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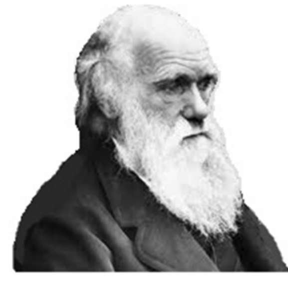

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Application of wood rot wild mushrooms in bioethanol production from sawdust of sawmills of Oromia Forest and Wildlife Enterprise, Ethiopia

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ABSTRACT

In this research sawdust samples of *Ecalyptus globulus* and *Cupressus lusitanica* were evaluated for bioethanol productions. The sawdust samples were first pretreated with three white rot fungi alone and also by combining the white rot fungi with mild NaOH and steam. Both, the fungal and combined pretreated samples were then hydrolyzed with hydrolytic enzymes from three cellulolytic wood rot fungi. Finally, the resulting sugars were fermented into bioethanol using *S. cerevisae* in anaerobic conditions. Results obtained, in general, indicated that bioethanol amount produced in all cases of sawdust management was significantly higher than the amount obtained from the un-pretreated sawdust samples ($p<0.05$). In both fungal alone and combined pretreated sawdust samples, higher ethanol yield was obtained from *E. globulus* than from *C. lusitanica*. Similarly, combination with NaOH showed better bioethanol yield over combination with steam. The highest alcohol concentration was obtained when pretreated NaOH-006-2G and hydrolyzed with enzymes from 033-1G, and followed by results when pretreated with 005-1G and 003-2G, respectively, and hydrolyzed with enzymes from 033-1G.

***Keywords*:** Bio-ethanol, Lignocellulosic biomass, wood rot wild mushrooms, pretreatment, hydrolysis, sawdust, *Ecalyptus globulus*, *Cupressus lusitanica*, Oromia Forest and Wildlife Enterprise

1. INTRODUCTION

There is an increasing search for an alternative biofuel energy sources in the world due to the rising energy demands, rising price of petroleum and increasing GHG (Greenhous Gas)

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emission concerns. Currently, ethanol is being used as an alternative fuel representing a sustainable substitute for gasoline dependent vehicles [1]. First generation bioethanol production has been reported to compete with food supply in developing countries [2]. Despite its abundance, biomass based bioethanol production, which is expected to replace first generation bioethanol production, currently is much below its projected scale [3]. The bottleneck problem of utilizing biomass as a feedstock for bioethanol production is its recalcitrance nature. But still there is a need to give attention to biomass wastes as bioethanol feedstock since food and feed crops, sources of first generation feedstocks, are essential to mankind [4]. Though different efforts are made so far it has been shown that wider bioethanol production from lignocellulosic celluloses is not as such developed yet [5].

Waste lignocelluloses, being largely underutilized, are the most abundant potential source for different industrial uses [6]. One of these is the requirement of fermentable sugars for large scale biofuel productions. Though, sawdust has been given attention as a feedstock for different industrial applications such as sugar productions over the past decade [7, 8], the lignin-hemicellulose matrix surrounding the cellulose microfibrils prevents the microbial enzymes' access to cellulose and inhibits its hydrolysis. This is usually solved by the removals or modifications of lignin and/or hemicelluloses [9].

Both, alkali and steam based pretreatments could modify the lignin structure of lignocellulosic materials so that the fungal enzymes reach the cellulose portion for hydrolysis. NaOH causes various structural alterations such as the depletion of lignin barrier and solvation of hemicelluloses [10]. It has also been observed to decrease cellulose crystallinity and the degree of polymerization [11] thus increasing access for enzymes [12]. Steam has also been implemented by using pressure to keep water in the liquid state at higher temperature to pretreat sawdust [13].

It changes the biomass native structure by the removal of its hemicellulose content alongside transformations of the lignin structure, which make the cellulose more accessible to the further enzymatic hydrolysis step [14]. On the other hand, biological pretreatment is a complex process involving multiple enzymes that work synergistically. It is typically carried out by wood rotting basidiomycetes [15]. The main enzymes involved in lignin degradation are Laccase, LiP and MnP [16, 17].

Combination of wood rot fungal pretreatment with other pretreatment methods has been reported to increase enzymatic hydrolysis yield [18, 19]. A mild alkali pretreatment of corn stalks with *I. lacteus* for 15 days significantly facilitated lignin degradation by wood rot fungi [20]. Similarly, Ma et al. [21] reported an increased saccharification yield compared to a single pretreatment when pretreating beech wood with *P. chrysosporium* and then steam explosion. The objective of this research work was, therefore, to evaluate the bioethanol productions after pretreatments and hydrolyses of *E. globulus* and *C. lusitanica* sawdust samples.

2. MATERIALS AND METHODS

2. 1. Sawdust sample source and preparation

Fresh sawdust samples of *E. globulus* and *C. lusitanica* used for this research were collected from Arsi branch of Oromia Forest and Wildlife Enterprise, Dagaga site, Ethiopia. The sawdust samples were dried, ground and passed through 5 mm pore size sieve and kept in plastic bags for further utilization.

2. 2. Fungal selection and inoculum preparation

For the pretreatment experiment, three efficient ligninolytic wild mushrooms (003-2G, *Pholiota squarrosa*; 006-2G, *Ganoderma aplanatum*, 005-1G, *Polyporus giganteus*) (**Table 1**) were selected for pretreatments based on their enzymatic assay reports of Megersa *et al*. [22] and Megersa *et al*. [23]. For the hydrolyses experiment, again three hydrolytic wild mushrooms (030-1D, *Phellinus tremulae;* 026-2D, *Pholiota adipose*; 033-1G, *Armilleria mellea*) (Table 1) were selected based on their enzymatic assay reports made by Megersa and Gure [24].

Inocula of the selected ligninolytic and hydrolytic wild mushrooms were prepared using the standard medium of Altaf *et al*. [25] containing 10.0 g glucose, 3.0 g yeast extract, 3.0 g peptone, 1.0 g KH_2PO_4, and 0.5 g $MgSO_4 \cdot 7H_2O$ per liter of distilled water. Four disks (ϕ5 mm) of each isolate were inoculated and grown on a rotary shaker at 150 rpm and at room temperature in 250 mL flasks containing 100 mL of the medium. After six days of fungal cultivation mycelial pellets were homogenized and used as inocula.

Table 1. List of wild mushrooms selected for pretreatment and hydrolysis experiments

Ligninolytic wild mushrooms		
Photo	**Code**	**Scientific name**
	003-2G	*Pholiota squarrosa*
	006-2G	*Ganoderma aplanatum*
	005-1G	*Polyporus giganteus*

Hydrolytic wild mushrooms		
Photo	Code	Scientific name
	030-1D	*Phellinus tremulae*
	026-2D	*Pholiota adipose*
	033-1G	*Armilleria mellea*

2. 3. Fungal pretreatments

For the fungal pretreatment, twenty gram of dry samples from raw sawdust samples was placed in 100 mL Erlenmeyer flasks in triplicates. The sawdust in each flask was conditioned with distilled water to obtain moisture content of 75%. The flasks containing wet sawdust were autoclaved at 121 ºC for 15 minutes, cooled and inoculated with a 10 mL inoculum on the top of the substrate. The inoculated flasks were incubated at 30 ºC in static conditions for 60 days and then washed with distilled water (30 mL) at 180 rpm for one hour, and filtered under vacuum to remove the water soluble components. The solid fractions from fungal pretreated and combined pretreated sawdust were dried in an oven at 65 ºC and then used for hydrolyses experiments.

2. 4. Combined pretreatments

During the combined pretreatments, raw sawdust samples were first pretreated with NaOH or steam and then further pretreated with the selected ligninolytic wild mushrooms. The raw sawdust samples were pretreated with alkali following the procedures of Mirahmadi *et al.*

[12]. 6% NaOH (w/w) with a ratio of 1:10 w/v was used in 250 Erlenmeyer flasks. The flasks were kept at room temperature for 2 hours by shaking at 150 rpm. Following alkali pretreatment, sawdust mixture samples were washed several times to neutral pH, filtered and solid mass collected for further use. On the other hand, raw sawdust samples (1:5 w/v in distilled water) were autoclaved for 2 h according to Ogunbayo *et al*. [26]. Following this steam pretreatments, the sawdust mixture samples were washed several times to neutral pH, filtered and solid mass collected for further use.

Twenty gram of dry samples from the NaOH and steam pretreated ones were placed in 100 mL Erlenmeyer flasks in triplicates. The sawdust in each flask was conditioned with distilled water to obtain moisture content of 75%. The flasks containing wet sawdust were autoclaved at 121 ºC for 15 minutes, cooled and inoculated with a 10 mL inoculum on the top of the substrate. The inoculated flasks were incubated at 30 ºC in static conditions for 60 days and then washed with distilled water (30 mL) at 180 rpm for one hour, and filtered under vacuum to remove the water soluble components. The solid fractions from fungal pretreated and combined pretreated sawdust were dried in an oven at 65 ºC and then used for hydrolyses experiments.

2. 5. Hydrolyses of sawdust samples

The hydrolytic enzymes of the selected fungal isolates were produced using the optimized medium of Hussain *et al*. [27]. The flasks were inoculated with 5 mL of the fungal inocula and incubated at 30 ºC for 12 days based on the optimized enzyme production for these fungal species [24]. Finally, 50 mL of 0.05 M citrate buffer (pH 5.0) was added to each flask and left for one hour shaking on a rotary shaker at 150 rpm. The samples were then filtered through clean muslin cloth and the filtrates were centrifuged at 4,000 rpm for 15 min. The supernatants (crude enzyme extracts) were used for hydrolyses experiments.

Twenty gram of the pretreated sawdust was added to each 100 mL flasks and autoclaved at 121 ºC for 15 minutes and then loaded with crude enzyme extracts at 5% (v/w). A citrate buffer solution of 0.05 M was added to the flasks to achieve and maintain a pH of 5.0 [7]. Following the addition of the enzyme, the flasks were sealed and placed at 40 ºC and at 150 rpm. The flasks were sampled after 72 h of incubation. Then samples were submerged in a water bath at 100 ºC for 5 minutes, followed by an ice bath and centrifuged at 4,000 rpm for 15 minutes. The supernatants were maintained for bioethanol production.

2. 6. Fermentation experiments

The hydrolysates were subsequently fermented with *Saccharomyces cerevisiae*. The yeast (0.5 g/L inoculum) was grown in 50 mL of the liquid medium at 32 ºC and 200 rpm for 24 h in 250 mL Erlenmeyer flasks for inoculum preparation [28]. The liquid medium contained 20.0 g glucose, 3.0 g yeast extract and 5.0 g peptone per liter of distilled water. 100 mL of the sawdust hydrolysate samples from the 72 h of enzymatic hydrolysis were inoculated with 10 mL of the yeast suspension in 200 mL flasks. The flasks were sealed with rubber plugs for anaerobic condition and connected to other flask with water through rubber tubes and incubated at room temperature and 200 rpm. Bioethanol contents were measured at 72 h of incubation. At each fermentation period, fermentation content was centrifuged at 4,000 rpm for 15 min. The quantity of bioethanol was determined using the calibration curve method [29].

Ethanol standard curve

The quantity of ethanol derived was determined using the calibration curve method [29]. 100 mL anhydrous ethanol was diluted with distilled water in test tube (0%, 25%, 50%, 75%, and 100%) (**Table 2**). The mass and volume of each dilution was measured and densities were calculated. Standard calibration graph of the calculated densities against percentage volume of dilutions was made. The dilution data and standard curve are shown below (**Fig. 1**).

Table 2. Dilutions of ethanol for making standard curve

Volume of anhydrous ethanol (ml)	Volume of water (ml)	Volume of anhydrous ethanol (%)	Mass of ethanol-water mixture (g)	Density of ethanol-water mixture (g/mL)
0	10	0	10.25	1.03
2.5	7.5	25	9.27	0.93
5.0	5.0	50	9.11	0.91
7.5	2.5	75	7.87	0.79
10	0	100	6.89	0.69

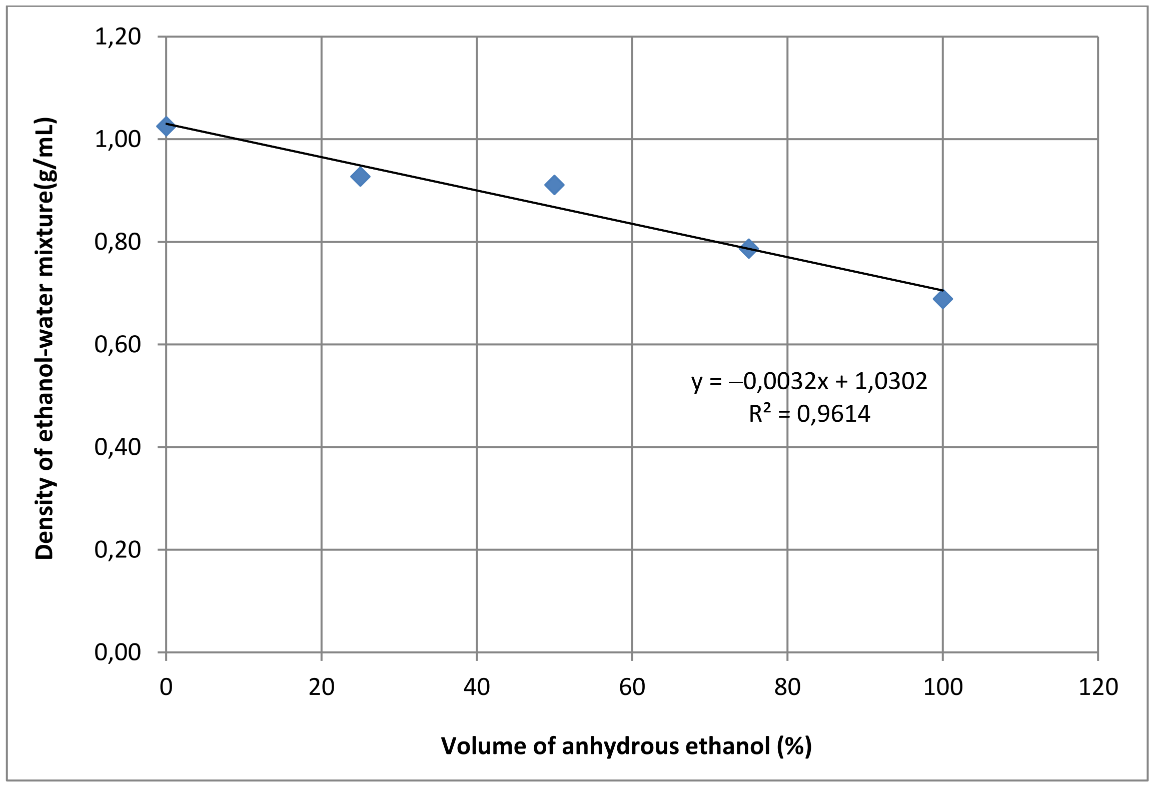

Fig. 1. Change in density dependent on dilution

Each 10 mL sample was weighed and its density calculated. Using calculated density of each sample, percentage ethanol was calculated from the regression line Y = –0.0032 + 1.0302. Example, if mass of 10 mL sample is 1.003 g/mL, then X = 8.5%. Therefore, 8.5% of 1.003 g/mL is found to be 0.085 g/mL or 85 mg/mL.

2. 7. Statistical analysis

All bioethanol yield experiments were carried out in triplicate and average values were obtained. The effects of pretreatments and hydrolyses of the sawdust samples on bioethanol productions were evaluated using SPSS for analysis of variance and significance tests at 95% confidence level. Tukey simultaneous test was performed to assess statistical differences between pretreatment and hydrolyses means.

3. RESULTS AND DISCUSSION

3. 1. Bioethanol yield from hydrolysates of WRF-pretreated and enzymatically hydrolyzed sawdust

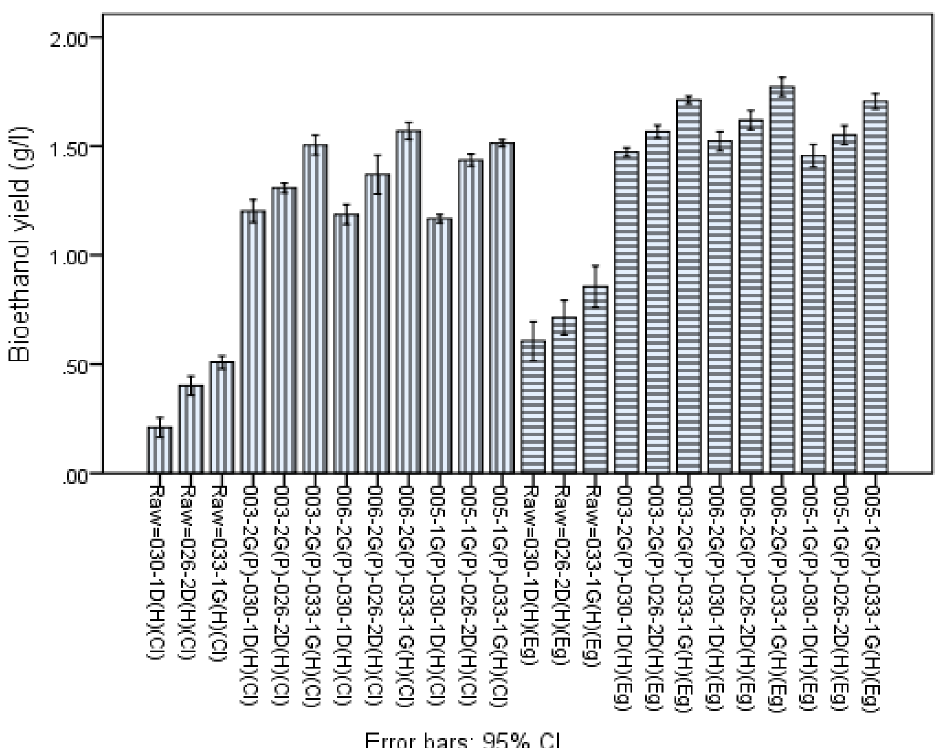

Fig. 2. Bioethanol yield from hydrolysates of WRF-pretreated and enzymatically hydrolysed sawdust samples

Hydrolysates from WRF (Weather Research and Forecasting) pretreated and enzymatically hydrolyzed *E. globulus* and *C. lusitanica* sawdust samples were fermented and considerable bioethanol yields were obtained. In *E. globulus* sawdust, the highest amount of bioethanol (1.77 g/L) was obtained from the 006-2G (*Ganoderma aplanatum*) pretreated and

033-1G (*Armilleria mellea*) hydrolyzed sawdust (**Fig. 2**). 003-2G (*Pholiota squarrosa*) pretreated and 033-1G hydrolyzed *E. globulus* sawdust showed the second highest bioethanol yield but this yield was not significantly differing from the one pretreated with 006-2G and hydrolyzed with 033-1G. Similarly, the highest bioethanol amount was produced from the 006-2G pretreated and 033-1G hydrolyzed *C. lusitanica* sawdust displaying 1.57 g/L bioethanol yield (Fig. 2). The second and the third highest bioethanol amounts, not significantly differ, were displayed by the 005-1G and 003-2G pretreated and 033-1D hydrolyzed *C. lusitanica* sawdust, respectively. Udhayaraja and Narayanan [30] fermented hydrolyzate from crude enzymes hydrolysis of sorghum stover and reported 4.0 g/L bioethanol yield. The nature of carbohydrates in lignocellulosic biomass is complex because of the presence of both six-carbon and five-carbon sugars [31]. Since baker's yeast (Saccharomyces cerevisiae) depends on hexoses, it is not possible to obtain bioethanol from pentose sugars which could be formed during pretreatment and hydrolyses experiments. This actually lowers the amount of bioethanol expected to be obtained from lignocellulosic materials.

3. 2. Bioethanol yield from hydrolysates of two way-pretreated and enzymatically hydrolyzed sawdust

NaOH-WRF pretreated and enzymatically hydrolysed

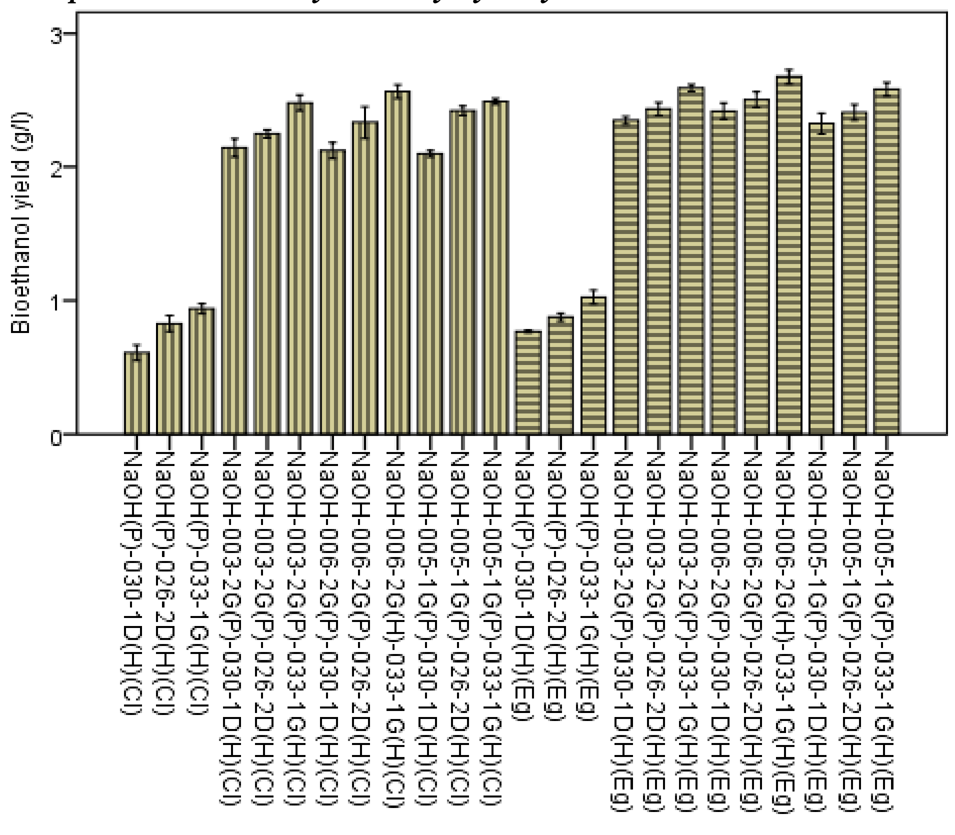

Fig. 3. Bioethanol yield from hydrolysates of NaOH-WRF-pretreated and enzymatically hydrolysed sawdust samples

In *E. globulus* sawdust, the highest amount of bioethanol (2.68 g/L) was obtained when pretreated from the NaOH-006-2G (**Fig. 3**) pretreated and 033-1G hydrolyzed sawdust and followed by the bioethanol yield of 2.59 g/L and 2.58 g/L by hydrolysis of the NaOH-003-2G and NaOH-005-1G pretreated sawdust of the species by enzyme of isolate 033-1G. Similarly, the highest bioethanol yields of 2.57 g/L, 2.49 g/L, and 2.48 g/L were recorded by hydrolyzing the NaOH-006-2G, NaOH-005-1G and NaOH-003-2G pretreated *C. lusitanica* sawdust with enzyme from isolate 033-1G, respectively, after 72 h of fermentation.

Steam-WRF pretreated and enzymatically hydrolyzed

The highest amount of bioethanol (2.347 g/L) was obtained from the Steam-006-2G pretreated and 033-1G hydrolyzed sawdust and followed by the bioethanol yields of 2.287 g/L and 2.253 g/L obtained by hydrolysis of the Steam-003-2G and Steam-005-1G pretreated sawdust of *E. globulus* by enzyme of isolate 033-1G. On the other hand, the highest bioethanol yields of 2.278 g/L, 2.206 g/L, and 2.195 g/L were recorded by hydrolyzing the Steam-006-2G, Steam-005-1G and Steam-003-2G pretreated *C. lusitanica* sawdust with enzyme from isolate 033-1G, respectively, after 72 h of fermentation (**Fig. 4**).

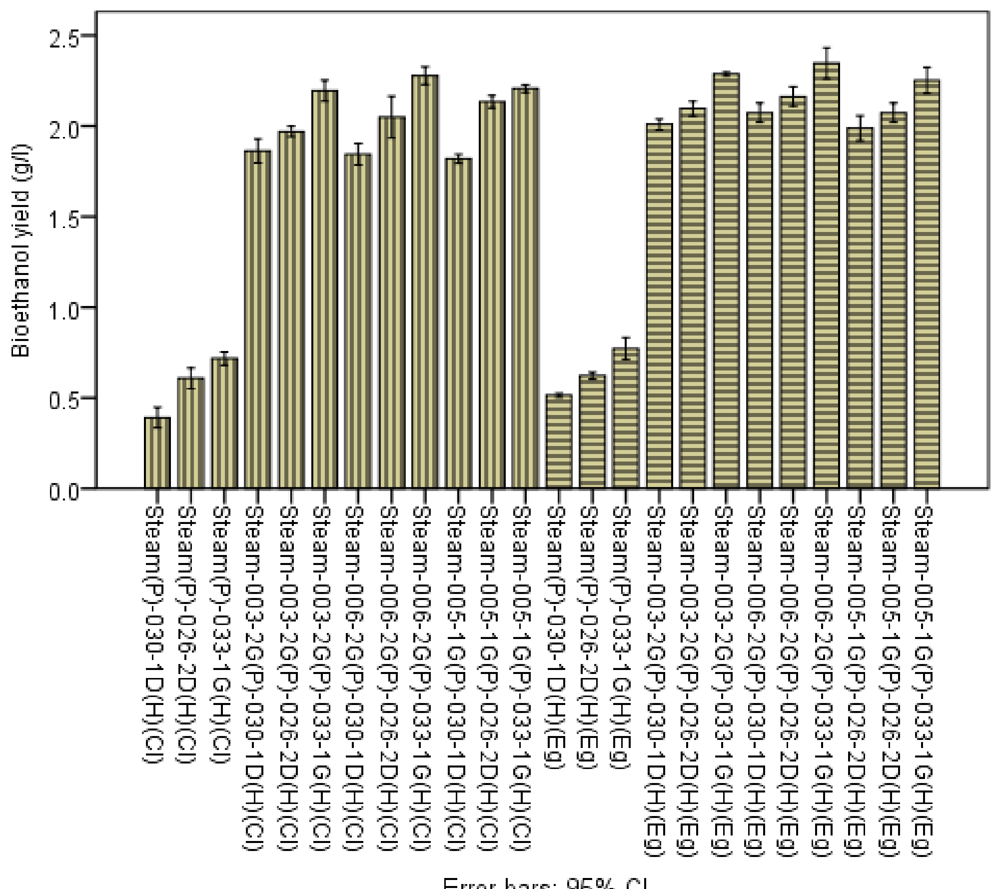

Fig. 4. Bioethanol yield from hydrolysates of Steam-WRF-pretreated and enzymatically hydrolysed sawdust samples

Etonihu and Idoko [32] pretreated sawdust collected from Timber processing facilities with 5% NaOH and reported bioethanol productions of 1.49-3.12 g/L during the 1-10 day fermentation periods. The bioethanol production of 7.2g/L was noted in the sawdust processed with concentrated phosphoric acid [33]. Kathiresan *et al*. [33] pretreated sawdust with 4% sodium hydroxide and fermented the hydrolysate with different species of marine yeasts and reported the maximum bioethanol yield of 8.1 g/L. Ali and Jamaludin [34] pretreated *Moringa oleifera* pod husk with NaOH obtained the maximum bioethanol production of 8.4 g/L and introduced as a new material for bioethanol production in Malaysia.

4. CONCLUSIONS

The present study aimed at obtaining higher bioethanol yields from sawdust by pretreating with wood rot fungi with and without combining with NaOH and Steam and hydrolyzing with enzymes from hydrolytic fungi. Both strategies of pretreatment of sawdust samples from *E. globulus* and *C. lusitanica* improved bioethanol yields. The results obtained showed higher bioethanol yields compared to control. Fungal pretreatment for 60 days and enzymatic hydrolysis for 72 h yielded the highest bioethanol amounts. Bioconversion offers a safe method of disposing the sawdust waste accumulation round sawmills and also it has the potential to convert sawdust waste into usable forms such as reducing sugars that could be used for bioethanol production. Hence the conversion of sawdust into bioethanol could contribute toward the energy generation and serve as a sustainable solid waste management strategy.

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References

[1] Ezeoha, S.L., Anyanwu, C.N., and Nwakaire, J.N. (2017). The prospects, impacts and research challenges of enhanced cellulosic ethanol production: a review. *Nigerian Journal of Technology* 36 (1): 267-275.

[2] Valentine, J., Clifton-brown, J., Hastings, A., Robson, P., Allison, G., and Mith, P. (2012). Food vs. fuel: the use of land for lignocellulosic ‘next generation’ energy crops that minimize competition with primary food production. *GCB Bioenergy* 4: 1-19.

[3] Saini, J.K., Saini, R., and Tewari, L. (2015). Lignocellulosic agriculture wastes as biomass feedstocks for second-generation bioethanol production: concepts and recent developments. *3 Biotech* 5: 337-353.

[4] Branco, R.H.R, Serafim, L.S., and Xavier, A.M.R.B. (2019). Second Generation Bioethanol Production: On the Use of Pulp and Paper Industry Wastes as Feedstock: Review. *Fermentation* 5, 4.

[5] Robak, K. and Balcerek, M. (2018). Review of Second Generation Bioethanol Production from Residual Biomass. *Food Technol. Biotechnol*. 56 (2): 174-187.

[6] Mishra, S., Singh, P.K., Dash, S., and Pattnaik, R. (2018). Microbial pretreatment of lignocellulosic biomass for enhanced biomethanation and waste management. *3 Biotech.* 8: 458.

[7] Trevorah, R.M. and Othman, M.Z. (2015). Alkali pretreatment and enzymatic hydrolysis of Australian timber mill Sawdust for biofuel production. *J. Renewable Energy*, Article ID 284250, 9 pages.

[8] Safarian, S. and Unnthorsson, R. (2018). An Assessment of the Sustainability of lignocellulosic bioethanol production from wastes in Iceland. *Energies* 11*:* 1493.

[9] Amezcua-Allieri, M.A., Durán, T.S., and Aburto, J. (2017). Study of Chemical and Enzymatic Hydrolysis of Cellulosic Material to Obtain Fermentable Sugars. *Journal of Chemistry,* Article ID 5680105.

[10] Ibrahim, M.M., El-Zawawy, W.K., Abdel-Fattah, Y.R., Soliman, N.A., and Agblevor, F.A. (2011). Comparison of alkaline pulping with steam explosion for glucose production from rice straw. *Carbohydrate Polymers* 83: 720-726.

[11] Rojo, E., Alonso, M.V., Dom´ınguez, J.C., Saz-Orozco, B.D., Oliet, M., and Rodriguez, F. (2013). Alkali treatment of viscose cellulosic fibers from eucalyptus wood: structural, morphological, and thermal analysis. *J. Appl. Polymer Sci.* 130(3): 2198-2204.

[12] Mirahmadi, M., Kabir, M.M., Jeihanipour, A., Karimi, K., and Taherzadeh, M.J. (2010). Alkaline pretreatment of spruce and birch to improve bioethanol and biogas production. *BioResources* 5(2): 928-938.

[13] Kamdem, I., Jacquet, N., Tiappi, F.M., Hiligsmann, S., Vanderghem, C., Richel, A., Jacques, P., and Thonart, P. (2015). Comparative biochemical analysis after steam pretreatment of lignocellulosic agricultural waste biomass from Williams Cavendish banana plant (Triploid Musa AAA group). *Waste Management and Research* 33 (11): 1022-1032.

[14] Alvira, P., Tomás-Pejó, E., Ballesteros, M., and Negro, M.J. (2010). Pretreatments technologies for an efficient bioethanol production process based on enzymatic hydrolysis: A review. *Bioresour. Technol.* 101: 4851-4861.

[15] Qin, D., Ren, H., Zhang, L., and Li, Z. (2015). Enhancing enzymatic digestibility of bamboo by fungal pretreatment. *Wood Research* 60(1): 25-32.

[16] Nazarpour, F., Abdullah, D.K., Abdullah, N., and Zamiri, R. (2013). Evaluation of biological pretreatment of rubber wood with white rot fungi for enzymatic hydrolysis. *Materials* 6*:* 2059-2073.

[17] Anwar, Z., Gulfraz, M., and Muhammad, I. (2014). Agro-industrial lignocellulosic biomass a key to unlock the future bio-energy: A brief review. *J. Rad. Res. and Appl. Sci.* 7: 163-173.

[18] Alexandropoulou, M., Antonopoulou, G., Ntaikou, I., and Lyberatos, G. (2017). Fungal Pretreatment of willow sawdust with *Abortiporus biennis* for anaerobic digestion: Impact of an external nitrogen source. *Sustainability* 9: 130.

[19] Martín-Sampedro, R., López-Linares, J.C., Fillat, U., Gea-Izquierdo, G., Ibarra, D., Castro, E., and Eugenio, M.E. (2017). Endophytic fungi as pretreatment to enhance enzymatic hydrolysis of Olive tree pruning. *BioMed Research International,* Article ID 9727581.

[20] Yu, J., Zhang, J., He, J., Liu, Z., and Yu, Z. (2009). Combinations of mild physical or chemical pretreatment with biological pretreatment for enzymatic hydrolysis of rice hull. *Bioresour Technol.* 100(2): 903-908.

[21] Ma F., Yang, N., Xu, C., Yu, H., Wu, J., and Zhang, X. (2010). Combination of biological pretreatment with mild acid pretreatment for enzymatic hydrolysis and ethanol production from water hyacinth. *Bioresour Technol.* 101: 9600-9604.

[22] Megersa, S., Gure, A., Alemu, M., and Feleke, S. (2017a). Qualitative assays and quantitative determinations of laccases of white rot fungi from plantation and natural forests of Arsi forest enterprise, Ethiopia. *World Scientific News* 67(2): 303-323.

[23] Megersa, S., Gure, A., Feleke, S., and Alemu, M. (2017b). Characterization of MnP and LiP from white rot fungi of plantation and natural forests of Arsi forest enterprise. *IJPSS* 7(5): 6-25.

[24] Megersa, S. and Gure, A. (2018). Qualitative assays and quantitative determinations of cellulolytic enzymes of wood rot fungi. *American-Eurasian J. Agric. and Environ. Sci.* 18 (6): 347-357.

[25] Altaf, S.A., Umar, D.M., and Muhammad, M.S. (2010). Production of xylanase enzyme by *Pleurotus eryngii* and *Flamulina velutipes* grown on different carbon sources under submerged fermentation. *W. Appl. Sci. J.* 8: 47-49.

[26] Ogunbayo, A.O, Olanipekun, O.O., and Babatunde D.E. (2016). Effect of pretreatment method on the hydrolysis of corn cob and sawdust. Anadolu Univ. *J. of Sci. and Technology - A - Appl. Sci. and Eng.* 17 (5): 795-811.

[27] Hussain, A., Shrivastav, A., Jain, S.K., Baghel, R.K., Rani, S., and Agrawale, M.K. (2012). Cellulolytic enzymatic activity of soft rot filamentous fungi *Paecilomyces variotii. Advances in Biores.* 3(3): 10-17.

[28] Salvachúa, D., Prieto, A., Lopes-Albelaiaras, M., Luchau, T., Martinezs, A.T., and Martinez, M.J. (2011). Fungal pretreatment: an alternative in second generation ethanol from wheat straw. *Bioresour. Technol*. 102(16): 7500-7506.

[29] Nwakaire, J.N., Ezeoha, S.L., and Ugwuishiwu, B.O. (2013). Production of cellulosic ethanol from wood sawdust. *Agri. Eng. Int.: CIGR J.* 15(3): 136-140.

[30] Udhayaraja, P. and J.S. Narayanan. (2012). Optimization for production of bioethanol using sorghum stovar by Saccharomyces cerevisiae. *International Journal of Research in Pure and Applied Microbiology* 2 (4): 64-67.

[31] Ezeoha, S.L., Anyanwu, C.N., and Nwakaire, J.N. (2017). The prospects, impacts, and research challenges of enhanced cellulosic ethanol production: Review. *Nigerian Journal of Technology* 36 (1): 267-275.

[32] Etonihu, A.C. and Idoko, O. (2014). Bioethanol production from lignocellulosic biomass wastes by fermentation of the hydrolyzates. *Standard Sci. Res. Essays* 9: 431-437.

[33] Kathiresan, K., Saravanakumar, K., and Senthilraja, S. (2011). Bio-ethanol production by marine yeasts isolated from coastal mangrove sediment. *Int. Multidi. Sci. R. J.* 1(1):19-24.

[34] Ali, E.N. and Jamaludin, M.Z. (2015). Possibility of producing ethanol from *Moringa oleifera* pod husk. *J. Advanced Res. Design* 5(1): 1-9.