripening of fruits including the avocado). The fungi and bacteria that can degrade rice husk and use it as a source of carbon and energy include a variety of aerobes and anaerobes, mesophiles and thermopiles. They are abundant in nature and play an important role in the carbon cycle by recycling CO_2 fixed by photosynthesis. These organisms occupy various ecological niches, where plant residues accumulate. As a general rule, fungi and bacteria found in such natural habitats constitute mixed populations including several species of both cellulolytic and non cellulolytic microorganisms that interact synergistically [18].

These interactions lead to efficient rice husks degradation with formation of CO_2 and H_2O in aerobiosis or CO_2 and CH_4 and H_2O in anaerobiosis. Of all the known lignocelluloses material, the most recalcitrant to microbial degradation is wood because it is highly lignified [19].

White rot-fungi such as the well studied Basidiomycete, *Phanerochaete chrysosporium,* can degrade both lignin and cellulose and play a major role in the degradation of lignocellulosic materials like rice husk. Other fungi are devoid of ligninase activity but are efficient cellulose degraders [20]. This is the case, for example, pf *Trichoderma reesei* whose cellulose system has been extensively studied. Aerobic cellulolytic bacteria have long been known to be present in soil. Cellulolytic bacteria found in soil include *Cellulomonas, Cytophaga, Pseudomonas, Bacilli* and a number of Actinomycetes [21]. Some cellulolytic actinomycetes are thermophilic such as *Thermomonosposa* and *Microbispora:* furthermore, several actinomycetes including *Nocardiae* and *Rhodococci* can attack lignocelluloses [22]. Cellolose rich materials are also found in anaerobic habitats including the rumen and intestinal tracts of animals, sewage sludge digesters, composts, fresh water and sea water muds and sediments [23].

MATERIALS AND METHODS

Sample Collection

Samples for isolation of rice husk degrading organisms were collected from an old rice husk dump that has decomposed. Samples were collected at three different points at the top of the dump at depths of about 6cm with sterile spoons and transferred to a sterile 500ml conical flask , covered immediately with cotton wool and transport to the laboratory in a covered ice pak container for analysis.

Sample Dilution

Ten grams (10g) of the soil sample was weighed into 250ml flask containing 100ml of sterile physiological saline and agitated for 20min to dislodge clumps. A six fold dilution was made from the supernatant from the mixture for aerobic bacteria isolation.

Physic-Chemical Analysis of the Soil Samples from the Old Rice Husk Dump

The following physicochemical parameters of the rice husk were tested: soil moisture content, soil pHI, Temperature, Organic carbon (combustion method), total nitrogen, Phosphorus content, Organic matter

Collection of Sample

Soil samples were collected from five different points in the sampling dump. 200g from each of the sampling points were collected, with sterile spoons and transferred to sterile 500 ml conical flask. It was then labeled and transported to the laboratory.

Determination of Soil Moisture Content

This was done on the basis of "oven-dry" soil. 10g of the soil sample were transferred into moisture tin and weighed. It was then dried at 100 ^{0}C until constant mass is reached. It was then cooled in a desiccators and weighed.

Calculation:

$$\text{Moisture \%} = \frac{\text{Weight of tin and soil sample before heating}}{\text{Weight of tin and oven dried soil sample}} \times 100\%$$

Determination of Soil pH

2.5g of the soil sample was placed in a sample bottle. 2.5ml of 0.01 mol/L solution of calcium chloride was added and shaked vigorously for 5 minutes. After shaking, it was allowed to stand for 2 hrs. the digital pH was calibrated (Model-152R) According to manufacturers manual. The temperature of the supernatant was measured making sure that it was the same that of the buffer. The pH was then measured in the supernatant and the value read after stabilization was reached and recorded (International Organization for Standardization. Soil quality (ISO) 10390, 1994).

Determination of Temperature

The temperatures of the different sampling sites in the dump were measured with a standard thermometer (Brannan 100mm). It was placed 3-4 cm into the soil. The thermometer was left for 4 minutes to stabilize and read before withdrawal. The time of measurement was between 12.30pm to 1.30pm.

Determination of Organic Carbon (Combustion Method)

10g of the soil sample was weighted into a crucible and 5 mls of 4 mol/L

hydrochloric acid added to it. It was allowed to wait for 4 hrs. It was then dried at 65^{0}C for 16 hrs. 2g of the sample was transferred to a crucible and heated at 900^{0}C for 4 hrs. CO_2 released was collected and the mass determined (ISO 10694, 1995).

Calculation

The organic carbon content was obtained by the formulae:

$$Wc,\ 0 = 1000\ x\ \frac{m_2}{m_1}\ \ x\ 0.2727$$

Where:
Wc, 0 = Organic Carbon Content (g/kg)
m_1 = mass (g) of test portion
m_2 = Mass (g) of release CO_2
0.2727 = Conversion factor for CO_2 to C

Determination of Total Nitrogen

The modifjied Kjedahl method as described by [24] was used. 0.5g of the soil sample was placed in the digestion flask. 4 ml of salicyclic/sulfuric acid was added and swirled until the acid was thoroughly mixed. It was stood for 12 hrs. 0.5g of sodium thiosulfate was added to the digestion flask and the mixture heated until frothing had ceased. The moisture was boiled for 3 hrs. The flask was allowed to cool after digestion and swirled to bring any insoluble material into suspension and the contents transferred to the distillation apparatus. 20 ml of sodium hydroxide was added. About 40 ml of the condensate was collected and used for titration. 3 drops of indicator was added to the distillate and was titrated with sulfuric acid a violet end point. A blank test was also carried out with the same procedure.
Calculation:
The total Nitrogen content was calculated using the formulae"

$$W_N\ =\ \frac{V_1 - V_0\ x\ c\left(H^+\right) x\ M_N}{m}\ \ x\ \frac{100\ +\ WH_2{}^0}{100}$$

Where:
W_N = Total Nitrogen Content (mg/g = g/kg)
V_1 = Volume of the sulfuric acid used in the titration of the sample (ml)
V0 = Volume of the sulfuric acid used in the titration of the blank sample (ml)
$C(H^+)$ = Concentration of H^+ in the sulfuric acid (moles/Litre)
M_N = Molar mass of nitrogen (= 14g/mol)
$WH_2{}^0$ = Water content of the soil sample based on oven-dried soil.

Determination of Phosphorus Content

Phosphorus determination was measured using vanadomolybdate. 0.5g of the soil sample was placed in a digestion flask. 5ml of digestion reagent (2parts Conc HNO3 and 1part Conc. HCL) was added. The mixture was heated over a laboratory sand bath until Nitrogen peroxide evolved and reached dryness. The procedure was repeated until only a white residue remained in the flask. (ISO 14254, 1994).

Determination of Organic Matter

The organic matter content of the soil samples was determined using the standard method for determination of moisture, ash and organic matter of peat and organic soils (ASTM D 2974, 1994).
A dry, empty and clean porcelain dish weighed and recorded. 10g of previously oven dried sample of the soil was placed in the porcelain dish. The dish was then placed in a muffle furnace and the temperature increased to 440^{0}C. It was left in the furnace overnight. The dish was then removed with tongs and allowed to cool. The weight of the dish containing the ash was determined.
Calculation:
Mass of organic matter = Mass of dry soil-Mass of ashed (burned) soil.
% organic matter =

$$\frac{\text{Mass of organic matter}}{\text{Mass of dry soil}}\ \text{x}\ 100$$

Bacterial Load of the Samples

Determination of Total Heterotrophic Bacteria

Total Heterotrophic bacterial count was done using pour plate method of [25]. 1g of the soil sample was added into 99ml sterile water blank and shaked vigorously for 5 minutes. It was then serially diluted to 10^6. With a sterile pipette, 1ml from each of the dilutions was transferred to sterile Petri plates. 15ml sterile nutrient

agar cooled to 45^{0}C was poured into each of the plates and allowed to cool. The plates were incubated at 35^{0}C for 5 days. Duplicating was performed for all the dilutions. After incubation, counts were made from plates that contained between 10 and 100 colonies.

Calculations:

Total heterotrophic bacteria (Cful/g) = number of colonies x dilution factor

Assessment of Isolates Ability to Degrade Rice Husk

Sample Collection

Samples for characterization of rice husk degrading bacteria were collected from current rice husk dump at Abakaliki rice mill, Ebonyi State. Samples were collected with clean polythene bags and transported to the laboratory.

Sample preparation

The fresh rice husk were ground with a pestle and then sieved with a 1mm sieve. The powdered rice husk was then used for hydrolysis test by the isolates from the old rice husk dump.

Assessment of Isolates ability to Degrade Rice Husk

Assessment of isolates ability to degrade rick husk was based on the isolates relative ability to release reducing sugars from the rice husk [26]. A minimal liquid medium was prepared according to the method of [27] and was used as the fermentation medium for the hydrolysis.

Ten grams (10g) of the rice husk was weighed out and added in a 250ml conical flask containing 200ml of the minimal medium. The flasks were plugged with cotton wool and sterilized at 15psi for 15min. each flask was inoculated with the isolates from the old rice husk dump. After inoculation, the flasks were incubated at 35^{0}C for 5 days on an orbital shaker (model: TR-201.BD) at 100rpm. After incubation, the media were filtered through what man filter paper No. 1.

Determination of Reducing Sugars in the Filtrates

The filtrates were used for the analysis of reducing sugars using the Dinitrosalicyclic acid method according to [28].

Procedure:

3mls of dinitrosalicyclic acid (DNS) reagent was added to 3ml of the filtrate sample in a lightly capped test tube.

The mixture was heated at 90^{0}C for 10 minutes to develop the color (red-brown).

1ml of a 40% potassium sodium tartrate solution was added.

Quantification of the Presence of Reducing Sugar

To quantify the reducing sugar in the filtrate samples. 0.1ml was measured out from the test tubes and added into the cuvette. 2.9ml of distilled water were also added to dilute the colour and the absorbance read at 540nm using a spectrophotometer (8610 UV-Grating, Jenway) and the value recorded. This was done for all the 18 isolates.

Estimation of Daily Degradation of Rice Husk

The six organisms that gave the highest reducing sugars from the batch hydrolysis were selected for another round of hydrolysis to determine the best rice husk degrading specie. The organisms were inoculated into conical flasks containing 10g of rice husks and 200 ml of the minimal medium and incubated at 25^{0}C on an oscillator for 5 days.

Determination of Time Course for Reducing Sugar Production

Starting from the first day of inoculation, samples were collected daily to test for the amount of reducing sugars in the culture flasks. This was done by aseptically pipetting out 5ml of the medium and filtered using Whatman filter paper No. 1.

3ml of the filtrate was then used to determine the presence of reducing sugars and spectrophotometer (8610 UV-Grating, Jenway) used to read the absorbance. This was done dauly for the 5 day incubation.

Cellulase Enzyme Assay

Cellulase activity assay was carried out on the six isolates: isolates 1, 3, 16, 19, 24., and 25. The cellulose activity was assayed by measuring the amount of glucose released from the rice husk following the action of cellulose secreted by the

isolates on powdered rick husk [29]. This was done for 4 days.

Principle of the Assay

Cellulase enzymes are groups of enzymes that work together to release glucose from cellulose [30]. Cellulose itself contains only glucose units, so the determination of the rate of release of glucose from cellulose will give the activity of the cellulose produced by the different isolates [31].

Estimation of glucose released was by the use of glucose determination kits obtained from Randox Laboratories Ltd. UK. [32].

RESULTS

Table 1: Morphological and Biochemical Characteristics of Mesophilic Bacteria Isolates

Isolate	Gram RXN	Cell shape	Cell Arrangement	Catalase	Motility	Oxidase	Spore Test	Indole
1	Tve	Small rods	Singles, paired and chains	+	+	-	+	-
2	Tve	Rods	Singles and paired	+	+	-	+	-
3	-ve	Small rods	Singles and scattered	+	-	+	-	-
4	-ve	Slender rods	Mostly singles few pairs	+	+	+	+	-
5	Tve	Rods	Long chains and clusters	+	+	-	-	-
6	Tve	Short rods	Singles and chains	+	+	+	-	-
7	Tve	Rods	Single and paired	+	+	-	+	-
8	Tve	Short	Single and paired	+	+	-	-	-
9	Tve	Rods	Single and paired	+	+	-	-	-
10	Tve	Short rod	Single and paired	+	+	-	+	-
11	Tve	Rods	Single paired and chains	+	+	-	-	-
12	-ve	Shot rods	Single	+	+	+	-	-
13	Tve	Rods	Single and mostly pairs	+	+	+	-	-
14	Tve	Shot rods	Pairs and short chains	+	-	-	-	-
15	Tve	Short rods	Single and paired	+	+	-	-	-
16	Tve	Rods	Single	+	+	+	+	-
17	Tve	Rods	Single and paired	+	+	+	+	-
18	Tve	Short small rods	Mostly in singles	+	-	+	-	-

Key:

Tve = Gram positive
-ve = Gram negative
+ = Positive reaction
- = Negative reaction

Table 2: Physico-Chemical Properties of the Soil Sample

S/N	Sampling site	pH	Temp. (°C)	Moisture Content (%)	Total Carbon Content (mg/g)	Total Nitrogen (%)	Total Phosphorus (%)	Total organic Matter (%)
1	Sampling site 1 (SSI)	6.5	39	10	1.3	0.09	0.007	2.2
2	Sampling site 2 (SS2)	6.5	38	11	1.4	0.04	0.005	2.4
3	Sampling site 3 (SS3)	6.8	37	10	1.6	0.08	0.009	2.6
4	Sampling site 4 (SS4)	6.3	38	10	1.7	0.03	0.006	2.0
5	Sampling site 5 (SS5)	6.5	39	11	1.4	0.09	0.009	2.4

Table 3: Prevalence of the Rice Husk Degrading Bacterial species

S/N	Bacteria	Number of Sampling Sites	Number occurred in sampling site	Percentage prevalence
1	*Bacillus brevis*	5	4	80
2	*Bacillus coagulans*	5	4	80
3	*Bacillus laterosporus*	5	5	100
4	*Bacillus firmus*	5	3	60
5	*Pseudomonas mallei*	5	5	100
6	*Pseudomonas putida*	5	4	80
7	*Cellulomonas fimi*	5	4	80
8	*Cellulomonas flavigena*	5	5	100

Table 4: Bacterial Load of the Sampling Sites

S/N	Bacteria	Number of Sampling Sites	Number occurred in sampling site	Percentage prevalence
1	Sampling Site 1	2.6	1.0	2.0
2	Sampling site 2	1.0	0.9	0.9
3	Sampling site 3	1.7	1.5	4.0
4	Sampling site 4	2.1	1.1	1.0
5	Sampling site 5	1.5	1.2	1.0

Table 5: Reducing Sugar Values of the Isolates after Batch Hydrolysis

Isolate	Reducing Sugar (mg/g)
1	29.1
2	9.14
3	30.0
4	18.3
5	15.1
6	6.90
7	15.4
8	11.4
9	15.7
10	14.9
11	12.9
12	29.7
13	14.6
14	29.7
15	21.7
16	21.7
17	28.9
18	31.4

Table 6: Characteristics of Rice Husk Degrading Isolates

Isolate	Acid fast	Starch hydrolysis	Casein decomposition	Ribose utilization	Citrate utilization	Glucose hydrolysis	Tryptophan hydrloysis	Minimum temp. of growth	Minimum temp. of growth	Nitrate reduction	Suspected organism
1	-	-	+	-	-	-	-	10°C	55°C	-	*Bacilus brevis*
3	-	-	-	-	-	+	-	10°C	45°C	-	*Pseudonomnas mallei*
4	-	+	-	-	-	+	-	5°C	35°C	-	*Pseudomonas putida*
13	-			-	+	+	-	5°C	40°C	+	*Cellulomons fimi*
15	-	+	-	-	-	+		15°C	55°C	-	*Bacilus coagulans*
16	-	-	+	-	-	+	-	15°C	45°C	-	*Bacilus laterospo*
17	-	+	+	-	-	+	-	5°C	45°C	-	*Bacilus firmus*
18	-	-	-	+	+	+	-	10°C	40°C	+	*Cellulons flavige*

Key:

Positive reaction.

Negative reaction

Table 7: Daily Analysis of Reducing Sugar by Rice Husk Degrading Species (mg/g)

Organism	Day 1	Day 2	Day 3	Day 4	Day 5
Bacillus brevis	24.6	24.2	35.4	31.4	27.4
Pseudomonas mallei	22.9	21.7	37.1	28.0	20.0
Cellulomonas fini	26.3	18.0	27.1	33.7	28.1
Bacillus coagulans	20.3	13.4	23.1	15.1	14.2
Bacillus firmus	20.6	18.0	34.3	32.3	27.1
Cellulomonas flavigena	18.6	14.9	33.7	22.0	20.6

DISCUSSION

The isolation of mesophilic bacteria from the rice husk dump showed that eighteen (18) bacterial species were recovered from the old rice husk dump (Table 1). The presence of these bacterial species was not surprising since bacteria and fungi have been known to be the major degraders of Agricultural wastes like rice husk and other lignocelluloses materials [33]. [34] reported the isolation of fungi and bacteria species implicated in the bioconversion of lignocelluloses materials.

The physic chemical properties showed that the rice husk modified the properties of the soil at the dump site (Table 2). Soils in the sampled sites in general were found to be neutral, that is, neither acidic nor alkaline (Table 2). The pH of a soil determine the biological and nutrient availability in that soil (Natural Resources Conservation Service (NARCS, 2001). Bacteria Are known to grow better at neutral pH. [35] reported the abundant growth of bacteria spp in a rice farm land that had a neutral pH value. The temperature values of the sampled sites in the dump revealed that it favoured the growth f mesophilic bacteria species, Mesophilic organisms are those organisms that grow between the temperature ranges of 20^0C and 45^0C while their optimum growth temperature is about 35^0C [36]. The moisture content of the sampled dump averaged 10.5 (Table 2). The soil organic matter of the dump which averaged 2.3% (Table 2) showed that it contained moderate organic matter. The organic matter content of the soil affects the soil fertility, structure, stability, nutrient, soil erosion and available water capacity (NARCS, 2001). The activities of the mesophilic bacteria on the rice husk at the dump site led to the partial decomposition of the rice husk and humification of the decomposed material leading to increase in the organic matter of the soil and hence the soil quality. [37] reported that one of the major assessment criteria for soil quality is the soil organic matter. The % total nitrogen (TN) of the soil indicated a moderate value (Table 2) and moderate soil fertility (NARCS, 2001). Total nitrogen in the soil is solicited because the nitrogen in the soils occurs in several forms and it takes into account all the nitrogen in organic and inorganic forms. Some scientists argued that TN dose not give good indication of soil fertility because only a small portion of TN is available to plants [38]. [39] from a study on forest soil fertility pointed out that organic and inorganic form of nitrogen are always interchangeable and it would be better to consider the total nitrogen to investigate soil quality. The presence of nitrifying bacteria group in the dump led to the mineralization of the rick husk (Table 4).

The total organic carbon expressed as percentage showed that it contained moderate organic carbon content (NARCS, 2001). The moderate value of organic carbon reported may be because rice husk as a lignclelulose material contained high carbon/nitrogen ratio [40]. During degradation, some of the carbon will be lost as CO_2 some will ne metabolized by the organisms for new cell synthesis and

growth while some will be converted into humus.

The average percentage phosphorus content of the soil is 0.0072 and was mostly from the activities of the decomposition of rice husk and mineralization by microorganisms (Table 2). Since phospgorus is not available in the mineral form, [41] stated that the redistribution of extractable pain agricultural soils is the direct effect of surface placement of manure and crop residue that leads to accumulation of soil organic matter and microbial biomass near the surface. Prevalence of rice husk degrading species showed that the two best rice husk degrading species, *Pseudomonas mallei* and *Cellulomonoas flavigena,* were recovered from all the sampling sites (Table 3). Also the percentage prevalence of other species was also high (Table 2). [42] reported that microorganisms able to utilize a particular substrate for growth is a particular environment, adapted to that environment. A though the sampling sites were in a single dump, the presence of these organisms in most of the sampling sites in the dump indicated that that have adapted to the environment and are not contaminants or were isolated by chance.

The heterotrophic bacterial count was higher than both the cellulolytic bacteria and the nitrifying bacteria (Table 4). They include those bacteria that use organic materials as sources of carbon and energy for growth. Although the main source of carbon and energy at the dump was rice husk, the heterotrophs that were not cellulolytic depend on the products of rice husk degradation like cellobiose and glucose produced by the cellulolytic species for their survival.

Cellulolytic bacterial count was higher than the nitrifying bacterial count (Table 4). Since rice husk is a lignocelluloses material, it is expected that a reasonable population of cellulolytic bacteria should be present to carry out the degradation. It is actually the activities of cellulolytic organisms in the dump cite that attracted the presence of other non rice husk degrading organisms. According to [5], significant synergistic lignocelluloses degradation can be achieved in mixed culture systems of cellulolytic bacteria and non cellulolytic bacteria, in which non-cellulolytic bacteria enhanced cellulolytic activity probably through consuming metabolites derived from cellulose as well as providing essential growth factors for cellulolytic bacteria.

The presence if nitrifying bacteria in the rice husk dump though lowest in number was not surprising since this group of organisms are involved in the mineralization of organic material [12].

Of the 18 isolates used for the hydrolysis, 8 of them turned red brown showing the presence of reducing sugar. The presence of reducing sugars indicated the degradation of rice husk by the isolates [33]. The remaining ten isolates did not produce the characteristic red brown colour of reducing sugar indicating their inability to degrade the rice husk. Although all the isolates had reducing sugar values (Table 5), it was found that only isolates that had values above 20.0mg/g were the ones that their test tubes turned to red brown colour of reducing sugars.

The presence of the 10 isolates that did not degrade rice husk in the dump was not surprising since these organisms have developed a mutual and synergistic relationship with rice husk degrading organisms. Products of cellulose hydrolysis are available as carbon and energy sources for cellulolytic microorganisms and other microbes living in the environment where cellulose is being degraded. In fact, this release of sugars is the main basis of microbial interactions occurring in such environments [1].

The reason the 10 isolates did not degrade the rice husk may be attributed to their inability to secrete lignocelluloses degrading enzymes [7].

The identification of rice husk degrading isolates showed that 4 species were of the genus *Bacillus,* 2 species of the genus *Pseudomonas* and 2 species of the genus *Cellulomonas* (Table 6).

The presence of *Bacillus* species in the dump sites once again lends credence to the versatility and metabolic diversity of this group of bacteria. According to [24] 48 species of the genus *Bacillus* have been identified and listed.

Also the presence of *Pseudomonas* species was not surprising since these are chemoorganotrophs that are able to metabolize varied types of substrates. [36] listed 29 species of the genus *Pseudomonas.*

The genus *Cellulomonas* are confirmed cellulolytic bacteria hence their presence attack cellulose.

All the bacteria genus characterizes in this work had been implicated in the biodegradation of lignocelluloses materials especially with respect to agricultural residues in the soil.

[31] reported the isolation of *Pseudomonas* spp and *Bacillus* spp while [11] reported the isolation of *Bacillus* spp and *Cellulomonas* spp respectively from soil where agricultural wastes were dumped.

Daily analysis of rice husk degrading species showed that day3 recorded the highest concentration of reducing sugars for most of the species for the 5 day period of the hydrolysis (Table 7). This result had a practical implication: this means that if these organism are to be used for the production of reducing sugars using rice husk as a substrate that hydrolysis/fermentation should stop at the 3rd day when the reducing sugar value will be highest and the reducing sugar harvested and purified from the culture broth.

All the rice husk degrading species produced cellulose with activity at its peak on the first day of hydrolysis (Fig. 7). After day one, the cellulose activity for all the organisms decreased.

The decrease in cellulose activity after the period of the highest activity may be attributed to some factors: cumulative effects of cellobiose, a dimmer of glucose is known to inhibit both endoglucanase and β-glucosidase [19]. Decrease in enzymes activity may also suggest that delignification produces aromatic water soluble products that repress the cellulolytic action of the nzyme [24]. This is supported by the findings of [40] who reported the inhibitory effects f accumulated cellobiose and cellodextrin. Also, depletion of carbon and nitrogen sources causes starvation which may inhibit the growth of the organism and hence cellulose activity since cellulose activity is growth released [31].

The analysis of variance result of reducing sugar production by the organisms showed that Feal was greater than Ftan which led to the acceptance of the alternative hypothesis (HI) that stated that there was significant difference in the rate or reducing sugar production by the organisms (Table 8).

For the days, statistical analysis also showed that there were significant differences in the value of reducing sugar produced daily. As stated earlier, day 3 had the highest mean value of 31.8mg/g (Table 8).

The least significant difference ($LSD_{0.05}$) in the production of reducing sugar by the organisms indicated that there were no significant difference between the reducing sugar produced by *Bacillus brevis. Cellulomonas fimi, Pseudomonas mallei* and *Bacillus firmus* which there were significant difference between the reducing sugar produced by the *Bacillus brevis. Cellulomonas fimi, Pseudomonas mallei , Bacillus firmus* and that of *Cellulomonas flavigena* and *Bacillus coagulans* (Table 11). It was therefore concluded that the best rice husk degrading organisms were *Bacillus brevis. Cellulomonas fimi, Pseudomonas mallei* and *Bacillus firmus.*

The least significant difference for the days showed that there were significant difference in the reducing sugar production for day 3 and that of other days except day 4 (Table 12).

The statistical analysis of glucose production by the organisms showed that Fcal was greater than Ftab meaning that there were significant differences in glucose production by the organisms (Table 9).

For the days, statistical analysis showed that there were significant differences in the value of glucose produced daily.

The least significant difference for the organisms indicated that there were significant differences in the glucose produced by *Pseudomonas mallei* compared to the other organisms (Table 13).

The least significant difference for days showed that there were significant difference in glucose production for day I compared to other days (Table 14).

The analysis of variance result of celllulase activity by the organisms showed that Fcal was greater than Ftab thereby causing the alternative hypothesis (HI) that stated that there was significant difference in cellulose activity by the organisms (Table 10) to become acceptable. For the days, statistical showed that there were significant differences in daily cellulose activity.

The least significant difference in cellulose activity by the organisms indicated that there were significant differences in cellulose activity of *Pseudomonas mallei* compared to the other organisms (Table 150). There was on significant differences between the cellulose activities of the other organisms. It was therefore concluded that *Pseudomonas mallei* produced the highest cellulose activity in the course of this study.

The least significant difference for daily cellulose production indicated that there were significant differences in cellulose activity for day I compared to other days (Table 16). There was no significant difference for the other days. In conclusion, day I produced the highest cellulose activity for all the organisms (Table 16).

CONCLUSION

Of the eighteen mesophilic bacteria isolated from an old rice husk dump site at Abakaliki Rice Mill, Ebonyi State, eight were shown to degrade fresh rice husk. The ability to degrade rice husks was indicated by the presence of reducing sugars in the culture filtrates of the organisms. The highest value of reducing sugar in this work was 37.1mg/g recorded at the 3rd day of hydrolysis by *Pseudomonas mallei* (Table 7). This value may seem very small but considering the quantity of rice husk produced daily at Abakaliki rice mill, many tons of reducing sugar that would have been used for the production of useful compounds like reducing sugar are lost annually. According to UNIDO (2005), about forty four thousand nine hundred (44,900) tones of rice husk is produced annually at Abakaliki Rice Mill. Hence, thousand of kilograms of reducing sugar can be recovered from the rice husk if there is commercial production of reducing sugar using rice husk as a substrate and these organisms.

Also, *Pseudomonas mallei* and *Cellulomonas flavgena* because of their high cellulose yield and prolonged cellulose secretion (Fig. 7) have potential in the production of cellulase using rick husk as a substrate. It was therefore recommended that further research on the cellulase producing ability of these organisms on different lignocelluloses materials like corn stubs, maize cobs, wood pellets etc be carried out. Cellulase, with its immense importance is being improted for use in Nigeria at very high cost. The local production of such enzyme using locally available agricultural wastes like rice husk as substrates may therefore reduce the cost of importation of the enzyme and encourage self reliance.

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