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PRODUCTION OF BIOETHANOL FROM RICE HUSK USING *Aspergillus niger* AND *Trichoderma harzianum*

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ABSTRACT
The feasibility of bioethanol production from rice husk as an important sustainable alternative source of biofuel with a view to minimize both the emission of green house gases and management of agricultural wastes was undertaken. The content of cellulose and hemicelluloses from the rice husk collected from rice processing site was evaluated followed by ethanol production. The process for bioethanol production involves three basic steps: pretreatment with an alkali, enzymatic hydrolysis using Aspergillus niger and Trichoderma harzianum and sugar fermentation by Saccharomyces cerevisiae, and all carried out in accordance to standard procedures. Results collected revealed that the rice husk contains 38% cellulose and 35% hemicelluloses. The result also revealed that there is significance difference (p<0.05) in the yields of the reducing sugar obtained from the substrate (5g) using Aspergillus niger (2.81g\L) and Trichoderma harzianum (2.77g\L) after 120hrs of hydrolysis. After fermentation of the substrates at 30^{0}C, pH 3.5 for 6 days, it was evidently clear that there was significance difference (p<0.05) in the yields of bioethanol produced by Aspergillus niger (6.99%) and Trichoderma harzianum (6.25%). Aspergillus niger proved to be a better choice in bioethanol production using rice husk substrate when compared to Trichoderma harzianum.

Keywords: Bioethanol, Rice husk, Aspergillus niger, Trichoderma harzianum, Saccharomyces cerevisiae

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INTRODUCTION

Agricultural wastes are among the causes of environmental pollution; their conversion into useful products may ameliorate the problem they cause. Ethanol productions from cellulosic agricultural waste materials offer a solution to some of the recent environmental, economic, and energy problems facing worldwide. Nationally, energy costs are on the rise and forecasts of petroleum supply disruptions are once again making new (Ajeet *et al.*, 2014) .

Rice husk consist on 36–40% cellulose, and 12–19% hemicelluloses (Banerjee *et al.*, 2009). Generally, a large amount of rice husk is dumped as waste which results in waste disposal problem and methane emissions. Moreover, the low density of rice husk can cause it to be air-borne easily resulting in breathing problems if inhaled (Nyachaka *et al.*, 2013). The present study was set up with the aim of using rice husk as a substrate for bioethanol production in view of the present call by government and other organizations for conversion of waste to wealth

MATERIALS AND METHODS

Collection of Samples

Rice husk (fito rice) was collected from local rice processer at Dawanau, Dawakin tofa local government, Kano state Nigeria. Sample rice was packed in a poly-ethane bag and was taken to laboratory for analysis.

Pretreatment of samples

The pretreatment was carried out using NaOH concentration 5% (w/v) (Tutt *et al.*, 2012)

Microorganisms used

Trichoderma harzianum and *Aspergillus niger* were isolated from Nigerian Stored Products Research Institute (NSPRI) soil located at Nasarawa, Kano state. Morphological appearances and microscopic observations were made for the pattern of conidiation and hyphal branching of the pure fungal isolates. Fungal isolates were identified using standard reference manuals and appropriate publications by wet mount preparation (Frazier and Westhoff, 1995; Gams and Bissette 1998; Dubey and Maheshwari, 2004;McClenny, 2005; International Submission on *Trichoderma* and Hypocrea Taxonomy(2015).

Inoculum Preparation

Fungal cultures were inoculated onto potato dextrose agar (PDA) medium in the petri dish, after 72 hrs, the spores were harvested using sterilized water with 0.1% Tween 80 (Ajeet et al., 2014). Spore count was measured with Heamatocytometer (Zakpaa *et al.*, 2009).

Enzyme Hydrolysis

Mineral salt medium (basal medium) was prepared as described by Ali *et al.,* (1991). About 5g per 100ml (basal medium) of the substrate was taken in 250ml conical flask. The flasks were sterilized and inoculated with 2mililiters of inoculums of fungal strains *(*4.0×10^6 cfu *T. harzianum* and *A. niger*) and incubated at 35^0C for 6 days in an orbital shaker at 100 rpm. Uninoculated flasks were used as control. All experiments were done in triplicates (Ajeet *et al.*, 2014).

Fermentation of the hydrolysate to ethanol

Fermentation was carried out using *S. cerevisiae* to ferment the hydrolysed samples to ethanol and carbon-dioxide. A 10ml of inoculum culture of yeast containing 3.8×10^6 cells\ml of *S.cerevisiae* was added to the hydrolysed samples and were incubated at 37^0C for 6days(Zakpaa *et al.*,2009). Whatman filter paper was used to separate the ethanol from residue after fermentation and the bioethanol was distilled (Wong and Sanggari, 2012).

Analytical Methods

Cellulose hemicelluloses and lignin content of the untreated and pretreated substrates were determined using AOAC(2000) method. The amount of reducing sugars in pretreated substrates in the culture broth were determined by dinitrosalicylic acid (DNS) method with glucose as standard. Determination of ethanol content was done using potassium dichromate and a standard calibration graph or Beer's plot of density against percentage volume of ethanol in solution was used in measuring percentage of ethanol in sample (Nwakaire *et al.*, 2013). All the data were subjected to analysis of variance and sample means were tested for significant differences using the Duncan multiple range test (Abdullahi *et al.*, 2014).

RESULTS AND DISCUSSION

Cellulose, hemicelluloses and lignin are the major component of lignocellulosic substrates. After pretreatment of substrates with dilute NaOH, cellulose content increases from 38% to 41% (Table 1). Dilute sodium hydroxide pretreatment of the substrates was found to cause swelling leading to an increase in internal surface area and disruption of the lignin structure which makes cellulose available for enzyme action, this make the lignin content of the substrate to reduce from 20% to 15% after pretreatment (Table 1). Alkali pre-treated sawdust was reported to induce the production of cellulases with high activity in *A. niger* (Acharya *et al.,* 2008). Cold alkaline pre-treatment or in combination with heat treatment by boiling or autoclaving has been reported (Vyas *et al.*, 2005; Ja'afaru and Fagade, 2007).

The reducing sugar concentrations after hydrolysis of rice husk by both organisms showed an exponential increase in yield within 120hrs of incubation. The reducing sugars production increased gradually and significantly ($p\leq0.05$) with time of fermentation from 0hr to 120hrs. A maximum yield of 2.81g m-L of reducing sugar was obtained after 120 hours of incubation. Hydrolysis with of *T. harzianum* produced the yield of $0.20gm^-L$ at 0hr and $2.77g\ m^-L$ after 120 hours (Table 2). The results are compared with those obtained with non inoculated medium (non-inoculated controls) incubated in the respective media ($0.14gm^-L$). Akpan *et al.* (2005) reported maximum glucose production from the highest substrate concentration after 120hr of incubation. *A. niger* was among the three fungi that were found to elaborate higher exoglucanase and endoglucanase activities than those of the fungal strains of the genus *Trichoderma* (Peciulyte, 2007). Various yield of sugar from cellulosic materials has been reported (Jaafaru and Fagade, 2007; Omojasola *et al.,* 2008; Zakpaa, 2009).

The yeast, *Saccharomyces cerevisiae* fermented the sugar produced by hydrolysates to bioethanol and carbondioxide. The concentration of bioethanol increased significantly ($p\geq0.05$) with the increase of fermentation time from 0hr to 120hrs using both fungal isolates after which it decreases significantly ($p\geq0.05$) (Table 3). The reduction of ethanol production may be due to the toxic effect on growth of yeast. Fermentation of the product of waste biomass hydrolysis by the yeast *S.cerevisiae* yielded ethanol in accordance with the sugar concentration obtained from hydrolysis. Hydrolysates with high sugar concentration yielded the highest concentration of bioethanol. This is an indication that the yield of ethanol is directly proportional to concentration of sugar in the fermenting fluid. This result is similar to the findings of Nyachaka *et al.,* (2013) were they reported that the highest ethanol yield of 9.2ml was obtained in the fermentation of hydrolyzate prepared under the optimal hydrolysis conditions for about six days. It also agreed with Yusuf *et al.*, (2008), they reported maximum ethanol concentration was obtained after 6days of incubation.

The maximum of bioethanol was recorded at pH 3.5 and decrease marginally above this value, the minimum production of bioethanol was recorded at pH 5 (Table 4).These results are in agreement with Kamwanna *et al.* (1987), Zohri and Mostafa (2000) showed that the best pH was 3.5 followed by 4.5 for ethanol production. Roukas (1994) studied the effect of pH on ethanol production from carob pod by *S.*

cerevisiae and found that the maximum ethanol concentration, ethanol yield, and fermentation efficiency were obtained at pH 3.5

The optimum temperature for maximal ethanol production was found to be 30°C. The ethanol yield decreased significantly ($p \geq 0.05$) at temperature values lower or higher than 30°C (Table 5). Zohri and Mostafa (2000) reported that the best ethanol concentrations were 9.161 % and 9.104% followed by the most suitable temperatures 30°C and 35°C. Varying the temperature of the fermentation process improves the effective utilization of corn cobs sugars for bioethanol production can be achieved (Yah *et al.*, 2010). However, Afifi *et al.*, (2011) reported that maximum ethanol concentration from solid potato waste was obtained at 25°C.

Table 1: Percentage Cellulose, Hemicelluloses and Lignin Content in of Untreated and Pretreated Samples of Rice Husk.

Composition	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Untreated rice husk	38	35	20
Pretreated rice husk	41	32	15

Table 2: Reducing Sugar Concentration ($g^{-}L$) After Hydrolysis of Rice Husk with *A. niger* and *T. harzianum* at varying Incubation Time

	Time (hrs)					
Organism used	0	24	48	72	96	120
A. niger	0.80 ± 0.02^{a}	1.72 ± 0.03^{c}	2.57 ± 0.03^{c}	2.66 ± 0.01^{d}	2.70 ± 0.05^{d}	2.81 ± 0.04^{e}
T. harzianum	0.77 ± 0.03^{a}	1.54 ± 0.04^{b}	2.71 ± 0.06^{c}	2.58 ± 0.01^{cd}	2.64 ± 0.01^{ef}	2.77 ± 0.02^{f}
Control	0.14	0.14	0.14	0.14	0.14	0.14

Each value represents mean of three independent tests ± standard error. Means displayed with homogenous superscript within the same column are not significantly different p > 0.05

Table 3: Percentage (%) Bioethanol Produced From Hydrolysates at Varying Fermentation Time

	Time (hrs)						
Fungal isolate	0	24	48	72	96	120	144
A. niger	0.0^{a}	2.93 ± 0.04^{b}	3.16 ± 0.04^{c}	4.44 ± 0.00^{d}	6.02 ± 0.00^{c}	6.47 ± 0.00^{f}	6.35 ± 0.11^{f}
T. harzianum	0.0^{a}	2.77 ± 0.03^{b}	3.06 ± 0.04^{c}	4.33 ± 0.00^{d}	4.67 ± 0.11^{f}	6.02 ± 0.01^{f}	5.91 ± 0.01^{g}
Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Each value represents mean of three independent tests ± standard error. Means displayed with homogenous superscript within the same column are not significantly different p > 0.05

Table 4: Effect of pH on Percentage Bioethanol Production from the Hydrolysate Using *A. niger* and *T. harzianum* for 120hrs

	pH				
Isolates	3	3.5	4	4.5	5
A.niger	5.54 ± 0.13^{c}	6.77 ± 0.08^{d}	6.19 ± 0.01^{b}	5.28 ± 0.01^{b}	4.25 ± 0.07^{a}
T.harzianum	5.22 ± 0.02^{c}	6.02 ± 0.05^{c}	5.50 ± 0.12^{d}	4.57 ± 0.08^{b}	3.90 ± 0.01^{a}

Table 5: Effect of Temperature on Percentage Bioethanol Production from the Hydrolysates using *A. niger* and *T. harzianum* at pH of 3.5 for 120hrs

	Temperature				
Isolates	20	25	30	35	40
A.niger	3.13 ± 0.04^{b}	5.67 ± 0.01^{c}	6.99 ± 0.02^{e}	6.28 ± 0.00^{d}	2.76 ± 0.21^{a}
T.harzianum	2.28 ± 0.04^{a}	4.94 ± 0.06^{b}	6.25 ± 0.00^{d}	5.85 ± 0.03^{c}	2.31 ± 0.11^{a}

Table 6: Optimum Physiochemical Parameters for Bioethanol Production using *A. niger* and *T. harzianum*

Isolates	Time (hrs)	pH	Temperature (^{0}C)	Percentage bioethanol
A. niger	120	3.5	30	6.99 ± 0.02^{e}
T. harzianum	120	3.5	30	6.25 ± 0.00^{d}

CONCLUSION

In the present study, bioethanol production process from rice husk using local fungal and yeast isolate was established. Rice husk hydrolysed by *A. niger* yielded the highest concentration of sugar and consequently produced the highest bioethanol yield on fermentation with *S. cerevisiae* at optimal conditions compared to using *T. harzianum*. *A. niger* proved to be a better choice in bioethanol production using rice husk than *T. harzianum*.

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