ESTIMATION OF TOTAL CARBOHYDRATE AND SUGAR CONTENTS OF FUNGI TREATED RICE HUSKS

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Abstract: Fungi isolates were obtained from decomposing rice husk using potato dextrose agar (PDA). These isolateswere *Aspergillus fumigatus* (AF), *Aspergillus niger*(AN), *Aspergillus oryzae* (AO), *Trichophyton mentagrophyte* (TM), *Trichophyton rubrum* (TR) and *Trichophyton soudanense* (TS). Pure strain of each fungal culture was obtained and identified. Measured quantities of freshly processed rice husk in Mandle's medium were heat pre-treated in an autoclave at 121^{o}C for 20 minutes. The isolated fungi as monoculture and co-culture combinations were inoculated into each of the pre-treated rice husks with the exception of two controls (heated and non fungal treated rice husk -C1 and non-heated and non-fungal treated rice husk -C2). A total of 23 rice husk treated groups were hydrolyzed for seven days and total carbohydrate, reducing sugar and non-reducing sugar contents were estimated. Data analysis was by analysis of variance (ANOVA). The rice husks treated with *Trichophyton soudanense* and *Trichophyton rubrum* (TS + TR) co-culture; *Aspergillus fumigatus* (AF) monoculture; *Aspergillus oryzae* and *Aspergillus niger* (AO + AN) co-culture yielded the highest carbohydrate contents of 20.53 ± 2.73 %, 19.52 ± 10.05 % and 18.80 ± 3.59%, respectively. The highest soluble reducing sugar contents of 2.66 ± 0.14 % and 2.61 ± 0.30 % were obtained from the rice husks treated with monocultures of *Trichophyton mentagrophyte* (TM) and *Aspergillus fumigatus* (AF), respectively. The highest soluble non reducing sugar contents of 18.08 ± 2.61 % and 16.92±9.75 % were obtained from the combination of *Trichophyton soudanese* and *Trichophyton rubrum* (TS+TR) and *Aspergillus fumigatus* (AF) treated rice husks, respectively.

Keywords: Carbohydrate, co-culture, fungi, monoculture, rice husk, sugar, treatment.

Introduction

To develop the carbohydrate potential of bio-waste materials, their cellulose content has to be converted into sugars such as glucose that can be used as starting compounds in the biosynthesis of many bioproducts (Okonko *et al*., 2009).Biological degradation of cellulose to soluble sugars has long been considered an alternative to the use of starch feedstocks for bioethanol production (Chen *et al*., 2010). Natural cellulose is an ordered, linear polymer of thousands of d-glucose residues linked by β-1,4-glucosidic bonds. Most chemical and biological treatment processes of rice husk and other agricultural grain processed wastes are met with great challenges such as relatively low yield of sugar, difficult reaction conditions (high temperature, uncertain varied pH and high pressure), accompanied by large capital investment, high processing costs, and great investment risks. Despite this, the search for alternative sources of biofuel such as from carbohydrate renewable sources other than from petroleum feed stock which is non-renewable must be carried out.

Cellulose plant material represents a huge source of untapped fermentable sugars for significant use, especially non-food lignocellulosic waste products like rice husk, rice straw, wheat straw, baggasse etc (Patel *et al*., 2007). Since lignocelluloses are components of lignin, cellulose and hemicelluloses, the lignin which is a major barrier in the utilization of the carbohydrate available in the cellulose and hemicellulose must be removed to obtain sugar. Effectively releasing the locked polysaccharides from recalcitrant lignocellulose to fermentable sugars is among the greatest technical and economic barriers to the realization of lignocellulose biorefineries. The reason for this is that leading lignocellulose pre-treatment technologies suffer from low sugar yields, severe reaction conditions, and high cellulase use in addition to narrow substrate applicability and high capital investment (Zhang *et al*., 2007).

The essence of heat treatment is to reduce the highly ordered crystalline structures of cellulose to the amorphous form. Amorphous cellulose will be much simpler to hydrolyse with enzymes than its crystalline form, since hydrolysis of amorphous cellulose can be conducted by only endoglucanase and β-glucosidase without exoglucanase (Zhang and Lynd, 2006). Pretreatment is required to alter the structures of cellulosic biomass to make more accessible to the enzymes that convert the carbohydrate polymers into fermentable sugars (Monsier *et al*., 2005). The hydrolysis of cellulose can make available the sugar component which will eventually be converted to fine products such as ethanol and other organic acids through fermentation by yeast (Ezeonu *et al*., 2014). This research evaluated the quantity of carbohydrate and sugar present in rice husk when heat treated as well as when hydrolyzed with enzymes from fungi.

Materials and Methods

Plant sample (rice husk)

Freshly processed rice husks and 8 months decomposing rice husk were collected from Adani Rice Integrated Resources Nig. Ltd., Adani in Uzo-Uwani Local Government Area of Enugu State, Nigeria. All samples were kept in air tight cellophane bags before use.

Isolation, screening and characterization of fungi

Decomposing rice husk (1g) was added to 9ml of sterile distilled water in a beaker and mixed thoroughly. This served as the stock for the isolation of the fungi. Serial dilution of the sample was carried out by pipetting 1ml of

the stock solution into another 9ml of distilled water. The sample suspension was further diluted to 10^{-6}. From 10^{-6} serial diluted (fungal sources) stock, 0.1ml was pipetted into five different petri dishes containing freshly prepared potato dextrose agar with inclusion of streptomycin/chloramphenol (Antibiotics) at 50°C on an alcohol sterilized bench. Spreading of inoculum was done by the pour plate method followed by gentle agitation to enable uniform spread. This was carried out using standard sterilization techniques in the presence of gentle Bunsen flame. The inoculated plates were incubated in a laboratory incubator at room temperature of 38±0.06°C for 5 days. Growth was monitored daily and identification of the various fungal colonies carried out. Subcultures (3 times for each identified colony) from the various plates were carried out by aseptically transferring each independently identified colony isolate into other potato dextrose agar slants (containing antibiotics) until pure fungal strains were obtained (for any batch the incubation was at room temperature recorded for 5 days). Pure fungal isolates were stored in culture tubes plugged with cotton wool in a refrigerator at 4°C for further use. Subsequent culturing of the identified pure fungal strains were carried out using PDA in Petri dishes with the inclusion of streptomycin/chloramphenol (mixture of 6g in 10 ml) afterautoclaving which was followed by incubation at room temperature for 6 days before use.

Preparation of culturing and fermentation medium

For the cultivation of the fungi and hydrolysis of the rice husk, Mendle's medium was prepared as reported by Patel *et al.* (2007). One litre of Mandle's medium contained the following mineral salt ingredients: $(NH_4)_2SO_4$(1.4g), KH_2PO_4(2.0g), Urea(0.3g),$CaCl_2.2H_2O$(0.4g), $MgSO_4.7H_20$(0.6g),$MnSO_4.H_2O$ (1.0mg),$ZnSO_47H_2O$ (1.4mg),$FeSO_4.7H_2O$(5.0mg), $CoCl_2.6H_2O$(3.7mg),Protease-peptone (0.75g),Tween 80(2.0mg).The medium (rice husk in Mandle's medium) was sterilized at 121°C for 20 min and the pH adjusted to 5.5.

Experimental design for the fungal treatment of rice husk

The experimental method was modification of Patel *et al.* (2007). Into each 500ml conical flasks used in the experiment, 20g of rice husks were weighed (total of 23 samples) and 400ml of Mandle's medium introduced. Sterilization of the various conical flasks plugged with cotton and covered with aluminium foil was carried out using an autoclave at 121°C for 20 min and cooled. Each conical flask except the controls (C1= non fungal but heat treated sample; C2 = non fungal, non heat treated sample) was inoculated with the fungi by addition of 10ml of 0.1% Tween 80 into PDA Petri dishes of pure fungal isolates both as monoculture and co-culture (inoculates) as shown in the treatments and controls to give the arranged in Table 1 below. This was achieved by proper labelling and aseptic transfer of fungi conidia and spores into sterile tubes with the aid of sterilized cotton swabs.

Table 1: Experimental groups and treatments

Group	Treatment
Group 1	*Aspergillus fumigatus* treated rice husk (AF)
Group 2	*Aspergillus orizae* treated rice husk (AO)
Group 3	*Aspergillus niger* treated rice husk (AN)
Group 4	*Trichophyton soudanense* treated rice husk (TS)
Group 5	*Trichophyton mentagrophyte* treated rice husk (TM)
Group 6	*Trichophyton rubrum* treated rice husk (TR)
Group 7	*Aspergillus fumigatus* and *Aspergillus orizae* treated rice husk (AF+AO)
Group 8	*Aspergillus fumigatus* and *Aspergillus niger* treated rice husk (AF+AN)
Group 9	*Aspergillus fumigatus* and *Trichophyton soudanense* treated rice husk (AF+TS)
Group 10	*Aspergillus fumigatus* and *Trichophyton mentagrophyte* treated rice husk (AF+TM)
Group 11	*Aspergillus fumigatus* and *Trichophyton rubrum* treated rice husk (AF+TR)
Group 12	*Aspergillus orizae* and *Aspergillus niger* treated rice husk (AO+AN)
Group 13	*Aspergillus orizae and Trichophyton soudanense* treated rice husk (AO+TS)
Group 14	*Aspergillus orizae* and *Trichophyton mentagrophyte* treated rice husk (AO+TM)
Group 15	*Aspergillus orizae* and *Trichophyton rubrum* treated rice husk (AO+TR)
Group 16	*Aspergillus niger* and *Trichophyton soudanense* treated rice husk (AN+TS)
Group 17	*Aspergillus niger* and *Trichophyton mentagrophyte* treated rice husk (AN+TM)
Group 18	*Aspergillus niger* and *Trichophyton rubrum* treated rice husk (AN+TR)
Group 19	*Trichophyton soudanense* and *Trichophyton mentagrophyte* treated rice husk (TS+TM)
Group 20	*Trichophyton soudanense* and *Trichophyton rubrum* treated rice husk (TS+TR)
Group 21	*Trichophyton mentagrophyte* and *Trichophyton rubrum* treated rice husk (TM+TR)
Group 22	Heated but non fungal treated rice husk(C1)
Group 23	Heated and non fungal treated rice husk (C2)

From each sterile tube, 1ml fungal suspension was used for the inoculation. The flasks were incubated at room temperature for 7 days with 90 minutes daily agitation. The mycelium were separated by filtration through Whatman filter paper No. 1 and discarded. The filtrate was recovered while the treated rice husk residues were dried on filter paper using an oven temperature of 105°C for 10 min. From each treatment, 1g was used to determine carbohydrate, reducing sugar and non-reducing sugar contents in triplicates.

Estimation of reducing sugar

This was determined by the Dinitrosalicylic acid (DNS) method as described by Miller (1959) and previously reported by Ezeonu *et al.* (2014).

Calculation

Absorbance corresponding to 0.1mL of test = x mg of glucose

10 mL contains = $\left(\frac{\chi}{0.1} X100mg\right)$ of glucose = % of reducing sugars.

Estimation of total sugar (carbohydrate)

The carbohydrate content was determined by the phenol sulphuric acid method as described by Dubois *et al*. (1956). In hot acidic medium glucose is dehydrated to hydroxymethyl furfural. This forms a green coloured product with phenol and has absorption maximum at 490nm. The method used in this study was the modification of the method described by Dubois as reported by Ezeonu *et al.* (2014).

Calculation

Absorbance corresponds to 0.1 mL of the test = *x* mg of glucose.

10 mL of the sample solution contains = $\left(\frac{\chi}{0.1} X100mg\right)$ of glucose = % of total carbohydrate present.

Non reducing sugar: This was obtained by subtracting the values of the reducing sugar from the total sugar.

Statistical analysis

The statistical package used is mean percentage and analysis of variance (ANOVA)using SPSS version16.

Results and Discussion

The total carbohydrate contents of the heat and fungal hydrolyzed rice husks are shown in Fig.1. The fungal treated rice husks had carbohydrate contents that were not significantly different from those of the two controls; Group 22 (heated but non fungal treated rice husk (C1) and Group 23 (non heated and non-fungal treated rice husk (C2). The carbohydrate contents of some of the fungal treated rice husks were: Group 3 =*Aspergillus niger* (AN) with 12.49 ± 2.75%, Group 4 = *Trichophyton soudanense* (TS) with14.50 ± 7.90%, Group 5 = *Trichophyton mentagrophyte* (TM) with 13.06 ± 1.87%, Group 13 = *Aspergillus oryzae* and *Trichophyton soudanense* (AO+TS) with 13.63 ± 2.16%, Group 15 = *Aspergillus oryzae* and *Trichophyton rubrum* (AO+TR) with 13.06± 2.15% and Group 14 = *Aspergillus oryzae* and *Trichophyton mentagrophyte* (AO + TM) with 13.20 ± 2.01%. The fungal treatments with higher values of carbohydrate yield that still had no significant difference at P >0.05 level of significance include: Group 20 = *Trichophyton soudanense* and *Trichophyton rubrum* (TS + TR) with 20.53 ± 2.73%, group 1= *Aspergillus fumigatus* (AF) with 19.52 ± 10.05%., Group 12 = *Aspergillus oryzae* and *Aspergillus niger* (AO + AN) with 18.80 ± 3.59%, Group 18 = *Aspergillus niger* and *Trichophyton rubrum* (AN + TR) with 18.23 ± 1.30%, Group 21 = *Trichophyton mentagrophyte* and *Trichophyton rubrum* (TM + TR) with 17.08 ± 5.60%, group 6 = *Trichophyton rubrum* (TR) with 16.79 ± 6.46% and group 2 = *Aspergillus oryzae* with 16.08 ± 7.46%. From the statistical observations therefore, heat treatments alone is quite inefficient and heat treatment and fungal treatment gave improved yield of carbohydrate which is statistically not significant at P > 0.05 level of significance.

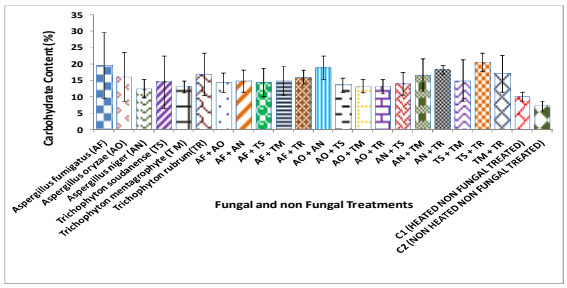

Fig. 1: Total carbohydrate content of fungal-treated rice husks

The results showed that heat treatment alone was inadequate in generating carbohydrate from the rice husks. Heat treatment and fungal treatment of rice husk were also inadequate in generating high amount of carbohydrate from rice husks. The result of this experiment also gave higher value. The total sugar content (39.00 ± 1.00%) obtained in this study was higher than that of 25 mg/g for *Aspergillus niger* treated rice husk reported by Patel *et al*. (2007). The reason for the non significant increase in the quantity of carbohydrate released from rice husk in the majority of fungal treatments could be due to the explanation by Fan *et al*. (1987) that crystallinity and lignification could limit the accessibility and susceptibility of cellulose to cellulolytic enzymes and other hydrolytic agents.

Heat treatment alone of Group 22 (heated but non fungal treated rice husk control - C1) gave reducing sugar value of 1.61 ± 0.21% which was quite insignificant when compared to that of group 23 (Non heated and non fungal treated rice husk - C2) (Fig. 2) with a value of 1.21 ± 0.05%. Therefore, C1 value did not show any significant increase at P >0.05 level of significance when compared to C2. Thus heating alone was not enough to release the reducing sugar in appreciable quantities from the rice husk. The entire fungal treated rice husk gave reducing sugar values which showed statistical significant increase at P<0.05 against the controls C1 and C2. The Fig. 2 indicates that group 5 = *Trichophyton mentagrophyte* (TM) and group 1 = *Aspergillus fumigatus* (AF) treated rice husk with values of 2.66 ± 0.14% and 2.60 ± 0.30% respectively gave the highest amount of reducing sugar. This is in agreement with the work of Patel *et al*. (2007) in which rice husk treated with two fungi *Aspergillus awamori* and *Pleurotus sajor-caju* gave good reducing sugar yield of 14.3 mg/g and 15.35 mg/g which were significantly different at P < 0.05 in comparison to the control (untreated rice husk) which had a value of 2.6 mg/g.

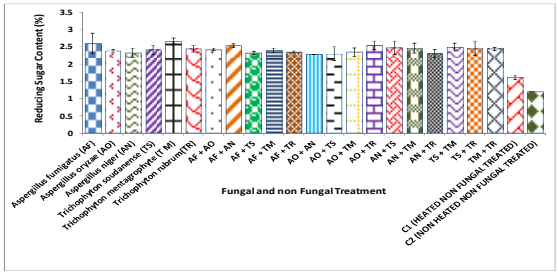

Fig. 2: Reducing sugar content of fungal-treated rice husk

The lowest value of reducing sugar was that obtained from treatments of rice husk with *Aspergillus oryzae* and *Aspergillus niger* (AO + AN) = group 12 with a value of 2.28 ± 0.07%. The general yield of reducing sugar from the estimate is as shown in Fig. 2. Thus, heat treatment followed by fungal treatment of the rice husk gave statistically significant increased values ($P < 0.05$) of soluble reducing sugar from the research. Nguyen *et al*. (2000) and Quiroz-Castañeda *et al*. (2009) 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. This is likely to be the cause of the soluble reducing sugar content obtained by the use of these two methods of rice husk treatment in this research work.

Fig. 3 gave the values of the highest mean percentage contents of non reducing sugar from the treatments in the following order: 18.08 ± 2.61% from group 20 = *Trichophyton soudanense* and *Trichophyton rubrum* (TS + TR), 16.00 ± 9.75% from group 1 = *Aspergillus fumigatus* (AF), 16.52 ± 3.53% from group 12 = *Aspergillus oryzae* and *Aspergillus niger* (AO + AN) and 15.93 ± 1.10% from group 18 = *Aspergillus niger* and *Trichophyton rubrum* (AN + TR) treated rice husk. The least value of the non reducing sugar from the experiment was 10.17 ± 2.62% from group 3 = *Aspergillus niger* (AN) treated rice husk. This result is not in concordance with the work carried out by Patel *et al.* (2007) in which the values of the reducing sugar was higher than those of the non reducing sugar. In the experiment most fungal treated rice husk especially those listed above with high mean percentage contents of non reducing sugar values showed statistical significant increase at $P < 0.05$ level of significance.

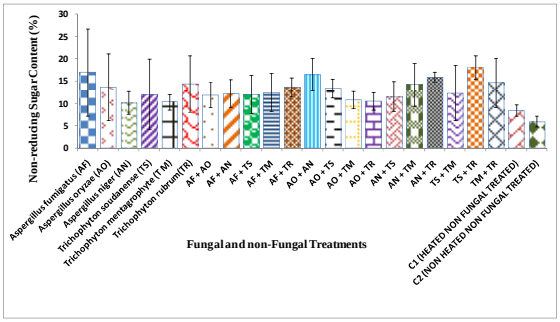

Fig. 3: Non-reducing sugar content of fungal-treated rice husk

The higher contents of non reducing sugar compared to the reducing sugar was expected as explained by Okaforagu and Nzelibe (2006) that for reducing sugar to be formed concerted efforts of three enzymes with specific functions must come into play. For instance Endo-β-glucanase (1,4-β-D-glucan glucanohydrolase) acts randomly on cellulose chains yielding glucose (reducing sugar) and cello-oligosaccharides. Due to this random action less reducing sugar may likely be generated. Also Exo-β-glucanase (1,4-β-D-glucan cellobiohydrolase or Avicellase) attacks the non-reducing end of cellulose yielding higher quantities of cellobiose (non-reducing sugar). Thus for more reducing sugar to result which was the limitation of the enzymes acting from the fungal treated rice in this experiment, enzymes such as β-glucosidase (cellobiase) must be robust and plentiful in the fungal treated rice husk broth so as to finally hydrolyse cellobiose to glucose. Heat treatment no doubt helped in the hydrolysis of non-reducing sugar to reducing sugar in the experiment, but the rate was likely minimal to gain appreciable quantity of reducing sugar.

Conclusion

Heat treatment alone of rice husk was inadequate in generating significant quantities of carbohydrate, reducing sugar and non reducing sugar. Also heat treatment and fungal treatments of rice husk was inadequate in causing significant increase in carbohydrate content ($p > 0.05$). However, depending on the type of fungi used, most of the rice husks had significant ($p<0.05$) increases in both non reducing and reducing sugars, especially when treated with *Trichophyton soudanense* and *Trichophyton rubrum* (TS + TR), *Aspergillus fumigatus* (AF) or *Aspergillus oryzae* and *Aspergillus niger* (AO + AN).

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Conflict of Interest

Researchers hereby declare that there is no conflict of interest whatsoever in this research.

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