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Bioethanol Production from Fungal Treated Rice Husks Fermented with Bakers *Saccharomyces cerevisiae* and Yeast Isolates from Palm Wine

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Abstract

This research was necessitated by the quest to create a useful product from wastes (bio-ethanol from rice husk). This if successful will serve two purposes; first, help reduce wastes in the environment and to create wealth from waste. Separated isolates of *Trichophyton soudanense*, *Trichophyton rubrum*, *Trichophyton mentagrophyte*, *Aspergillus oryzae*, *Aspergillus niger* and *Aspergillus fumigatus* were obtained from husks of processed rice undergoing decomposition for more than 8 months. Husks of freshly processed rice were pretreated by autoclave boiling for 20 minutes at a temperature of 121°C after mixing in Mandle's media. The experimental test samples consisted of co-culture combinations and monocultures individually inoculated into various measured heat treated husks; additional control groups were also made. Non-reducing sugar, reducing sugar and total sugar were assayed at the seventh day following hydrolysis. The resulting filtrates of the various husks (treated and control experimental units) were subjected to 7 days fermentation with yeasts from palm wine as well as bakers' yeasts. Values of the result indicated highest trends in the following treatments: *T. mentagrophyte* treated husks with soluble reducing sugar value of 2.66 ± 0.14 g/L, *A. fumigatus* treated husk with soluble non reducing sugar value of 18.08 ± 2.61%, co-culture of *T. soudanense* and *T. rubrum* treated husks gave total sugar value of 20.53 ± 2.73%. Fermented *A. oryzae* treated husk filtrate inoculated with palmwine yeasts had optimal bio-ethanol yield (120.82 ± 0.39 g/L) followed by *A. oryzae* and *T. soudanense* treated husks fermented with bakers yeasts with 60.60 ± 0.10 g/L bio-ethanol. Recognizable yields of bioethanol from palm wine yeast fermented husk as well as sugar from other treated husks were obtained.

Keywords: Carbohydrate; Ethanol; Fungi; Rice husk; Sugar

Introduction

In biological waste treatments, whole organisms or enzymes are used in lignocellulosic wastes pre-treatment. Filamentous fungi in particular are the best studied in relation to submerged saccharified fermentation because of their hyphal growth [1]. Also introduction of yeast can give a reasonable quantity of bio-ethanol if the hydrolysis of the lignocellulosic waste had been carried out successfully by the fungi or their enzymes. The basic features in the production of bio-ethanol from the lignocellulosic materials are: pre-treatment, hydrolysis and fermentation. The pre-treatment methods include: biomass fragmentation, particle size reduction, heat treatment, lignin separation as well as hemicellulose removal [2]. Husk biomass components as well as biofuels were obtained from Aspergillus spp and heat treated rice husks [3] and yeast fermentation.

Also suitable conditions such as pH 5 and 30°C, enhanced enzyme production in fungal treated rice husks [3]. Biofuel generation from total sugar sources may be the perfect substitute for the crude oil source. Rice husk is abundant in all tropical environments thus making it an agro processed waste of choice for research purposes. The goal of this work was to discover the possibilities of obtaining biofuel and other useful components in rice husk after varied hydrolytic treatments.

Materials and Methods

Plant material (Rice husk)

Husks (decomposing and fresh processed) were obtained from Enugu (Adani Rice Integrated Resources Nig. Ltd.,) Nigeria; stored in sealed polythene bags prior to research.

Fungal separation and identification

Sterile distilled water (9 ml) in a beaker and a gram of fungal degraded rice husk were thoroughly stirred to serve as the stock solution for fungal isolation. Into 9 ml of distilled water was added 1 ml from the stock solution. Serial dilution was then carried out to a value of 10^{-6}. On a bench sterilized with alcohol was dispensed 0.1 ml of the fungal solution into 5 separate petri dishes containing potato dextrose agar with chloramphenicol/streptomycin at 45°C. This was incubated for 5 days at 38 ± 0.06°C. pure fungal strains were gotten by aseptically sub culturing up to 3 times from each colony of fungal isolate independently identified.

Culture and fermentation medium preparation

Patel et al. [4] described a medium (Mendel's) which was adopted in this research. Combination of Mandle's medium and rice husk was sterilized for 20 min at 121°C and the pH of 5.5 was retained.

Design of experiment

Twenty one experimental test units of husks, each of 20 g in 400 ml Mandle's solution were prepared, sterilized at 121°C for 20 min, cooled and inoculated with conidia and spores of selected fungi. Control samples: C1 (heat treated husks) and C2 (unheated husks) were also prepared. Both monoculture and co-culture (inoculates) were transferred from PDA petri dishes into sterile tubes using 10 ml of 0.1% Tween 80 solution. Fungal suspensions (1 ml from each sterile tube) were used for each inoculation. All samples were properly labeled. Following successful inoculation, with daily agitation of 90 minutes, the flasks containing the hydrolyzing rice husk, were incubated for 7 days; after which reducing sugar, non-reducing sugar and total sugar were assayed from 1 g of sample residues. Recovered filtrates from each experimental unit was fermented with Saccharomyces spp (Bakers' yeast and Palm wine) for seven days.

Reducing sugar content determination

Dinitrosalicylic acid (DNS) method by Miller [5] was used to determine reducing sugar.

Total sugar (Carbohydrate) content determination

Dubois et al. [6] described sulphuric acid method of total sugar content determination used in this experiment.

Non reducing sugar content determination

Reducing sugar subtracted from total sugar (carbohydrates) gave the non-reducing sugar content from husks.

Bio-ethanol production using bakers' yeast and yeast from palm wine

Filtrates of the husks were fermented for 7 days with Saccharomyces spp (Palm Wine and Bakers' yeast). Sandhu et al. [7] described bio-ethanol recovery method through distillation; which was adopted in this research.

Results and Discussion

Rice husk hydrolyzed with fungi (Figure 1) gave carbohydrate yield which at P>0.05 showed no significant yield compared with the other standards: heated husk (C1) and unheated husk (C2); both without inclusion of fungi.

Although with no significant difference (P>0.05) in yield of total sugar in comparison with each other, fungal hydrolyzed husk with greatest significant yield in ascending order are *A. fumigatus* (19.52 ± 10.05%) and co-culture of *T. soudanense* with *T. rubrum* (20.53 ± 2.73%) respectively. Statistically, hydrolysis of rice husks with heat only was not very effective, however, double hydrolysis (heat followed with fungi) gave added total sugar yield even though there was no significant yield (P>0.05).

Rice husk hydrolyzed with *A. niger* reported by Patel et al. [4] gave 25 mg/g of total sugar which was lesser than the value of 12.49 ± 2.75% gotten from this research.

Fan et al. [8] explained that poor susceptibility and accessibility of cellulolytic enzymes and other hydrolytic agents to cellulose is due to crystallinity and lignification found in the sample.

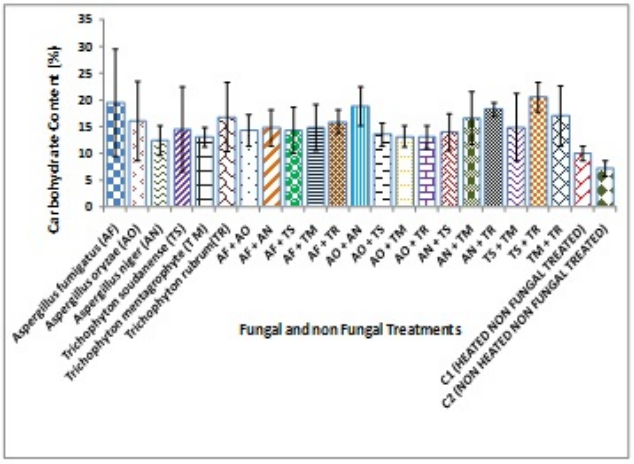

Figure 1: Carbohydrates (Total Sugar) content from fungal-treated rice husk.

This could be the reason for low total sugar released from most of the fungal treated husk.

A. fumigatus and *T. mentagrophyte* hydrolyzed rice husks (2.60 ± 0.30% and 2.66 ± 0.14% respectively) in Figure 2 gave the greatest amount of reducing sugar in this research; in comparison with previous research Patel et al. [4] consisting of *Aspergillus awamori* and *Pleurotus sajor-caju* treated rice husk with values of 14.3 mg/g and 15.35 mg/g reducing sugar there was significant difference (P<0.05) in which values from this work had higher yields. *A. oryzae* and *A. niger* treated husks yielded 2.28 ± 0.07% reducing sugar as the least value recorded.

The general yield of reducing sugar from the estimate can be appreciated as shown in Figure 2.

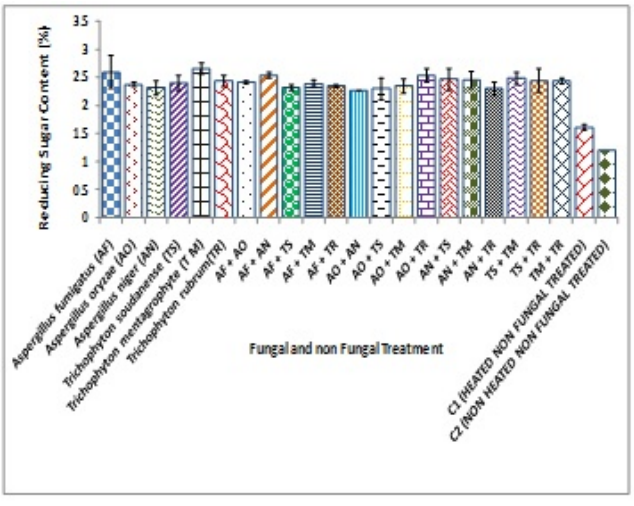

Figure 2: Reducing sugar content from fungal-treated rice husk.

Thus, heat treatment followed by fungal treatment of the rice husk gave statistically significant increased values of soluble reducing sugar

(P<0.05) from the experiment. Nguyen, et al. [9] and Quiroz-Castañeda, et al. [10] explained that pre-treatment (heat and enzymes) also decreases the recalcitrance of crystalline cellulose by generating pores on its surface and making it more accessible to hydrolytic enzyme attack. Reasons alluded above are probably why there was limited soluble reducing sugar by the two methods of hydrolysis used in this research.

For non-reducing sugars (Figure 3), maximum values were obtained from rice husk hydrolyzed with co-cultures of *A. niger* and *T. rubrum* (15.93 ± 1.10%), *A. oryzae* and *A. niger* (16.52 ± 3.53%), *T. soudanense* and *T. rubrum* (18.08 ± 2.61%) as well as monoculture of *A. fumigatus* (16.00 ± 9.75%).

According to Okafoagu and Nzelibe [11], concerted effort of β-glucosidase (cellobiase) and Endo-β-glucanase (1,4-β-D-glucan glucanohydrolase) randomly acts on cellulose (cello-oligosaccharides) giving up its glucose (reducing sugar) as well as Exo-β-glucanase (1,4-β-D-glucan glucanohydrolase or Avicellase) thereby attacking the non-reducing end of cellulose, thus producing cellobiose (non-reducing sugar). This explanation above may be the reason for more non-reducing sugar obtained in this research when compared to the reducing one.

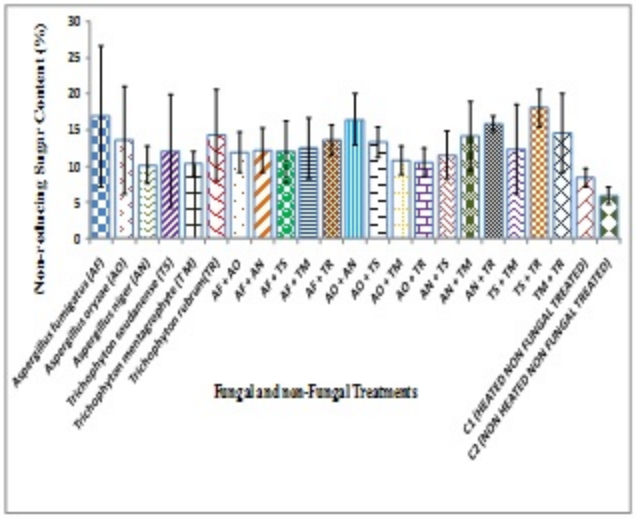

Figure 3: Non-reducing sugar content from fungal-treated rice husk.

Hydrolysis of the husks with fungi (Table 1) followed by bioethanol fermentation with yeast of palmwine source indicated that good bioethanol yield of 120.82 ± 0.39 g/L and 110.11 ± 0.15 g/L was achieved in rice husk hydrolyzed with A. oryzae (group 2) and T. soudanense (group 4) respectively; these values were the highest bioethanol yield in all the treatments.

Table 1 also illustrated appreciable bioethanol yields in the following: *A. niger* and *T. mentagrophyte* (70.01 ± 0.12 g/L), *A. niger* and *T. soudanense* (60.87 ± 0.03 g/L), *A. fumigatus* (60.60 ± 0.48 g/L), *A. niger* (60.46 ± 0.39 g/L), *A. fumigatus* and *T. soudanense* (50.68 ± 0.38 g/L), *A. oryzae* and *T. mentagrophyte* (40.65 ± 0.20 g/L), *A. fumigatus* and *A. oryzae* (40.65 ± 0.17 g/L), *T. soudanense* and *T. mentagrophyte* (40.56 ± 0.20 g/L)

belonging to groups 17, 16, 1, 3, 9, 14, 7, 19 as well as group 21 – *T. mentagrophyte* and *T. rubrum* (40.18 ± 0.11 g/L) respectively. The bio-ethanol obtained from the above mentioned treatments showed significant bio-ethanol increase at P<0.05 when compared to 30.16 ± 0.03 g/L from heated rice husk C1 (group 22). Additionally in Table 1, the following palm wine yeast fermented fungal hydrolyzed husk and their bioethanol yields: *T. mentagrophyte* (30.75 ± 0.35 g/L), *T. rubrum* (30.52 ± 0.36 g/L), *A. fumigatus* and *A. niger* (30.65 ± 0.49 g/L), *A. fumigatus* and *T. rubrum* (30.92 ± 0.21 g/L), *A. oryzae* and *A. niger* (30.97 ± 0.09 g/L), *A. oryzae* and *T. soudanense* (30.52 ± 0.22 g/L), *A. oryzae* and *T. rubrum* (30.67 ± 0.49 g/L), *A. niger* and *T. rubrum* (30.71 ± 0.34 g/L); representing groups 5, 6, 8, 11, 12, 13, 15, 18 and group 20 – *T. soudanense* and *T. rubrum* (3.60 ± 0.13%) did not show statistically significant bio-ethanol yield at P>0.05 when compared to heated rice husk-C1, but showed statistical significant difference at P<0.05 level of significance when compared to C2. Therefore from the result of the experiment generally, yeast from palm wine gave good yield of alcohol (bio-ethanol). Moreover, the amount of reducing sugar produced should translate to the percentage of bio-ethanol generated which is the case in this research. The result obtained in this experiment gave higher yield in comparison to that of Moonjai, et al. [12], in which ethanol production by the simultaneous saccharified fermentation (SSF) of fungal pre-treated rice husk and rice polish were carried out using *L. polychrous* Lev. LP-PT-01 cellulase and *S. cerevisae* cells. In their finding pre-treatment of 100% rice husk with white rot fungi resulted in a low amount of reducing sugar in fermentation medium. However, the concentration of reducing sugars produced on enzymatic hydrolysis increased with increasing rice polish percentage added. Maximum ethanol yield according to their experiment was 0.50, 0.70, 1.14 and 1.53 g ethanol/100 g original dry substrate in SSF experiments with 100% rice husk, 90% rice husk + 10% polished rice, 80% rice husk + 20% polished rice and 70% rice husk + 30% polished rice. Also Patel, et al. [4] in a similar research using *A. niger* to hydrolyze rice husk obtained 1 g/litre of ethanol indicating lesser yield to that obtained in current research. This could be due to difference in fermentation methods or environmental factors such as soil type, different methods used in cultivation of the rice as well as the difference in biological formation of the biomass contents such as cellulose, hemicellulose and sugar contents which varies between their rice husk and those used in this experiment. Moreover, the aforementioned researchers used bakers' yeast (*S. cerevisae*) in their fermentation. Using Saccharomyces (yeast) from palm wine in production of bio-ethanol from fungal hydrolyzed rice husk has been established as the best means of encouraging maximal yield as shown by the results in this research. Perhaps, this is due to easy adjustment to the environmental conditions of the rice husk which is similar to the palm tree from which they were originally sourced.

Table 1 clearly illustrated that mono and co-culture fungal hydrolyzed rice husk treatments fermented with *S. cerevisae* (Bakers' yeast) gave values with statistically significant increase in bioethanol production at P<0.05 level of significance in comparison to heated rice husk (C1) and unheated rice husk (C2) (control groups 22 and 23). Between heated rice husks (C1) and unheated rice husks fermented with bakers' yeast, the heated rice husk gave statistically significant bioethanol yield (P<0.05).

Groups	Treatments shown below indicates rice husk hydeolysed with various fungi and controls viz:	Palm wine yeast (g/L) bioethanol Mean ± SEM	Baker's yeast (g/L) bioethanol ± SEM mean ± SEM
1	*A. Fumigatus*	60.6 ± 0.48*b	50.60 ± 0.42*b
2	*A. orizae*	120.82 ± 0.39*a	40.85 ± 0.03*b
3	*A. niger*	60.64 ± 0.39	40.37 ± 0.02*b
4	*T. soudanense*	110.11 ± 0.15*a	40.11 ± 0.09*b
5	*T. mentagrophyte*	30.75 ± 0.35c	50.14 ± 0.10*b
6	*T. rubrum* treated rice husk	30.52 ± 0.35c	40.95 ± 0.15*b
7	*A. Fumigatus* and *A. orizae*	40.65 ± 0.17*b	40.50 ± 0.33*b
8	*A. Fumigatus* and *A. Niger*	30.65 ± 0.49c	40.86 ± 0.16*b
9	*A. Fumigatus* and *T. soudanese*	50.68 ± 0.38*b	40.70 ± 0.17*b
10	*A. Fumigatus* and *T. mentagrophyte*	40.47 ± 0.13*b	40.38 ± 0.24*b
11	*A. Fumigatus* and *T. rubrum*	30.92 ± 0.21c	40.79 ± 0.21*b
12	*A. orizae* and *A. niger*	30.97 ± 0.09c	60.02 ± 0.14*b
13	*A. orizae* and *T. soudanense*	30.52 ± 0.22c	60.56 ± 0.1.*b
14	*A. orizae* and *T. mentagrophyte*	40.65 ± 0.26*b	60.15 ± 0.08*b
15	*A. orizae* and *T. rubrum*	30.67 ± 0.49c	50.89 ± 0.25*b
16	*A. niger* and *T. soudanense*	60.87 ± 0.03*b	40.55 ± 0.12*b
17	*A. niger* and *T. mentagrophyte*	70.01 ± 0.12*b	50.46 ± 0.40*b
18	*A. niger* and *T. rubrum*	30.71 ± 0.34c	30.98 ± 0.15*b
19	*T. soudanense* and *T. mentagrophyte*	40.56 ± 0.20*b	50.43 ± 0.39*b
20	*T. soudanense* and *T. rubrum*	30.60 ± 0.13c	40.03 ± 0.13*b
21	*T. mentagrophyte* and *T. rubrum*	40.18 ± 0.11*b	40.31 ± 0.02*b
22	Heated rice husk	30.16 ± 0.03c	30.05 ± 0.03c
23	Unheated rice husk	20.40 ± 0.08d	20.24 ± 0.12d
Note: Percentage mean with different alphabets (a,b,c,d) differ significantly at P<0.05. The groups with asterisks (*) shows significant yield of ethanol at P<0.05			

Table 1: Percentage ethanol yield by the various rice husk treated groups fermented with yeast from palm wine and Bakers' yeast.

Inference drawn from the above result is that heated rice husks and rice husks hydrolyzed by heat and subsequently by fungi when fermented with bakers' yeast will likely yield appreciable quantity of bioethanol. *A. oryzae* and *T. soudanense* hydrolized rice husk as well as *A. oryzae* and *T. soudanense* hydrolized rice husk (groups 13 and 14) each fermented with bakers' yeast gave highest yield of bioethanol (60.56 ± 0.10 g/L and 60.56 ± 0.10 g/L) in that group. This is similar to the results obtained by Patel, et al. (2007) collaborated this finding when using *Apergillus awamori* and *Pleurotus sajor-caju* in hydrolyzing rice husk and bagasse and fermenting with bakers' yeast achieved good ethanol yield of 8.5 g/L and 9.8 g/L respectively. Rice husks hydrolysates with fungal co-cultures containing *A. oryzae* in combination with other fungi as depicted in Table 1; fermented with bakers' yeast, gave the best bioethanol yield in this research. From the foregoing, *A. oryzae* clearly is a choice fungus for hydrolysis of carbohydrates for ease of fermentation to bioethanol. *A. fumigatus* and *T. mentagrophyte* (groups 1 and 5) hydrolyzed rice husks fermented with bakers' yeast gave 50.60 ± 0.42 g/L and 50.14 ± 0.10 g/L bioethanol yield; the highest obtained among the monoculture hydrolyzed rice husk.

Conclusion

Discoveries from this research showed that rice husks hydrolyzed by heating and further with fungi released harness-able soluble sugar. Carefully fermenting these released sugar with selected yeasts as described in this research will give an appreciable yield of bioethanol. Thus, scaling this up to industrial level will give commercial quantity of bioethanol. Since rice husk may be gotten at little or no cost, the quantity used for obtaining bioethanol may be high but yet economically feasible. Yeast from palm wine source showed more

usefulness in generating bioethanol from rice husk than that from bakers' yeast.

Heat hydrolysis of rice husk alone was not sufficient in generating significant quantities of carbohydrate, reducing sugar and non-reducing sugar. Rice husks hydrolyzed with *A. oryzae* and its co-cultures fermented with baker's yeast gave acceptable yield of bio-ethanol.

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References

1. Toor Y, Ilyas U (2014) Optimization of cellulose production by Aspergillus ornatus by the solid state fermentation of Cicer arietinum. Am J Res Commun 2: 125-141.
2. Taherzadeh MJ, Karimi K (2008) Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: A review. Int J Mol Sci 9: 621-1651.
3. Ezeonu CS, Otitoju O, Onwurah INE, Ejikeme CM, Ugbogu OC, et al. (2014) Enhanced availability of biofuel and biomass components in Aspergillus niger and Aspergillus fumigatus treated rice husk. Eur Sci J 10: 97-117.
4. Patel SJ, Onkarappa R, Shobha KS (2007) Comparative study of ethanol production from microbial pretreated agricultural residues. J Appl Sci Environ Manage 11: 137-141.
5. Miller GL (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugars. Anal Chem 31: 426-429.
6. Dubois M, Gilles KA, Rebers PA, Smith F (1956) Phenol sulphuric acid method for total carbohydrate. Anal Chem 25: 350.
7. Sandhu H, Bajaj KL, Arneja JS (1998) Biochemical studies on bioconversion of rice straw to ethanol. Indian Journal Ecology 25: 62-65.
8. Fan LT, Gharpuray MM, Lee YH (1987) Cellulose Hydrolysis. Berlin, Germany: Springer-Verlag 3: 1-68.
9. Nguyen QA, Tucker MP, Keller FA, Eddy FP (2000) Two-stage dilute-acid pretreatment of softwoods. App Biochem Biotechnol 84-86: 561-576.
10. Quiroz-Castañeda RE, Balcázar-Lopez E, Martinez A, Folch-Mallol J, Anaya CM (2008) Characterization of cellulolytic activities of Bjerkandera adusta and Pycnoporus sanguineus on solid wheat straw medium. Electron J Biotechnol 12: 1-8.
11. Okafoagu CU, Nzelibe HC (2006) Effect of acid hydrolysis of Garcinia kola (bitter kola) pulp waste on the production of CM-cellulase and ß-glucosidase using Aspergillus niger. Afr J Biotechnol 5: 819-822.
12. Moonjai N, Pukahuta C, Salubchua J (ND) Simultaneous saccharificatiion and fermentation of fungal bio-pretreated rice husk and rice polish to ethanol. Department of Biological Sciences, Faculty of Sciences, Ubon Ratchathani University, Ubon Ratchathani 34190, Thailand.