MATTER: International Journal of Science and Technology
ISSN 2454-5880

Ezeonu, Chukwuma Stephen, 2016

Volume 2 Issue 1, pp. 21-31

Year of Publication: 2016

DOI- http://dx.doi.org/10.20319/mijst.2016.21.2131

This paper can be cited as: Stephen, E. C. (2016). Lignocelluloses, Saccharides and Bio-Ethanol Yields in Fungal Di-Culture Treated Rice Husk. MATTER: International Journal of Science and Technology, 2(1), 21-31.

LIGNOCELLULOSES, SACCHARIDES AND BIO-ETHANOL YIELDS IN FUNGAL DI-CULTURE TREATED RICE HUSK

Ezeonu, Chukwuma Stephen
Department of Biochemistry, Federal University Wukari, Taraba State, Nigeria,
chuksmaristos@yahoo.com

Abstract

Pure monoculture strains of Aspergillus fumigates (AF), Aspergillus niger (AN), Aspergillus oryzae (AO), Trichophyton mentagrophyte (TM), Trichophyton rubrum (TR) and Trichophyton Sudanese (TS) were isolated from decomposing rice husk and di-culture combination in equal proportion used for treatment of fresh heat treated rice husk. Freshly processed rice husk in Mantle's medium, were heat pre-treated using an autoclave at 121^{o}C for 20 minutes. The isolated fungi di-culture combinations were inoculated into each of the pre-heated rice husk with the exception of two controls (heated rice husk C1 and untreated rice husk C2). Seven days rice husk fungal di-culture hydrolysis was followed by estimation of saccharine, lignocelluloses and bio-ethanol yields. Fungal did-culture treated rice husks were left to ferment for 7 days with introduction of both bakers' and palm wine yeast. The result (in percent ± standard error of mean) obtained in the work gave the highest carbohydrate (20.53 ± 1.39 %) from rice husks treated with TS + TR did-culture. Other highest values obtained were as follows: soluble reducing sugar percentage yield (2.54 ± 0.37 %) was obtained from AF + AN as well as AO +

TR; soluble non reducing sugar (18.08 ± 1.10 %) was from TS + TR; total lignin percentage yield of 26.00 ± 2.06 % was from rice husk treated with AN+TR; cellulose yield of 66.00±0.96 and hemicelluloses yield of 32.50±1.78% were obtained from AN+TS and AO+TR fungal did-culture treated rice husks respectively. The introduction of yeasts from palm wine gave the highest bio-ethanol yield (14.18 ± 0.13 %) from TM + TR treated rice husk broth, while 6.56 ± 0.26 % of bio-ethanol was from AO + TS treated rice husk fermented with baker's yeast. The selective treatment of rice husk with the fungal did-cultures illustrated to give the highest yield in each of the components carried out in this study will be ideal for optimal production of the parameters studied from rice husk.

Keywords

Fungi, Rice Husk, Di-culture, Saccharine, Lignocelluloses, Bio-Ethanol

1. Introduction

Lignocelluloses are the most abundant biomass on earth readily available in every environment. Certainly, rice husk which is a basic waste from processed rice is a good example of wastes from agro-processed grains. These wastes in addition to their lignocelluloses components also contain the saccharine (carbohydrates, reducing sugar and non reducing sugar components). As long as these saccharine could be fermented using suitable yeasts they will readily yield bioethanol. Srivastava *et al.* (2014) states that ethanol productions from cellulosic materials offer a solution to some of the recent environmental, economic, and energy problems facing worldwide. Cereal straw, one of the most abundant renewable lignocelluloses resources which possess valuable components, has gradually become the research hot spot as a promising substitute for both the fossil fuel resource and petroleum based industry with the increasing calling for befoul and green chemistry (Srivastava *et al*. 2014). 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). Fungi with restricted metabolic capabilities develop mutuality relationships in degrading cellulose, lignin etc. (Rayners and Buddy, 1988; Malherbe and Colet, 2002). Most fungi are capable cellulose degraders and such fungi produce active polymer degrading enzymes, including celluloses and

xylanases (Hodrova *et al*., 1998; Malherbe and Colet, 2002). The research is aimed at combining fungal cells and enzymes in hydrolysis of rice husk and to study their effect in biomass components such as saccharine, lignocelluloses. The bio-ethanol yield was also studied.

2. Materials and Methods

21 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.

22 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}. 0.1ml was pipette into five different petri dishes containing freshly prepared potato dextrose agar with inclusion of streptomycin/chloramphenol at 50 ^{o}C on an alcohol sterilized bench. Spreading of inoculums was done by the pour plate method. The inoculated plates were incubated in a microbial laboratory incubator at room temperature of 38 ± 0.06 ^{o}C for 5 days. 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).

23 Preparation of culturing and fermentation medium: For the cultivation of the fungal di-culture and hydrolysis of the rice husk, Mendel's medium was prepared as reported by Patel *et al*. (2007). The medium (rice husk in Mantle's medium) was sterilized at 121^{o}C for 20 min. and the pH adjusted to 5.5.

24 Experimental design for the fungal treatment of rice husk: Into each 500ml conical flasks used in the experiment, 20g of rice husks were weighed (total of 17 samples) and 400ml of Mantle's medium introduced. 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 (inoculates) after proper labeling and aseptically transferring their conidia and spores into sterile tubes with the aid of sterilized cotton swabs after autoclave at 121^{o}C for 20 minutes and cooled. From each sterile tube 0.5ml fungal suspension was used for the inoculation in such a manner that each combination (did-culture) total to 1ml. The flasks were incubated at room temperature for 7 days with 90 minutes daily agitation. Each filtrate (rice husk fungal did-culture treated broth) was recovered and fermented in duplicate for 7 days using baker's yeast and yeast from palm wine. From each treated residue, 1g was used to determine the saccharine: carbohydrate, reducing sugar and non-reducing sugar content in triplicates. Also 1g of treated rice husk was used to determine the Lignocelluloses: Cellulose, Hemicelluloses and Total lignin.

25 Estimation of reducing sugar: This was determined by the Dinitrosalicylic acid (DNS) method as described by Miller (1959) using glucose in establishing the standard curve.

26 Estimation of Total Sugar (Carbohydrate): The carbohydrate content was determined by the phenol sulphuric acid method as described by Dubois *et al*. (1956).

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

28 Measurement of Cellulose Content: Cellulose content of fungal and non-fungal treated rice husk was determined according to the modified gravimetric method of Marzieh and Marjan (2010).

The cellulose content was calculated from the following equation (Oakley, 1984; Ritter and Fleck, 1924).

$$\text{Cellulose}\,(\%) = \frac{\square\square\square\square\square\square\square\square\square\square\square\square}{\square\square\square\square h\,\square} \times 100$$

29 Estimation of Hemicelluloses: The estimation of hemicelluloses was done according to the Method of Goering and Vastest (1975)

210 Determination of lignin contents: This was by the Klason method.

***211* Fermentation for Ethanol Production Using Baker's Yeast and Yeast from Palm wine:** Culture filtrate of the fungal treated rice husk was inoculated with Baker's yeast and yeasts from palm wine (*Saccharomyces cerevisiae*) and allowed to ferment for seven days. The bio-ethanol was recovered through distillation as described by Sandhu *et al.* (1998).

3. Results and Discussions

The results in table 1 is a summary of the fact that all the fungal did-culture treated rice husk apart from *Aspergillus oryzae* and *Trichophyton rub rum* (AO + TR) with percentage value of 13.06 ± 1.23 % and *Aspergillums oryzae* and *Trichophyton mentagrophyte* (AO + TM) with value of 13.20 ± 1.19 % have carbohydrate values that have significant increase at $P < 0.05$ when compared to heated and non-fungal treated rice husk (C1) and untreated rice husk (C2). Among the values obtained, fungal did-culture treatments: *Trichophyton Sudanese* and *Trichophyton rubrum* (TS + TR), *Aspergillums oryzae* and *Aspergillums Niger* (AO + AN), *Aspergillums Niger* and *Trichophyton rub rum* (AN + TR) with percentage values of 20.58 ± 1.39 %, 18.80 ± 1.60 % and 18.23 ± 0.96 % had the highest percentage carbohydrate values compared with the rest. These values are higher and differ greatly from similar work carried out by Patel *et al* (2007) in which a diculture of *Aspergillums Niger* and *Trichophyton varied* (A + TV) treated rice husk gave total sugar content of 41 mgg^{-1}. Thus, these three fungal did-culture combined treatments as explained above in this work are effective in the production of carbohydrate from pre-heated rice husk. Reducing sugar values as illustrated in table 1 shows that the entire fungal di-culture treated rice husk has significant increase at $P < 0.05$ level of significance compared to the two controls (C1 and C2). However, the sugar yield did not show any percentage significant increase in yield at $P > 0.05$ level of significance. The highest reducing sugar yields were from the following did-cultures: *Aspergillums fumigates* and *Aspergillums Niger* (AF + AN) and *Aspergillum oryzae* and *Trichophyton rub rum* (AO + TR), both with percentage values of 2.54 ±

0.37 % respectively. The reducing sugar value as illustrated above, despite not having significant increase in quantity is still higher than the value gotten by Khan and Dahot (2010) in which rice husk treated with a monoculture of *Penicillium expanse* after being pretreated with 0.6N H_2SO_4 and has cultural growth for 240 hours with initial pH 4.0 at 28 ± 2°C. Their work showed a reducing sugar value of 0.86 mg/ml.

All the percentage non-reducing sugar values from the entire fungal did-culture treated rice husk showed both significant increase and significant yield compared to the controls (C1 and C2). However, *Trichophyton Sudanese* and *Trichophyton rub rum* (TS + TR), *Aspergillum oryzae* and *Aspergillum Niger* (AO + AN), as well as *Aspergillum Niger* and *Trichophyton rubrum* (AN + TR) treated rice husk with percentage values of 18.08 ± 1.10 %, 16.52 ± 1.38 % and 15.92 ± 0.86 % has the highest significant increase in yield at $P < 0.05$ level of significance compared to the other treatments. These values are higher than those reported by Patel *et al* (2007) in which *Trichoderma reesei and Trichoderma viride* diculture where used in treating rice husk, the non-reducing sugar value obtained was 15.5mgg^{-1}. Total lignin values showed a statistical analysis in which *Trichophyton mentagrophyte* and *Trichiphyton rubrum* (TM ± TR), *Trichophyton soudanense* and *Trichophyton mentagrophyte* (TS ± TM) having values of 19.00 ± 0.84 % and 19.00 ± 2.52 % had no significant increase in total lignin yield at $P > 0.05$. The entire fungal di-culture treated rice husk showed significant increase at $P < 0.05$ level of significance in total lignin compared to the controls (C1 and C2), with the highest lignin percentage value of 26.00 ± 2.06 % obtained from *Aspergillus niger* and *Trichophyton rubrum* (AN + TR) di-culture treated rice husk. The cellulose contents shows that apart from *Aspergillus fumigatus* and *Aspergillus niger* (AF + AN) treated rice husk with percentage value of 33.00 ± 5.44 %, all the fungal di-culture treated rice husk showed significant increase in yield at $P < 0.05$ level of significance with the following exceptional increase in percentage yields of 66.00 ± 0.94 %, 65.00 ± 2.37 %, 63.00 ± 1.44 % and 61.00 ± 8.96 % from *Aspergillus niger* and *Trichophyton Sudanese* (AN + TS), *Aspergillus niger* and *Trichophyton rubrum* (AN + TR), *Aspergillus fumigatus* and *Aspergillus oryzae* (AF + AO) and Aspergillus oryzae and Trichophyton rubrum (AO + TR). However, the following fungal di-culture rice husk treatments: *Trichophyton mentagrophyte* and *Trichophyton rubrum* (TM + TR), *Trichophyton soudanense* and *Trichophyton mentagrophyte* (TS + TM), *Aspergillus oryzae* and *Trichophyton mentagrophyte*

(AO + TM) as well as *Trichophyton mentagrophyte* and *Trichophyton rubrum* (TM + TR) with percentage cellulose values of 44.00±2.49, 42.00±2.83, 41.00±7.91 and 38.00 ± 8.06 % showed significant increase in yield but not significant increase in percentage value at $P < 0.05$ level of significance when compared to the heated but non fungal treated rice husk (C1) and the untreated rice husk (C2). The cellulose values obtained in this work (Table 1) are in agreement with the works carried out by Below and Badalona (2009); Laval and Unhooked (2010) and Srivastava *et al.* (2014) in which the values of cellulose from their work ranged from 33 – 43%.

Rice husk treated with di-culture of *Aspergillums Niger* and *Trichophyton Sudanese* (AN + TS) with hemicelluloses yield value of 14.50 ± 0.59 % was the only fungal treated rice husk with no significant increase at $P > 0.05$ level of significance. The highest yields were from *Aspergillums oryzae* and *Trichophyton rub rum* (AO + TR) as well as from *Aspergillum niger* and *Trichophyton mentagrophyte* (AN + TM) with percentage hemicelluloses values of 32.50 ± 1.78 and 28.00 ± 1.19 % respectively. These values are higher than those obtained by Belewu and Badalona (2009) who recorded 19.05 % hemicelluloses from rice husk treated with *Rhizopus oligosporum* as against the initial hemicelluloses content of 14.67 % of the untreated rice husk, but similar to the work by Srivastava *et al.* (2014) with values of 20 -35% from rice husk treated with Trichoderma reseed (MTCC-4876).

Bio-ethanol yield from the fungal did-culture treated rice husk broth showed that combined *Trichophyton mentagrophyte* and *Trichophyton rub rum* treated rice husk broth fermented with palm wine yeast gave the highest percentage value of 14.18 ± 0.59 %. Apart from the following: AO + AN (3.97±0.25%), AF + AN (3.92±0.38%), AN + TR(3.71±0.49%), AO + TR (3.67±0.59%), AF + AN (3.65±0.59%) and TS + TR (3.60±0.30%) fungal di-culture treated rice husk broth fermented with palm wine yeast, all the other fungal di-culture treated rice husk broth fermented with palm wine yeast as seen in table 1, gave percentage significant increase in values and yields at $P < 0.05$ level of significance when compared with the controls (C1 and C2). Similarly, apart from AN + TR fungal di-culture treated rice husk broth fermented with baker's yeast with a percentage bio-ethanol yield of 3.98±0.33 % the entire fungal di-culture treated rice husk broth fermented with baker's yeast as shown in table 1 gave significant increase in bio-ethanol values and yields at $P < 0.05$ level of significance when compared with values of both the heat treated rice husk (C1) and untreated rice husk (C2) broth fermented with

baker's yeast. Additionally, The highest bio-ethanol yield from fungal did-culture treated rice husk broth fermented with baker's yeast was 6.56 ± 0.26 % from *Aspergillum oryzae* and *Trichophyton soudanense* (AO + TS) group followed by 6.15 ± 0.24 % from *Aspergillum oryzae* and *Trichophyton mentagrophyte* (AO + TM) group and 6.02 ± 0.31 % from *Aspergillum oryzae* and *Aspergillus niger* (AO + AN). The value of bioethanol obtained in this work is higher than values of 3.20 ± 0.36 g/l or 0.27 g/g total sugar reported by Srivastava *et al*. (2014) in rice husk treated with *Trichoderma reseei* before fermenting with *Saccharomyces cerevisiae* for 7 days. All relevant results involved in bio-ethanol yield from fungal di-cultures is as depicted in Table 1.

Table 1: *Table Showing Percentage Yields of various constituents of Saccharine, Lignocelluloses and Bio-Ethanol yield from Rice Husk Treatment*

FUNGAL DICULTURE TREATMENTS & CONTROLS	SACCHARIDES (%±STANDARD ERROR OF MEAN)			LIGNOCELLULOSES (% ± SEM)			BIO-ETHANOL (% ± SEM)	
	CARBOHYDRATES (TOTAL SUGAR)	REDUCING SUGAR	NON-REDUCING SUGAR	TOTAL LIGNIN	CELLULOSE	HEMICELLULOSE	PALMWINE YEAST	BAKERS YEARST
AF + AO	14.38±1.42^{*b}	2.42±0.21^{b}	13.86±1.31^{*b}	21.00±1.46^{*b}	63.00±1.44^{*a}	26.50±1.33^{*b}	4.65±0.35^{*b}	4.50±0.48^{*b}
AF + AN	14.78±1.42^{*b}	2.54±0.37^{b}	12.24±1.16^{*b}	21.50±0.50^{*b}	33.00±5.44^{c}	28.00±0.00^{*a}	3.65±0.59 c	4.86±0.34^{*b}
AF + TS	14.36±1.74^{*b}	2.32±0.13^{b}	12.04±1.61^{*b}	22.50±1.03^{*b}	51.00±0.54^{*b}	23.50±1.33^{*b}	5.68±0.52^{*b}	4.70±0.35^{*b}
AF + TM	14.93±1.74^{*b}	2.40±0.18^{b}	12.53±1.56^{*b}	23.00±0.84^{*b}	56.00±0.94^{*b}	22.00±1.19^{*b}	4.47±0.30^{*b}	4.38±0.41^{*b}
AF + TR	15.93±1.23^{*b}	2.35±0.06^{b}	13.58±1.17^{*b}	20.50±1.03^{*b}	49.00±1.09^{*b}	27.00±0.84^{*b}	3.92±0.38^{c}	4.79±0.39^{*b}
AO + AN	18.80±1.60^{*a}	2.28±0.22^{b}	16.52±1.38^{*a}	20.00±0.00^{*b}	49.00±1.09^{*b}	23.00±1.19^{*b}	3.97±0.25^{c}	6.02±0.31^{*b}
AO + TS	13.64±1.23^{*b}	2.30±0.13^{b}	11.34±1.10^{*b}	22.00±1.19^{*b}	38.00±8.06^{*c}	24.50±1.33^{*b}	3.52±0.39^{c}	6.56±0.26^{*b}
AO + TM	13.20±1.19^{c}	2.35±0.00^{b}	10.85±1.19^{*b}	20.50±1.33^{*b}	41.00±7.91^{*c}	25.00±0.00^{*b}	4.65±0.42^{*b}	6.15±0.24^{*b}
AO + TR	13.06±1.23^{c}	2.54±0.37^{b}	10.52±0.86^{*b}	18.50±1.33^{*a}	61.00±8.96^{*a}	32.50±1.78^{*a}	3.67±0.59^{c}	5.89±0.42^{*b}
AN + TS	14.07±1.56^{*b}	2.47±0.29^{b}	11.60±1.27^{*b}	21.00±0.84^{*b}	66.00±0.94^{*a}	14.50±0.59^{c}	6.87±0.15^{*b}	4.55±0.29^{*b}
AN + TM	16.65±1.86^{*b}	2.46±0.29^{b}	14.19±1.57^{*b}	23.50±1.03^{*b}	56.00±0.94^{*a}	28.00±1.19^{*a}	7. 01±0.29^{*b}	5.46±0.53^{*b}
AN + TR	18.23±0.96^{*a}	2.31±0.	15.92±0.8	26.00±2.0	65.00±2.3	23.00±0.84^{*b}	3.71±0.49	3.98±0.3

		10[b]	6[*a]	6[*a]	7[*a]		[c]	3[c]
TS + TM	14.93±2.11[*b]	2.49±0.33[b]	11.44±1.78[*b]	19.00±2.52[c]	42.00±2.83[*c]	25.50±2.45[*b]	4.56±0.38[*b]	5.43±0.53[*b]
TS + TR	20.53±1.39[*b]	2.45±0.29[b]	18.08±1.10[*a]	21.00±1.19[*b]	50.00±0.94[*b]	22.50±1.33[*b]	3.60±0.30[c]	4.03±0.03[*b]
TM + TR	17.08±1.99[*a]	2.44±0.28[b]	14.64±1.71[*b]	19.00±0.84[c]	44.00±2.49[*c]	23.00±0.00[*b]	14.18±0.13[*a]	3.05±0.15[c]
C1	10.05±1.01[c]	1.61±0.38[c]	8.44±0.63[c]	18.00±1.88[c]	34.00±5.44[c]	20.80±1.88[*b]	3.16±0.13[c]	3.05±0.15[c]
C2	7.18±1.01[d]	1.21±0.19[c]	5.97±0.82[c]	17.00±0.84[c]	32.00±1.63[c]	19.60±1.46[c]	2.40±0.24[d]	2.24±0.29[d]

Key: Aspergillum fumigates = AF, Aspergillums Niger = AN, Aspergillums oryzae = AO, Trichophyton mentagrophyte = TM, Trichophyton rub rum = TR and Trichophyton Sudanese = TS, Addition = +, C1 = Heated but non fungal treated rice husk, C2 = Non heated and non-fungal treated rice husk (Untreated) Legend: Percentage mean with different alphabets (a, b, c, d) differ significantly at $P < 0.05$. The groups with asterisks (*) shows significant yield of saccharine, lignocelluloses and ethanol at $P < 0.05$.

4. Conclusion

Treatment of rice husk with heat and fungal di-culture has good potential of releasing available saccharine. There was also appreciable increase in lignocelluloses by some of the fungal diculture treated rice husk. Selective use of yeast in fermentation of rice husk after hydrolysis with heat and fungal diculture as shown in the result of this experiment also gave an appreciable yield of bio-ethanol especially yeast from palm wine source.

5. Acknowledgement

The management of Godfrey Okayed University Enugu, Nigeria, is hereby acknowledged for provision of research facilities used in this work. The funding for the conference and publication of this paper is provided by Federal University Wukari, Taraba State, Nigeria courtesy Tertiary Education Trust Fund (TETFUND).

REFERENCES

Belewu, M. A. & Badalona, F.T. (2009). Nutrient enrichment of some waste agricultural residues after solid state fermentation using *Rhizopus oligosporus*. *Journal of Applied Biosciences*, 13, 695 - 699.

Dubois, M., Gilles, K.A., Reberts, P.A. & Smith, F. (1956) Phenol sulphuric acid method for total carbohydrate. *Analytical Chemistry*, 25, p 350. http://dx.doi.org/10.1 021/ac6 011 1a017

Ezeonu, C.S., Otitoju, O., Onwurah, I.N.E., Ejikeme, C.M., Ugbogu, O.C. & Anike, E.N. (2014). Enhanced availability of Biofuel and Biomass components in *Aspergillus niger* and *Aspergillus fumigatus* treated Rice Husk. *European Scientific Journal*, 10(18), 97-117.

Goering, H.D. & Vansoest, P.J. (1975). Forage Fibre Analysis, Dept. of Agriculture, Agricultural Research Service, Washington.

Hodrova, B., Kopecny, J. & Kas, J. (1998). Cellulolytic enzymes of rumen anaerobic fungi *Orpinomyces joyonii* and *Caecomyces communis*. *Res. Microbiol*., 149, 417-427. http://dx.doi.org/10.1016/S0923-2508(98)80324-0

Khan, M. Y. & Dahot, M. U. (2010) Effect of Various Agriculture Wastes and Pure Sugars on the Production of Single Cell Protein by Penicillium Expansum. *World Applied Sciences Journal* 8, 80-84.

Lawal, S. A. & Ugheoke, B. I. (2010). Investigation of Alpha-Cellulose content of Agro-waste Products as Alternatives for Paper Production. *Australian Journal of Technology*, 13(4), 258-260.

Malherbe, S. & Cloete, T. E. (2002). Lignocellulose biodegradation: Fundamentals and applications. *Reviews in Environmental Science and Biotechnology* 1(2),105-114. http://dx.doi.org/10.1023/A:1020858910646

Marzieh, M. N. & Marjan, M. N. (2010). Utilization of sugar beet pulp as a substrate for the fungal production of cellulase and bioethanol. *African Journal of Microbiology Research*, 4(23), 2556-2561.

Miller, G.L. (1959). Use of Dinitrosalicylic acid reagent for determination of reducing sugars. *Analytical Chemistry*, 31, 426-429. http://dx.doi.org/10.1021/ac60147a030

Oakley, E. T. (1984). Determination of cellulose index of tobacco. *Americal Chemical Society*, 32, 1192-1194. http://dx.doi.org/10.1021/jf00125a065

Patel, S.J., Onkarappa, R. & Shobha, K.S. (2007). Comparative Study of Ethanol Production frome Microbial Pretreated Agricultural Résiduels. *Journal of Apple Science and Environnemental Management*, 11(4), 137-141.

Rayners, A. D. M. & Buddy, L. (1988). Fungal communities in the decay of wood. *Adv. Microb. Ecol*., 10, 115-166. http://dx.doi.org/10.1007/978-1-4684-5409-3_4

Ritter, H. & Fleck, L. C. (1924). Determination of cellulose in wood. *Industial Engineering and Chemistry*, 16, 147-148. http://dx.doi.org/10.1021/ie50170a017

Sandhu, H., Bajaj, K. L. & Arneja, J. S. (1998). *Biochemical Analysis*, Kalyani Publishers, New Delhi.

Srivastava, A. K., Agrawal, P. and Rahiman, A. (2014). Delignification of Rice Husk and Production of Bioethanol. *International Journal of Innovative Research in Science, Engineering and Technology* (IJIRSET), 3(3), 10187-10194.