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Pretreatment of maize straw with *Pleurotus ostreatus* and *Lentinus squarrosulus* for bioethanol production using *Saccharomyces cerevisiae*

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Abstract

Maize straw (MS) is a lignocellulosic substrate that constitutes huge wastes in the environment. This work aimed to pretreat MS with mushroom alone as a biological agent, and with NaOH prior to mushroom treatment (combined chemical and biological), and subsequently converting the released reducing sugars (RS) to ethanol using *Saccharomyces cerevisiae*. MS was degraded by *Pleurotus ostreatus* (PO) and *Lentinus squarrosulus* singly and in combination for 35 d. Samples were collected every 7 d from the treated straw to determine the RS content. Moreover, MS samples were pretreated with NaOH prior to degradation by the selected mushroom (combined pretreatment), and then their sugar profiles were determined using High-performance liquid chromatography (HPLC). The RS recovered from the degraded MS samples were fermented using 2 molecularly-identified *S. cerevisiae* strains. The highest RS contents (16.79 mg/ g) were recorded when MS was pre-degraded by PO for 21 d compared to *Lentinus squarrosulus* (16.55 mg/ g), and with the consortium of the two fungal cultures (16.36 mg/ g). However, MS pretreated with NaOH and *Pleurotus ostreatus* gave better yield of RS (17.38 mg/ g), than treatment with *Pleurotus ostreatus* (16.79 mg/ g) alone. The sugar profiles of the NaOH-PO-pretreated MS (mg/ 100 g) included; glucose (850.60); xylose (837.04), fructose (754.29), arabinose (502.76), ribose (2.066×10^{-4}) and rhamnose (3.552×10^{-5}). The fermenting yeasts were molecularly identified by sequencing of ITS region as *S. cerevisiae* SA01 and *S. cerevisiae* SA02, and assigned Accession no. of MK038975 and MN491900, respectively. Equal concentration of bioethanol (1.58 g/ l) was recorded in PO and in NaOH-PO-pretreated MS, which were fermented by *S. cerevisiae* SA01. Accordingly, MS can be utilized as a substrate for fermentation and then bioethanol production.

Keywords: Bioethanol, Maize straw, *Pleurotus ostreatus*, *Saccharomyces cerevisiae*, Reducing sugars

1. Introduction

Lignocellulosic substrates are mainly made up of cellulose, hemicellulose and lignin, and are non-edible wastes utilized for the production of second generation biofuel. The cellulose and hemicellulose aspects are referred to as holocellulose, and are the plant parts that contain fermentable sugars, which can be fermented to bioethanol. However, lignin does not contain any reducing sugars (Cardona and Sanchez, 2007; Agbor *et al*., 2011). These reducing/ fermentable sugars needed for generating the bioethanol from the lignocellulosic substrates are not readily released (Wakil *et al*., 2017). Therefore, there is a need for pretreatment of lignocellulose to obtain simple sugars required for biofuel production.

There are different types of pretreatments such as physical; chemical and biological ones (Rezania *et al*., 2020; Nguyen *et al*., 2021). The cheapest of these pretreatment techniques is the biological pretreatment, which is also ecofriendly (Kumar and Sharma, 2017). Biological pretreatment makes use of microorganisms including white-rot; brown-rot and soft rot fungi and/or their metabolites to degrade the lignocellulose (Isroi *et al*., 2011). There are two different types of white rot fungi that are selective and non-selective. Selective white rot fungi biodegrade lignin and hemicellulose parts, while the cellulose portion is essentially not affected. On the other hand, the non-selective white rot fungi biodegrade all components of lignocellulose equally (Hatakka and Hammel, 2010; Isroi *et al*., 2011). Among the white rot fungi that have been used for biodegradation of lignocellulose are *Phanerochaete chrysoporium*; *Pleurotus ostreatus*, *Lentinus squarrosulus*, *Pycnoporus cinnarbariunus*, *Cyathus stercolerus*, *Ceriporiopsis subvermispora* and *Ceriporia lacerate*, as reported by the recent studies conducted by Kumar and Sharma, (2017); Ganash *et al*., (2021).

Nigeria is the largest producer of maize in Africa (Ogbeh, 2018). MS is a lignocellulose that is made up of plant residues, which are abandoned in the farmland after the harvest of maize. It is also referred to as corn straw; corn stover or maize stover (Heuze *et al*., 2019). Huge quantities of this MS are released as wastes to the environment annually. Conversion of this waste to bioethanol will reduce the quantity of wastes in the environment, and will also create new jobs for several individuals. The objectives of the current study were to pretreat MS biologically and chemically, and then fermentation of the released reducing sugars to bioethanol using *S. cerevisiae*.

2. Materials and methods

2.1. Collection of maize straw and fungi

Maize straw samples were collected from Okunlola's farm, Ilora (7.8188° N, 3.9088° E), Oyo state, Nigeria. Two white rot fungi namely; *Pleurotus ostreatus* and *Lentinus squarrosulus*, with potent abilities to produce cellulases; hemicellulase and lignase enzymes were obtained from the Department of Botany, University of Ibadan, Ibadan, Nigeria. Two isolates of *S. cerevisiae* were provided by the Department of Biological Sciences, Ajayi Crowther University, Oyo, Nigeria.

2.2. Molecular identification of *Saccharomyces cerevisiae*

Yeasts cells (100 mg) suspended in 200 µl of isotonic buffer were placed individually in lysis tubes, and then 750 µl of lysis solution was poured into each tube; bead fitted with 2 ml tube holder to secure each sample. These samples were processed for 5 min. at maximum speed. The tubes were centrifuged using a microcentrifuge (Germany) for 1 min. at 10000 × g. Approximately 400 µl of the supernatant was poured into a Zymo-Spin IV Spin filter in a collection tube, which were then centrifuged for 1 min. at 7000 × g. Afterwards, 1200 µl of fungal DNA binding buffer was poured to the filtrate. 800 µl of the this mixture

was poured into a Zymo-Spin IIC Column in a collection tube, and then centrifuged for 1 min. at 10000 × g. About 200 µl of the DNA pre-wash buffer was added into a new collection tube containing Zymo-Spin IIC Column, and then centrifuged for 1 min. at 10000 × g. Approximately 500 µl of the fungal DNA wash buffer was added to Zymo-Spin IIC Column, and then centrifuged for 1 min. at 10000 × g. An aliquot of 100 µl of DNA elution buffer was poured to the column matrix containing microcentrifuge of the Zymo-Spin IIC column, and then centrifuged for 30 sec at 10000 × g; in order to elute the DNA (Garner *et al*., 2010; Angelov *et al*., 2015).

The extracted DNA was amplified in PCR. The cocktail mix is made up of H_2O (3.1 µl), 10 ng/ µl DNA (2.0 µl), Taq 5 units/ µl (0.1 µl), 2.5 Mm DNTPs (0.8 µl), DMSO (1.0 µl), 5 pMol forward primer (0.5 µl), 5 pMol reverse primer (0.5 µl), 25 mM $MgCl_2$ (1.0 µl), and 10 × PCR buffer (1.0 µl). The total volume was 10 µl. The forward primer is ITS4 TCCTCCGCTTATTGACATGS, while the reverse primer is ITS5 GGAACTAAAAGTCGTAACAAGG. The initial denaturation was executed at 94 °C for 5 min., and another denaturation was carried out for 40 sec. Annealing was carried out at 54 °C for 40 sec, while extension was carried out at 70°C for 45 sec. There were 36 cycles and final extension was carried out at 72 °C for 7 min. and then held at 10 °C.

Amplicon of PCR was loaded on 1.5 % agarose gel. The ladder used was 1 kb from Invitrogen (USA). The PCR product was purified by adding 20 µl of absolute ethanol; incubated at room temperature for 15 min., and then spun down at 10,000 rpm for 15 min. The supernatant was decanted and then spun down at 10,000 rpm for 15 min. An aliquot of 40 µl of 70 % ethanol was added, the supernatant was decanted and then air dried. 10 µl of ultra-pure water was added and the amplicon was checked on 1.5 % agarose. The PCR product was used for the sequencing reaction. The sequencing product was purified, and then loaded on the 3130 × l analyzer from applied biosystems (USA) to get the sequences.

The sequence obtained was subjected to sequence comparison through BLAST nucleotide search tool, and then identified at the National Centre for Biotechnology Information (NCBI). The sequence was sent to GenBank of NCBI in order to get the Accession number. Molecular phylogenetic analysis was carried out by maximum likelihood method, in reference to Tamura and Nei, (1993); Kumar *et al*., (2018).

2.3. Pretreatment of maize straw with white rot fungi

About 200 g of dried and milled maize straw sample was mixed with 600 ml of dist. water and then packed separately in polythene bags. This preparation was sterilized at 121 °C for 15 min. After cooling, each bag was inoculated individually with a full plate cut aseptically into discs using a sterile cork borer from 7 d old culture of each white rot fungus i.e. *Pleurotus ostreatus* and *Lentinus squarrosulus*, whereas a half plate of each fungus was used during the combination treatment, and then allowed to undergo degradation at 28± 2°C for 35 d (Wakil *et al*., 2017). Samples were withdrawn every 7 d and the reducing sugar contents were estimated.

2.4. Pretreatment of maize straw with NaOH and *Pleurotus ostreatus*

Sodium hydroxide was used as a chemical pretreatment alkali. About of 2.5 % NaOH solution was added to MS (10 %) for 1 h, and then autoclaved at 121 °C for 30 min. The autoclaved sample was filtered using a muslin bag, and then washed several times with dist. water until the pH of the filtrate was about 7. The residue was dried at 105 °C until a constant weight was obtained (Irfan *et al*., 2011; Nadeem *et al*., 2015). The alkali-pretreated sample was then degraded with a mushroom (*Pleurotus ostreatus*), which already had a recorded better degradability in terms of the released reducing sugars for 21 d; as described before in the step of pretreatment of MS with the white rot fungi. After the degradation, the samples were dried at 105 °C until a

constant weight was obtained, and then kept in an airtight nylon until further use (Adenipekun and Fasidi, 2005; Nadeem *et al*., 2015). After drying, the reducing sugar contents and sugar profiles of the degraded samples were determined, and then these samples were used for bioethanol production.

2.5. Determination of reducing sugars content

Reducing sugars were extracted by adding 5 % of the degraded substrates into an acetate buffer (0.1 M, pH 5). This suspension was boiled for 10 min., filtered using Whatman filter paper no. 1, and then the filtrate was used to determine the reducing sugars content using Dinitrosalicylic acid (DNS) method of Miller, (1959). The reagent of the DNS method was prepared by adding 1 g of 3, 5-Dinitrosalicylic acid to 20 ml of dist. water, and then heated in boiling water until dissolved. About 20 ml of 2 M NaOH was added followed by 30 g of sodium-potassium tartrate, followed by stirring until complete dissolution. The volume was made up to 100 ml with dist. water; boiled and finally allowed to cool down. The degradation filtrate was added to the DNS reagent at the ratio of 1:1; boiled at 100 °C for 5 min., and then allowed to cool down. Absorbance was measured using a spectrophotometer (721G Visible Spectrophotometer, China) at 540 nm. Similarly, different concentrations of glucose was also prepared and mixed with DNS reagent at the ratio of 1:1 as a standard. Absorbance of the different concentrations of glucose was measured at 540 nm, and used to plot a standard curve. Reducing sugar concentrations of the degradation filtrates were extrapolated from the standard curve (Miller, 1959).

2.6. Sugars profile analysis of maize straw pretreated with NaOH and *Pleurotus ostreatus*

Sugar profiles of the combined pretreated MS were analysed through HPLC (Agilent 1200 series - USA), according to Valliyodan *et al*., (2015). Refractive index detector (RID) was used as a detector with an injection volume of 5.0 µl and flow rate of 1.0 ml/ min. at 25 °C. A membrane filter (0.45 µm) was used to filter and degas freshly prepared mobile phase (75 acetonitrile: 25 ultra-pure water) via vacuum filtration. A standard stock solution of approximately 8 g/ l was prepared and aliquots of this solution were used to make up the required standard concentration. Appropriate and accurate weights of the standards were added to a volumetric flask in order to prepare different calibrations standards using deionized water. Approximately 2.5 g of combined pretreated MS was weighed into a volumetric flask making up to a volume of 500 ml. Cellulose-acetate membrane filters were used to filter and degas the sample solution through vacuum filtration. Syringe filters were fitted on all the syringes before injecting into the column.

2.7. Fermentation of pretreated maize straw by the two strains of *S. cerevisiae*

A total of 5 % of pretreated *Pleurotus ostreatus* and NaOH-*Pleurotus ostreatus* MS was added to an acetate buffer (0.1 M, pH 5.0), boiled and then filtered with a muslin bag. The filtrates were sterilized at 121 °C for 15 min.; allowed to cool, and then inoculated individually with 2 % inoculum of 1.0 MacFarland standard of *S. cerevisiae* SA01 and *S. cerevisiae* SA02. All treatments were incubated at 30 °C for 5 d (Nadeem *et al*., 2015). An aliquot of 100 ml of the fermenting samples was withdrawn every 24 h for estimation of the reducing sugars and the bioethanol contents.

2.8. Determination of the bioethanol contents

Bioethanol content was determined by a gravimetric method, in reference to Wakil *et al*., (2013). About 60 ml of the fermented filtrates was distilled using a heating mantle, and the distillate was collected through a glass condenser. Equal volumes of the distillate and dist. water were added individually to empty and dried 25 ml density bottles, and then their weights were determined. The weights of the distillate and dist. water were determined by subtracting the weights of the empty bottles from weights of the distillate and dist. water within the bottles. The specific gravity of the distillate was determined by dividing the weight of the distillate by weight of the

dist. water. Specific gravity was used to extrapolate the bioethanol contents with ethyl alcohol conversion table (AOAC. 1990).

2.9. Statistical analysis

The experimental data was analysed using Analysis of Variance (ANOVA) to determine the means with SPSS version 23 and the level of significance was set at $p \leq 0.05$.

3. Results

3.1. Molecular identification of *S. cerevisiae*

The two strains of *S. cerevisiae* were identified as *S. cerevisiae* SA01 and *S. cerevisiae* SA02, assigned accession numbers of MK038975 and MN491900, respectively. Phylogenic analysis of both strains is shown in Fig. (1). Both *S. cerevisiae* strains were closely related to *S. cerevisiae* Wu-Y2, but have distant relationship with *Pichia membranifaciens* CBS107 and *Brettanomyces bruxellensis* CBS72.

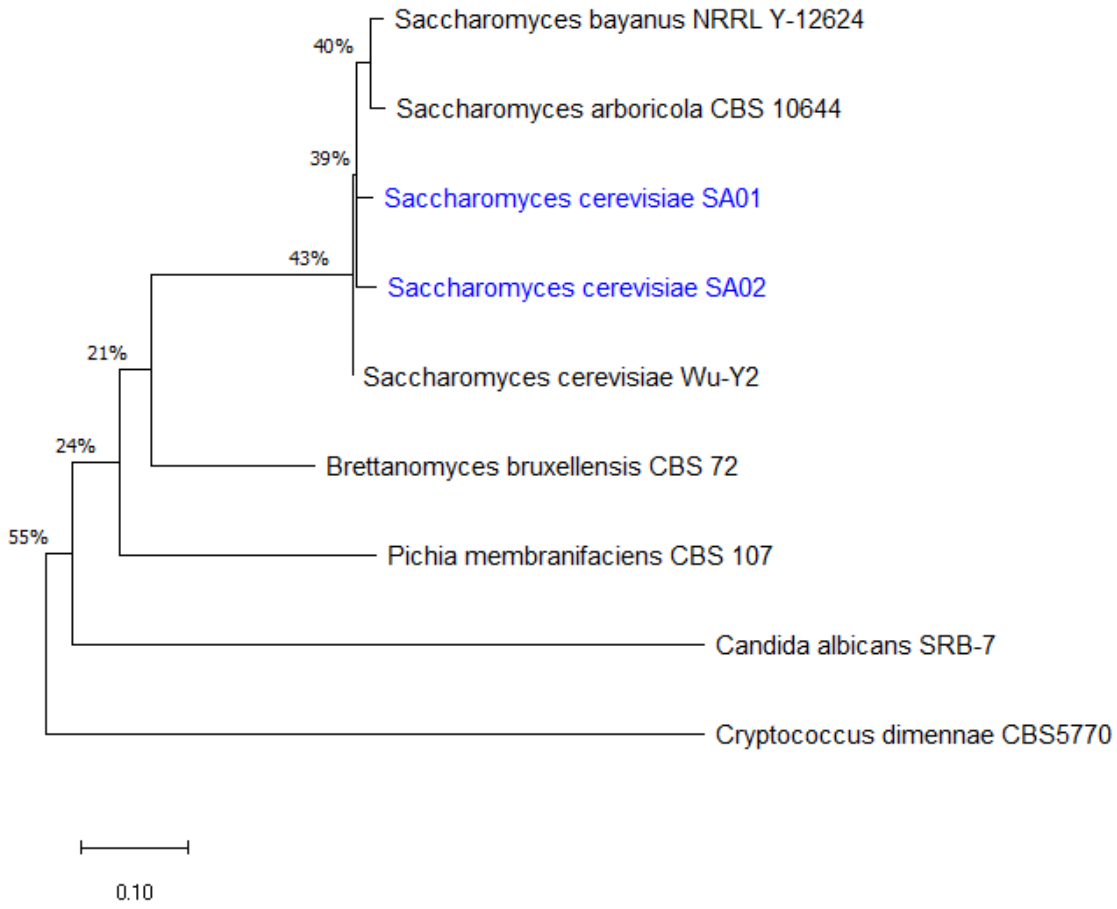

Fig. 1: Phylogenic analysis of *S. cerevisiae* SA01 and *S. cerevisiae* SA02

3.2. Pretreatment of maize straw with the white rot fungi

The amount of reducing sugars released during degradation of MS by *Pleurotus ostreatus* and *Lentinus squarrosulus* are shown in Table (1). The concentration of reducing sugars of MS degraded by *Pleurotus ostreatus* (PO) ranged from 15.81 mg/ g to 16.79 mg/ g; with the least values (15.81 mg/ g) recorded at 14 and 35 d of degradation, while the highest concentration (16.79 mg/ g) was expressed after 21 d of degradation. When MS was degraded by *Lentinus squarrosulus* (LS), the highest concentration of 16.55 mg/ g and the least concentration of 14.18 mg/ g were recorded at 35 and 28 d of degradation, respectively. Increase in reducing sugars concentration from 15.52 mg/ g (at 7 d of degradation) to 16.36 mg/ g (at 28 d of degradation) were observed when MS was degraded by a consortium of *Pleurotus ostreatus* and *Lentinus squarrosulus* (PO+LS). There was a significant difference ($p \leq 0.05$) in the released reducing sugars from MS with the increase in the degradation time. The highest concentration of reducing sugars (16.79 mg/ g) was recorded when MS was degraded by *Pleurotus ostreatus* for 21 d. Hence, MS degraded by *Pleurotus ostreatus* for 21 d was selected for further research.

Table 1: Amounts of reducing sugars released (mg/ g) during degradation of maize straw by *Pleurotus ostreatus* and *Lentinus squarrosulus*, within 35 d of degradation

Period of degradation (d)	PO	LS	PO+ LS
0	15.90^{ab}	15.90^{c}	15.90^{b}
7	15.86^{ab}	15.81^{c}	15.52^{a}
14	15.81^{a}	15.57^{b}	15.68^{a}
21	16.79^{c}	15.79^{c}	15.58^{a}
28	16.02^{b}	14.18^{a}	16.36^{d}
35	15.81^{a}	16.55^{d}	16.17^{c}

Mean values with different superscript letters along the column were significantly different ($p \leq 0.05$). Where; PO: *Pleurotus ostreatus*; LS: *Lentinus squarrosulus*; PO+ LS: Consortium of *Pleurotus ostreatus* and *Lentinus squarrosulus*

3.3. Pretreatment of maize straw with NaOH and *Pleurotus ostreatus*

The reducing sugars content of MS pretreated with *Pleurotus ostreatus*, and combined pretreatment with NaOH followed by *Pleurotus ostreatus* are demonstrated in Fig. (2). The concentration of reducing sugars content of the non-pretreated (NP) MS was 15.98 mg/ g. The reducing sugar content of MS pretreated with *Pleurotus ostreatus* (PO) only was 16.79 mg/ g, whereas a concentration of 17.38 mg/ g was recorded on combined pretreatment of straw with NaOH and *Pleurotus ostreatus* (NaOH+ PO). The quantities of reducing sugars with different pretreatment methods are represented in the descending order of NaOH + PO >PO >NP.

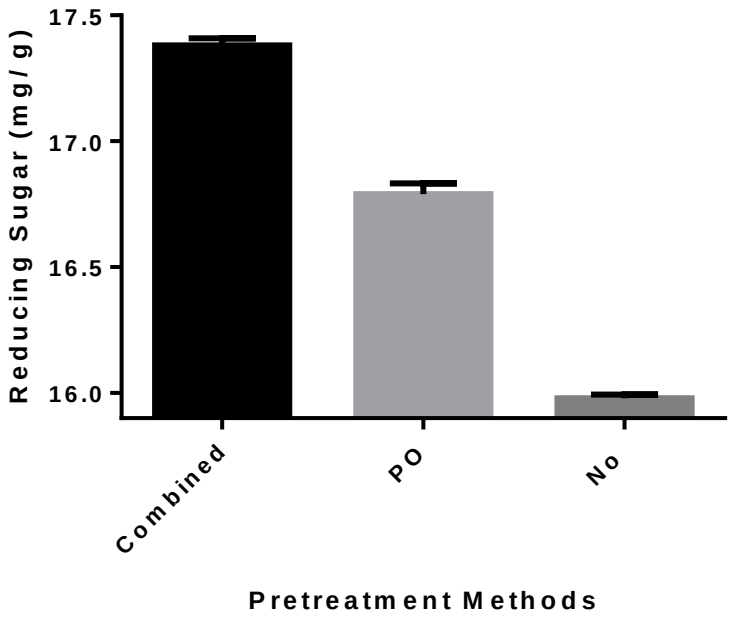

Fig. 2: Reducing sugars of single and combined pre-treatment of MS with the white rot fungi

Where; Combined: NaOH + *Pleurotus ostreatus*; PO: *Pleurotus ostreatus*; No: No treatment. The error bars represent the mean standard deviation ± SEM

3.4. Sugars profile analysis of maize straw pretreated with NaOH and *Pleurotus ostreatus*

The concentration of sugars recorded in the combined-pretreated MS is presented in Table (2). The recovered sugars in these combined pretreated MS were; ribose, xylose, arabinose, rhamnose, fructose, glucose, maltose, lactose, sucrose, furfural and hydroxyl-methylfurfural (HMF). Glucose recorded the highest sugar concentration of 850.599 mg/ 100 g, followed by the five-carbon xylose (837.043 mg/ 100 g); however, the least concentration of 3.552×10^{-5} mg/ 100 g was observed in rhamnose. Furfural and hydroxyl-methylfurfural were also detected in the samples with concentrations of 63.122 mg/ 100 g and 40.648 mg/ 100 g, respectively.

3.5. Fermentation and bioethanol production from the pretreated maize straw using *S. cerevisiae*

Bioethanol production and reducing sugars consumption from MS pretreated with NaOH + *Pleurotus ostreatus*, and with *Pleurotus ostreatus* alone, and then fermented by *S. cerevisiae* SA01 are demonstrated in Fig. (3). Before fermentation by the yeast strain, the initial concentration of reducing sugars released from combined (chemical + biological) pretreated straw samples (17.99 mg/ g) was higher than the concentration recorded from *Pleurotus ostreatus*-pretreated straw samples alone (16.85 mg/ g). Moreover, higher concentrations of reducing sugars were observed in the combined pretreated straw than *Pleurotus ostreatus* pretreated straw alone throughout the fermentation period.

Table 2: Sugars profile of the combined pretreated maize straw

Sugars	Concentration (mg/ 100 g)
Furfural	63.122^{e}
Hydroxymethylfurfural	40.648^{d}
Ribose	2.066×10^{-4c}
Xylose	837.043^{j}
Arabinose	502.763^{h}
Rhamnose	3.552×10^{-5a}
Fructose	754.392^{i}
Glucose	850.599^{k}
Maltose	418.964^{f}
Lactose	468.464^{g}
Sucrose	4.237×10^{-5b}

Where; The mean values with different superscript letters along the column were significantly different ($p \leq 0.05$)

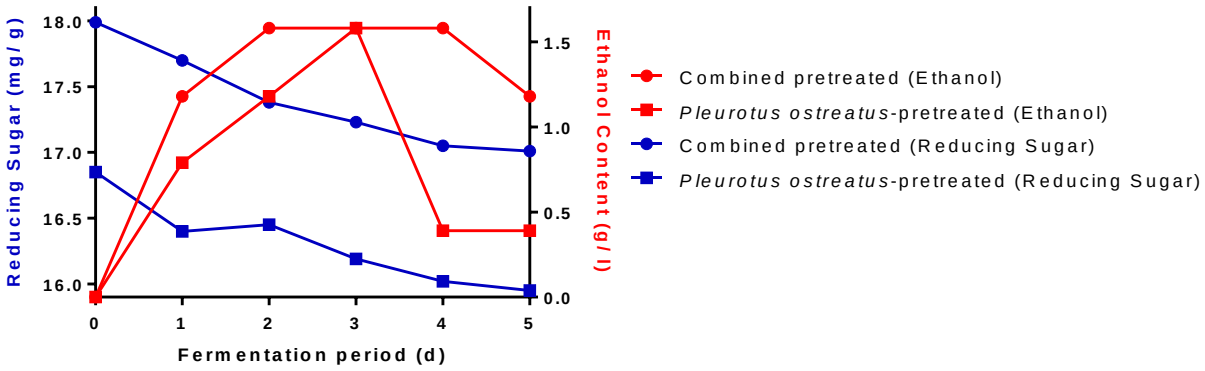

Fig. 3. Ethanol production and reducing sugars utilization by *S. cerevisiae* SA01 from pretreated maize straw

There was a decrease in concentration of the reducing sugars of both of the combined and *Pleurotus ostreatus*-pretreated MS fermented by *S. cerevisiae* SA01 with the increase in fermentation period from 17.99 to 17.01 mg/ g and 16.85 to 15.95 mg/ g, respectively. High bioethanol content of 1.58 g/ l was recorded in both of the combined and *Pleurotus ostreatus* – pretreated MS fermented by *S. cerevisiae* SA01. The fastest content of bioethanol (1.58 g/ l) was expressed at the 2nd d of fermentation in the combined pretreated MS.

The effect of fermentation period on bioethanol production by *S. cerevisiae* SA02 from combined (*Pleurotus ostreatus* + NaOH) pretreated, and *Pleurotus ostreatus*–pretreated MS is demonstrated in Fig. (4). Higher reducing sugar concentration of 17.99 mg/ g was observed in the unfermented combined-pretreated MS filtrate, whereas the least concentration (15.81 mg/ g) was recorded in 5-d fermented *Pleurotus ostreatus*-pretreated MS filtrate. Throughout the fermentation period, the concentration of reducing sugars content was higher in the combined pretreated MS than in *Pleurotus ostreatus*-pretreated MS. The content of bioethanol produced from *Pleurotus ostreatus*-pretreated MS fermented by *S. cerevisiae* SA02 ranged from 0.00 g/ l before fermentation to 1.18 g/ l after 1 and 2 d of fermentation. There was an increase in the bioethanol content of the combined-pretreated MS with increasing the fermentation period. The highest bioethanol content of 1.18 g/ l was recorded after 2 d of fermentation of the combined pretreated MS filtrate; maintained till the 4th d and thereafter declined.

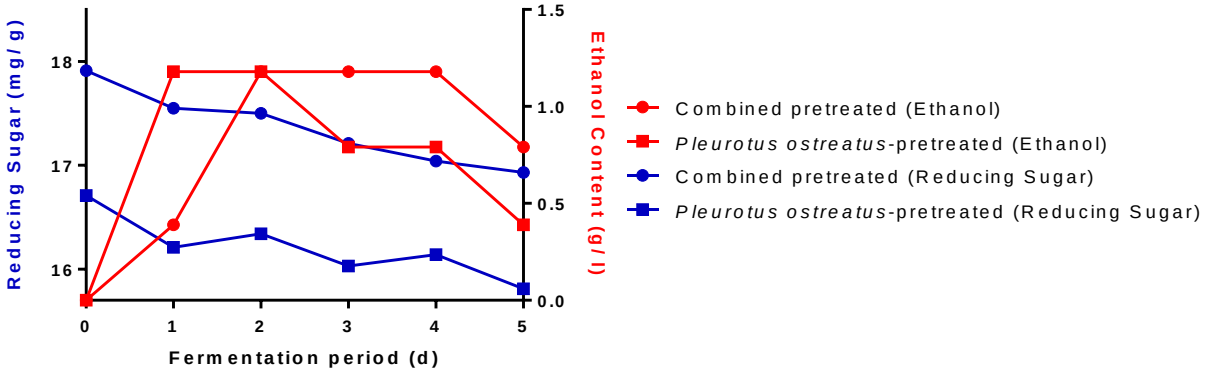

Fig. 4: Ethanol production and reducing sugars utilization by *S. cerevisiae* SA02 from pretreated maize straw

4. Discussion

In this study, *Pleurotus ostreatus* and *Lentinus squarrosulus* mushrooms degraded MS and released reducing sugars. The ability of these mushrooms to break down the lignocellulosic biomass has been long known (Wakil *et al*., 2017; Ravichandran *et al*., 2021), which could be attributed to their abilities to produce lignocellulose degrading enzymes including; cellulases, xylanases and lignases that break down the different components of lignocellulose. Many researchers have used *Pleurotus* and *Lentinus* species to degrade lignocellulose (Wakil *et al*., 2017; Ravichandran *et al*., 2021; Zamora *et al*., 2021). The observed higher reducing sugars in the monoculture-degraded substrates than in the co-cultured-degraded substrates could be due to the antagonistic effects of the co-cultured mushrooms on each other's. Similarly, a previous study conducted by Wang *et al*., (2014) reported better yield of reducing sugars in *Populus tomentosa* (Poplar wood) degraded by *Trametes orientalis* only, compared to that degraded with consortium of microorganisms. MS used in this study has also been used for bioethanol production by several previous researchers (Onoghwarite *et al*., 2016; Kolajo, 2021). According to Huang *et al*., (2017); (2019), the increase in reducing sugars contents observed in the biodegraded MS could be related to the breaking down of the different components of that straw by enzymes produced by the microorganisms. However, this could be influenced by both of the genetic make-up and the environmental conditions.

Suhardi *et al*., (2013); Yang and Wang, (2019) suggested that the observed higher reducing sugars concentrations in combined pretreated MS (NaOH and a fungus); compared to single pretreated straw could be attributed to the synergistic effects of the biological and chemical pretreatments. The possible breaking down of the recalcitrant lignin in the lignocellulosic substrates by pretreatment with NaOH might have better exposed the MS to the enzymes produced by the mushrooms used for biological pretreatment. In accordance, Saulnier *et al*., (2020); Kumar *et al*., (2021) recently reported that pretreatment of lignocellulose with acid/ base prior to inoculation with cellulase producing microorganisms allows better access of this enzyme to the cellulose in this substrate. In addition, Yang and Wang, (2019) revealed that combined pretreatment of grass resulted in higher yields of xylose and arabinose than single pretreated samples. Furthermore, Dai *et al*., (2015) observed that biological pretreatment of rice straw combined with NaOH had higher yield of sugars than the single pretreated substrate.

The yeasts including *S. cerevisiae* used for fermentation of reducing sugars recovered from pretreated MS have been known for their conversion abilities of hexoses to ethanol. Hexoses are fermented to ethanol through glycolysis and then pyruvate is converted to ethanol via the alcoholic fermentation pathway. This could be attributed to the ability of *S. cerevisiae* to produce pyruvate decarboxylase and alcohol dehydrogenase enzymes during the alcoholic fermentation, as reported recently by Zhang *et al*., (2021).

The observed decrease in reducing sugars contents recorded during fermentation of the degraded substrates might be attributed to the conversion of reducing sugars to ethanol during the process of fermentation (Kurambhatti *et al*., 2018; Zhao *et al*., 2018).

The recorded decline in the bioethanol yield after an initial increase during the first 72 h of fermentation of *Pleurotus ostreatus*-degraded MS by *S. cerevisiae* SA01 and *S. cerevisiae* SA02 could be attributed to the decline in the reducing sugars in the fermentation medium, in addition to the conversion of the produced bioethanol to other compounds (Adelabu *et al*., 2018; Huezo *et al*., 2019; Mori *et al*., 2019). In agreement with this observation, Mori *et al*., (2019) reported that exhaustion of fermentable sugars results in the oxidation of bioethanol to acetate by many microorganisms, which will later be converted into

acetyl-CoA, carbon dioxide and water during the Kreb's cycle. In this study, the highest yield of bioethanol observed at 72 h of fermentation is in accordance with the previous works conducted by Tsunatu *et al*., (2017); Adelabu *et al*., (2018), who also reported the same result. Despite that combined-pretreated MS had higher reducing sugars than single-pretreated one; the two treatments had equal higher bioethanol contents on fermentation. A previous study of Kupiainen *et al*., (2014) attributed this observation to the presence of furfural and hydroxymethylfurfural in the hydrolysed combined-pretreated MS, which could have prevented glucose from being available for bioethanol production. Lukajtis *et al*., (2018) reported that furfural and hydroxymethylfurfural have negative effects on the metabolism of microorganisms during fermentation.

Conclusion

In biological pretreatment, the highest reducing sugar (16.79 mg/ g) was released when MS was pretreated by *Pleurotus ostreatus* for 21 d, while combined (biological and chemical) pretreatment gave better reducing sugars yield (17.38 mg/ g). The highest content of bioethanol (1.58 g/ l) was attained through fermentation of both single (at 72 h) and combined (at 48 h) pretreated MS by *S. cerevisiae* SA01. Meanwhile, *S. cerevisiae* SA02 produced 1.18 g/ l of bioethanol from single and combined pretreated straw at 24 h and 48 h of fermentation, respectively. The characters of being a cheap and a readily available substrate made bioethanol production from MS highly attractive.

Conflict of interest

No conflicts of interest exist.

Funding source

There was no external funding source for this research.

Ethical approval

Not applicable.

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