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Degradation of Treated Fresh Rice Husk Varieties Using Fungi Isolates from Ebonyi State

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Abstract: Fungi were isolated from rice husk dump site in Abakaliki and identified. The organisms were used to degrade fresh rice husk varieties of R8, R15, R18, Faro 44 and Faro 54 treated mildly with 0.8% solution of sodium hydroxide (Na OH) at room temperature. Thus, chemical treatment is to remove the lignin content and the mild treatment is to generate cost effective method and obtain good results in order to reduce cost for the third world countries. The isolation and identification were done using standard procedure. The organisms used include: *Aspergilus flavus, Aspergillus nidulans, Aspergillus fumigates, Aspergillus terreus* and *Mucor.* The results showed that the organisms degraded the rice husk samples almost at the same rate without significant difference. A slight difference was observed with Faro 54 where there was about one degree difference. These organisms degrade rice husks and can therefore be used to eliminate the menace coursed by rice husks or convert them into other useful forms.

Key words: Fungi · Isolates · Rice Husk · Abakaliki · Ebonyi State

INTRODUCTION

Fungi are eukaryotic non-photosynthetic, heterotrophic-(having absorptive nutrition) micro-organisms, growing as a mass of branching interlacing filaments or as single cells with cell wall composed mainly of chitin or cellulose, glucan and mannan and reproduce sexually and asexually [1, 2]. Fungi are very important in the economy of nature. Without them, many insoluble organic matters may never be degraded in nature and these materials would litter and perhaps disrupt the ecosystem [3]. Fungi are widely distributed and are found wherever moisture is present [4]. They exist primarily as filamentous hyphae. Like some bacteria and protists, fungi digest insoluble organic matter by secreting exoenzymes, then absorbing the solubilised nutrient. In this way they play important role to humans in both beneficial and harmful ways. One of their beneficial activities is their ability to degrade materials of lignocellulosic origin or which contain lignin and cellulose; such as plant materials; rice husks, straw, guinea corn leaves and stalk, maize cobs, millet husk etc [5]. Cellulose is the major constituent of organic matter of plant origin. Lignocellulosic materials are the most abundant and renewable resources on earth. Fortunately, they can be transformed into other useful forms with the help of micro-organisms. Many species of fungi and bacteria that can degrade them have been isolated and more are still being isolated, yet many of these cellulose materials are still found as heaps and slitters in many parts of the world [6, 7]. Some of the species of fungi that have been used to degrade cellulose materials include: *Aspergillus* spp*, Fusarium* spp and *Chaetomiun* spp. Others include: *Trichoderma* spp, *Myrethecium* spp*, Pleurotus* spp and *Penicillium* species [8, 9].

The use of micro-organisms especially fungi to degrade cellulose materials yield a number of organic solvents. The chief among them is ethanol [4]. Bioethanol produced from renewable biomass; rice husks, millet husk, guinea corn leaves and stalks, wheat straw, rice straw, biogasses from local sugar factory, wood pulp etc, has received considerable attention in recent years. Using ethanol as gasoline fuel additive as well as transportation fuel helps alleviate the problems of global warming and

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environmental pollution. This has helped to change the way in which agricultural and some industrial wastes are managed.

MATERIALS AND METHODS

Sample Collection: Samples were collected from rice husk dump site in Abakaliki. Five sterilized conical flasks were covered with foils wrapped in a clean black polythene bag and taken to the site. Pair of hand gloves and a trowel was taken also.

The samples were collected at five different levels on the heap of the rice husk. The first was collected at the base of the heap then another one some distance upward until the top was reached. The levels were labeled; level 1 – level 5, (L1- L5). The samples were collected after removing the surface layer at each level, except at level 5 where the sample was collected on the surface of the heap. The conical flasks were corked immediately the samples were collected. They were taken to the laboratory for processing in order to isolate the fungi organisms involved in their degradation. The isolates will be used to degrade fresh rice husks.

Isolation of Fungi from the Samples: The samples were returned to the laboratory immediately for processing for the isolation of fungi organisms.

The bench was cleaned with 70% ethanol. A piece of foil was placed on the weighing balance and 10 g of the samples were measured into five different 100 ml sterilized beakers. Twenty ml sterile distilled water was added into each of them and stirred gently for 3 min with sterile glass rod. They were allowed to stand for about 5 min. Then 1 ml of each of the supernatant was collected using clean, fresh 5 ml syringes added into five sterilized test tubes containing 9 ml of sterile distilled water. They were shaked gently to mix. Then 0.5 ml of each sample from the test tubes was dispensed using fresh syringes on the surface of the already prepared medium (SDA). This was spread over the surface using sterile spreading rod and allowed to incubate at 25°C – on the table for 4-5 days.

The plates were checked every day for fungal growth. When any growth is observed, a sample of that colony is transferred into a new agar plate to get pure cultures. This was done until no new colony was observed. The new plates containing the cultures were incubated for 4-5 days. They were sub cultured into new agar plates until pure cultures were observed.

Collection and Processing of the Fresh Rice Husks: Fresh rice husks were collected from pure line of rice varieties cultivated by the Alliance for Green Revolution in Africa (AGRA), in collaboration with the Biotechnology Research Centre of Ebonyi State University Abakaliki. This was done through the assistance of AGRA representative in Ebonyi State University. The rice samples were parboiled differently and sun dried for two days on a white sack material. After the drying, they were pounded gradually in a wooden mortar with piston until the rice seeds were removed from the husks and the husks were separated from the rice seeds. The rice husks were further processed by grinding them with dry electric grinding machine. The rice husks used were from five varieties which include: R8, R15, R18, Faro 44 and Faro 54. Care was taken to avoid collecting mixed samples by dismantling and cleaning the machine thoroughly before another sample is introduced into the machine. Then the husks were taken to the laboratory and sieved to equal sizes using a sieve of 0.5mm of pore size.

Chemical Treatment of the Fresh Rice Husks: After sieving the rice husk samples to equal size, some of the five rice husks were treated chemically by soaking 20g of the sieved husks in 100ml of the 0.8% NaOH in 250ml beakers. They were allowed to stand for 24hrs on the table. Then they were washed with tap water using a sieve of 0.2mm of pore size in order to avoid losing most of the small size wet rice husk samples. They were then spread on clean trays and dried under the sun for 2days. The treatment was done to reduce the effect of lignin and hemicelluloses content of the rice husks to the available cellulose component, according to Larry and Judy [2]. However, low percentage of 0.8% NaOH was used in order to establish the effect of low percentage chemical treatment and to keep them close to their natural state.

Determination of Reducing Sugars in the Filtrates: The filtrate from the fermentation was used to determine the amount of reducing sugar released into the medium by the organism(s). The fungi worked on the rice husks, using them as source of carbon. In the process, the rice husks are broken down and the cellulose subunits which are reducing sugars are released into the medium. The amount or the concentration of the reducing sugar in the sample indicates the rate of degradation of the rice husks by the organisms. In this way, the degradation of the rice husks will be determined. Samples were taken from the filtrates of the degradation every day for four days

and tested for reducing sugar. The dinitrosalicylic acid (DNS) method of Miller 1959 was used to determine the amount of reducing sugars in the filtrate.

Quantification of the Presence of Reducing Sugar: To quantify the presence of reducing sugar in the samples, 0.1ml of each of sample starting with the standard was measured from each test tube and added into a cuvette. Then 2.9ml distilled water was added to dilute the color. The cuvette was then returned to the spectrophotometer and the absorbance read at 540nm and the values recorded. The values of the standard and the tests were used to calculate the actual concentration of the reducing sugars in the samples.

Identification of the Organisms: The fungal isolates identified were the five fungi isolates used for the final degradation process after the preliminary degradation test run. To identify the fungi isolates, the microscopic and the macroscopic characteristics of the organisms were observed. For the microscopic observation, three procedures were carried out. First, the tease mount, second, the transparency tape preparation and thirdly, the microslide culture technique.

Determination of Reducing Sugar in the Filtrate: The concentration of the reducing sugar in the filtrate was derived using the absorbance of the filtrate read at 540nm and the concentration of the glucose standard (1000mg/ml). The results are shown in the tables below.
Standard determination of glucose concentration in a sample (conc of glucose standard = (1000mg/L).

$$\frac{\text{Absorbance of test}}{\text{Absorbance of glucose standard}} \text{ X} \frac{\text{Conc of test}}{\text{Conc of standard}}$$

$$\text{Conc of Test} = \frac{\text{Absorbance of test}}{\text{Absorbance of glucose standard}} \text{ X Conc of glucose standard}$$

RESULTS

A variety of fungi organisms were isolated from the rice husk dump, mostly the species of aspergillus and the molds. The isolation was done during the dry season. Five of the isolates were used in the final degradation process after the preliminary test on them for their ability to degrade the rice husk. They are *Aspergilus flavus, Aspergillus nidulans, Aspergillus fumigates, Aspergillus terreus* and *Mucor.*

Table 1: Mean and P values for treated R15 with 0.8% NaOH

Where sample 1=*Mucor*, 2=*Aspergillus fumigatus*, 3= *Aspergillus flavus*, 4= *Aspergillus terreus*, 5= *Aspergillus nidulans* For R15

Organism	Day 1	Day 2	Day 3	Day 4
1	89.772	92.9765^{A}	96.5515	93.113
2	88.537	90.402^{A}	88.0515	94.7315
3	86.0575	90.521^{A}	92.684	79.87106
4	86.8455	90.1175^{A}	95.694	95.596
P-VALUE = 0.05	0.8039	0.9835	0.5775	0.8979
LSD	17.375	14.521	13.631	11.525

Table 2: Mean and P values for R18 treated with 0.8% NaOH.

Where 1 = *Mucor*, 2 = *Aspergillus fumigatus,* 3 = *Aspergillus flavus*, 4 = *Aspergillus terreus*, 5 = *Aspergillus nidulans* For R18

Organism	Day 1	Day 2	Day 3	Day 4
1	94.231	94.648^{A}	93.478	94.1175
2	90.129	86.8235^{BC}	94.3705	97.015
3	89.4	88.8935^{ABC}	91.257	91.607
4	90.895	92.0865^{AB}	93.9255	92.2285
5	88.3595	82.995^{C}	75.51807	94.152
PVALUE=0.05	0.9255	0.0379	0.8308	0.8176
LSD	17.966	6.7292	8.5706	12.515

Table 3: Mean and P values of Faro 54 treated with 0.8% NaOH

Where 1 = *Mucor*, 2 = *Aspergillus fumigatus*, 3 = *Aspergillus flavus*, 4= *Aspergillus terreus*, 5 = *Aspergillus nidulans* For Faro 54

Organisms	Day 1	Day 2	Day 3	Day 4
1	96.154^{A}	96.992^{A}	166.25^{A}	160.357^{A}
2	92.5895^{AB}	89.777^{B}	96.629^{B}	97.352^{B}
3	90.464^{AB}	97.4015^{A}	99.314^{B}	98.9365^{B}
4	88.385^{B}	92.463^{B}	95.6235^{B}	97.6845^{B}
5	89.022^{B}	90.471^{B}	94.6555^{B}	95.437^{B}
PVALUE= 0.05	0.1349	0.0103	<.0001	0.0003
LSD	6.6872	3.8894	6.6116	14.146

Means with the different letter superscripts are significantly different.

Table 4: Mean and P values of R8 treated with 0.8% NaOH

Where 1 = *Mucor*, 2 = *Aspergillus fumigatus*, 3 = *Aspergillus flavus*, 4= *Aspergillus terreus*, 5 = *Aspergillus nidulans* For R8

Organism	Day 1	Day 2	Day 3	Day 4
1	59.944^{B}	90.1615	96.226^{A}	92.342
2	90.0575^{A}	95.3495	98.169^{A}	97.9215
3	85.96^{A}	93.4375	95.53^{A}	92.882
4	86.7605^{A}	89.5295	96.574^{A}	96.225
5	91.3^{A}	92.7835	97.1135^{A}	96.938
PVALUE= 0.05	0.0003	0.5832	0.7289	0.4856
LSD	6.4547	9.8819	5.0161	7.8200

Means with the different letter superscripts are significantly different.

Table 5: Mean and P values of Faro 44 treated with 0.8% NaOH

Where 1 = *Mucor*, 2 = *Aspergillus fumigatus*, 3 = *Aspergillus flavus*, 4= *Aspergillus terreus*, 5 = *Aspergillus nidulans* For Faro 44

Organism	Day 1	Day 2	Day 3	Day 4
1	94.241	96.753^{A}	97.619	93.6455
2	93.2405	93.3755^{A}	97.2405	93.9065
3	88.6415	91.599^{A}	94.4625	98.2625
4	86.608	91.575^{A}	94.4315	93.442
5	90.918	91.541^{A}	90.801	92.974
PVALUE= 0.05	0.4445	0.8769	0.6284	0.7528
LSD	10.918	15.387	11.983	11.361

DISCUSSION

This research was carried out in Abakaliki Ebonyi State Nigeria to assess the possibility of isolating fungal organism(s) from rice husk dumping site which is able to break down or degrade rice husks in a bid to using them to degrade fresh rice husks, so that in future they will no longer be burnt or dumped anyhow to litter the environment but will become useful to us in Nigeria as it is to people in many other parts of the world. A total of 15 fungi organisms were isolated from the dump site during the period. Out of this number, five of them were used to carry out the degradation process. A pure line of rice husks (i.e. not mixed) were used in this research. The varieties of rice used are: faro 54, faro 44, R8, R15 and R18. The study showed that the organisms found on the dumps during the period (dry season) were mostly the species of *Aspergillus* and *Mold*. The organisms did not degrade the rice husks significantly as shown on the tables above as a result of the treatment method of 0.8% NaOH as shown in the tables above. This is in line with the findings of Ndazi *et al*., 2007 and Allen, 1989. They found out that the results obtained from proximate analysis of the alkali treated rice husks have revealed that degradation of lignin is certain and becomes very significant at room temperature when concentration of NaOH is at least 4% and progressively less significant below 2% alkali treatment. The above information was observed with the 0.8% NaOH treatment as the color change from yellow to reddish-brown took a long time to appear after the treatment with the dinitrosalycylic acid (DNS) method of Miller 1959. Therefore chemical treatment with NaOH at room temperature should always be above 2% and especially at 4%. However, the fungi were able to degrade the rice husk samples and can be used to eliminate them from the environment when applied with proper treatment.

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