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Ethanolic Fermentation of Saccharified Common Thatch Grass Hydrolysate by *Candida shehatae* AND *Saccharomyces cerevisiae* Species

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ABSTRACT

Fossil fuels, such as oil, coal and natural gases, still remain the prime sources of energy worldwide. These resources are likely to be depleted within the next few decades. Current environmental issues like global warming, acid rain and urban smog have led to a shift of focus to utilizing renewable energy sources, such as solar, wind,and biofuels, which are less environmentally harmful and are sustainable. Ethanol is one of the most promising alternative biofuel in this respect. A number of biomass feedstock have been considered for bioethanol production among which grasses have been suggested. In this study, bioethanol was produced by the fermentation of a hydrolysate obtained from the saccharification of pre-treated common-thatch grass (*Hyparrhenia spp*.). The grass is abundant in Southern Africa and can be used as feed during its early stages of development. Older grasses have low nutritional value due to lignification and is thus used for thatching, fences or is burnt to clear the veld. Size reduction was done to the grass after-which it was pre-treated with either alkali or acid to remove lignin which is a barrier to saccharification. The grass was enzymatically saccharified using a mixture of cellulases namely Celluclast™ and *Aspergillus niger* cellulase mixture to produce glucose and other fermentable sugars. *Candida shehatae* CSIR Y-0492 and *Saccharomyces cerevisiae* WBSA 1386 were utilised to convert the glucose in the hydrolysate to ethanol. The effect of adding a nutrient supplement on the production of ethanol was also investigated. The pre-treatment method and addition of nutrients had no significant effect on the amount of bioethanol produced from the grass hydrolysate. Both *S. cerevisiae* WBSA 1386 and *C. shehatae* CSIR-Y 0142 were able to produce ethanol above the theoretical expected value of 0.5 g/L, indicating the use of sugars other than glucose in the production of ethanol. Ethanol production was at an average of 8.3 g/L for both organisms. The results obtained indicate that pre-treated thatch grass, when saccharified by cellulases and xylanases, can potentially be utilized for production of bioethanol.

Key words: ethanolic fermentation, bioethanol, saccharification, thatch-grass, hydrolysate

1.0 INTRODUCTION

Ethanol produced from various bio-based sources (bioethanol) has been gaining high attention lately due to its potential to cut down net emissions of atmospheric carbon dioxide (Lu *et al.,* 2021*)*. The search for cleaner fuels has promoted the research and development of alternatives for replacing fossil resources as the feedstock of fuels and chemicals (Robak and Balcerek, 2020; Medina and Magalhaes, 2021). Renewable lignocellulosic biomass is a sustainable feedstock for bioethanol production, and it shows immense potential to replace fossil fuels (Li *et al*., 2013; Singh *et al*., 2020). Among the various biomass feedstocks available are the sugar and starch-rich crops (first generation) and the lignocellulosic materials (second generation). Common thatch grass (*Hyparrhenia spp*.) is part of the lignocellulosic materials and is attractive due to its abundance, renewability, and potential as a sustainable resource for biofuel production. *Hyparrhenia* species is

widely distributed in East and Southern Africa and other tropical areas around the world. *Hyparrhenia* specimens are very difficult to identify to species level, as a result of hybridization, apomixis and polyploidy. It consists of a mosaic of intergrading species. The grass is a strongly rooted perennial grass that is mainly used as fodder and thatching material. It can also help in controlling erosion and is considered a weed in some regions. When the grass is still young, it is a valuable fodder that can be grazed by all classes of livestock or it can be cut to make hay or silage. Older plants are coarse and unpalatable and thus provide poor quality grazing. It is used maily as thatching material in Africa and weaving mats. *Hyperrhaenia* is drought- resistant and can be used to reclaim soil and control erosion (Kativu, 2011). In this study, common thatch grass (CTG) was used for bioethanol production. Common thatch grass has high lignocellulosic content and the complex structure of lignin, cellulose, and hemicellulose presents challenges for direct fermentation. To overcome these challenges, a two-step process is often employed: saccharification, which involves the breakdown of the plant's structural components into simpler sugars, followed by fermentation. Ethanolic fermentation is a biotechnological process that converts fermentable sugars into ethanol and carbon dioxide through the action of microorganisms, primarily yeast (Tse *et al*., 2021).

Pre-treatment of lignocellulosic materials such as CTG maximises the efficiency and effectiveness of the saccharification process (Tse *et al*., 2021; Lu *et al.,* 2021). Pre-treatment processes may be physical (e.g., milling, temperature, and ultrasonication), chemical (e.g., acid and alkaline treatments, organic solvent treatments) or biological (e.g. enzymatic hydrolysis) processes, among others (Lu *et al.,* 2021; Li *et al*., 2013). Alkali pre-treatment of common thatch grass (*Hyparrhenia spp*.), enhances its digestibility and increases the yield of fermentable sugars. The hydroxides of sodium, potassium, calcium and ammonium are often used for alkali pre-treatment, with sodium hydroxide being the most effective (Kim *et al*., 2016). A saponification reaction during the alkali pre-treatment process results in solubilisation of lignin and hemicellulose fragments due to breakdown of intermolecular ester linkages between hemicelluloses and lignin. The swelling of cellulose during alkali treatment causes a reduction in crystallinity and degree of polymerisation of lignocellulose structure thereby increasing the internal surface area (Lu *et al.,* 2021*)*. Uronic acid substitutions and acetyl removal in hemicelluloses during alkali treatment increases the accessibility of the carbohydrates during saccharification (Baruah *et al*., 2018; Singh *et al*., 2020). Acid pre-treatment has been widely used to solubilise hemicellulose and improve accessibility to cellulose prior to enzymatic digestion by interrupting lignin structure. Acid pre-treatment is an efficient process but sugar yield may be low and under poorly optimised conditions, fermentation inhibitors, such as furfural, 5-hydroxymethylfurfural (HMF), formic acid, acetic acid, levulinic acid, and phenolic compounds are formed (Robak and Balcerek, 2020; Singh *et al*., 2020).

Saccharification or hydrolysis of CTG to fermentable sugars is a critical step in bioethanol production. During saccharification, cellulose and hemicellulose are cleaved using cellulolytic and hemicellulolytic enzymes (Olivieri *et al*., 2021). Generally, cellulolytic enzymes consist of three categories of enzymes; endoglucanase which breaks β-1-4-glucosidic bonds cellulose chains; while, exoglucanases or cellobiohydrolases cleaves reducing and non-reducing ends of cellulose chains thus producing cellobiose units. The β-glucosidase breaks cellobiose to release glucose units (Keshav *et al*., 2021). Fermentation of lignocellulosic sugars produced from enzymatic saccharification of CTG is the last step in bioethanol production. The naturally occurring ethanologenic yeast and bacterial strains include *Saccharomyces cerevisiae, Kluyveromyces marxianus,*

Scheffersomyces stipitis, Candida shehatae, Pachysolen tannophilius, and Escherichia coli. Among these microbes, *S. cerevisiae* and *Z. mobilis* are normally employed in bioethanol fermentation of hexose sugars (Keshav *et al*., 2021). The organisms can also tolerate ethanol concentrations between 12 and 18% but are unable to utilize xylose sugars present in hydrolysate. On the other hand, *C. shehatae,* and *P. tannophilius* are known to be capable of fermenting xylose sugars, however their ethanol fermentation efficiencies are low and are more sensitive to higher concentrations of inhibitors and ethanol (Vohra *et al*., 2014). In this study, *S. cerevisiae* and *C. shehat*ae were used for fermentations to produce ethanol from the hydrolysates.

2.0 MATERIALS AND METHODS

2.1 Proximate analysis of Common thatch grass

Proximate analysis of CTG was done using the Dosi fiber method for acid detergent lignin, acid detergent fiber and neutral detergent fibre, crude protein, ash, nitrogen, crude fibre, moisture content and dry matter content. The detergent soluble hemicellulose was calculated as follows:

Hemicellulose = neutral detergent fibre – acid detergent fibre [1]

Detergent cellulose was calculated as follows:

Cellulose = acid detergent fibre – acid detergent lignin [2]

2.2 PREPARATION OF FERMENTATION SUBSTRATE

2.2.1 Size reduction of Common thatch grass

Common thatch grass (CTG) (*Hyparrhenia sp.*) was cut into pieces approximately 1cm long and dried in an oven at 100 °C overnight. The dried grass was ground into a fine textured powder using a commercial blender after which it was sieved using a kitchen sieve with an aperture of 1 mm. The sieved grass was then transferred into a sealed container and autoclaved for 15 minutes at 121 °C and 1 atmosphere. The autoclaved CTG was stored in a dark bottle at 4 °C.

2.2.2 Pre-treatment of common thatch grass

Ground CTG was subjected to either acid or alkaline pre-treatment. Acid pre-treatment was performed by addition of 100 ml of 0.5% H_2SO_4 to 7.83 g of CTG. The slurry was hydrolysed at 121 °C for one hour. pH was adjusted to pH 5 using 10 M NaOH (Saha *et al.*, 2005). The slurry was filtered using a 150 mm Buchner filter fitted with Whatman Chromatography paper. The filter paper containing the pre-treated grass was removed and dried at 50 °C until a constant weight was obtained.

Alkali pre-treatment was performed by adding 0.1 g $Ca(OH)_2$ to 1 g ground CTG. A slurry was produced by adding 10 ml of water to 1 g of ground CTG. The slurry was placed in an incubator at 50 °C overnight with shaking at 100 rpm. Glacial acetic acid was used to adjust the pH to 5. The pre-treated grass was dried at 50 °C until a constant weight was obtained. The pre-treated CTG was kept in a dark bottle at 4 °C until needed.

2.3 Production of cellulolytic enzymes in solid state fermentations

Aspergillus niger FGSC A733 was cultured in 100 ml Erlenmeyer flasks containing 7.5 g of *Jatropha* seedcake with 10% common thatch grass. The substrate was hydrated with 15 ml of water. The flasks were inoculated with 750 µl of the inoculum (1 x 10^6 spores/ml). The flasks were shaken by hand to mix the contents and the culture was incubated for 40 °C for 4 days.

2.4 Extraction of enzymes from solid state fermentations

After the incubation period, the crude enzyme was extracted by adding 5 ml of a 50 mM acetate buffer, pH 5, per gram of

fermented substrate. The mixture was shaken at 150 rpm and 4 °C for 2 hours and was then centrifuged at 1500 x g for 20 minutes to obtain a clear supernatant. The supernatant was kept in 50 ml aliquots at -20 °C and thawed at room temperature when required.

2.5 Saccharification of common thatch grass under optimized conditions

Enzymatic hydrolysis of CTG was performed according to the US National Renewable Energy Laboratory Procedure LAP 009 (Selig *et al*., 2008) with slight modifications. The hydrolysis mixture consisted of 7.5% (w/v) CTG, 15 ml of the enzyme mixture and 15 ml of 50 mM acetate buffer pH 5. The enzyme mixture had 10 mg/ml Celluclast™ solution and *Aspergillus* cellulase (crude culture supernatant) (2:1 ratio) (v/v) translating to Celluclast™ (12.27 FPU) to Aspergillus niger cellulase (1.32 FPU) mixture. Enzymatic hydrolysis of CTG was performed in 50 ml reaction tubes. The control was prepared by substituting the active enzyme with 15 ml of an enzyme previously boiled for 10 minutes.

The reaction mixture was incubated at 50 °C in a shaking water bath at 150 rpm for 24 hours. After the incubation period, the tubes were centrifuged for 10 min at 9 000 x g using a Beckman Coulter Allegra X22R centrifuge (Indiana, US). The supernatant was recovered and then filtered through a 0.2 µm syringe filter. The supernatant was analysed using the high-performance liquid chromatography (HPLC) determine the amount of sugars produced from the CTG by the enzyme mixture.

2.6 Analysis of sugars from the hydrolysis mixture

The CTG hydrolysate samples were analysed using a Shimadzu (Kyoto, Japan) HPLC equipped with either an Aminex HPX-87C column (300 x 7.8 mm, Ca^{2+}, particle size 9 µm) (Biorad, Hercules, CA, USA) or the Rezex ROA-organic acid column (300 x 7.8 mm, H^{+}, particle size 8 µm) (Phenomenex, USA) as described by Scarlata and Hyman (2010). The Aminex HPX-87C was fitted with an Aminex Resin Microguard, Carbo C column (Biorad, Richmond CA, USA) while the Rezex ROA-organic acid column was fitted with a 5-micron Rezex organic guard column (Phenomenex, USA). The detection of eluents was done using an RID 10A refractive index detector (Shimadzu, Kyoto, Japan). The samples were filtered through a 0.2 µm syringe filter. Glucose, maltose, mannose, fructose, xylose and melezitose were used as standards to determine the retention time of sugars in the samples separated on the columns. The Aminex HPX-87C column temperature was maintained at 85 °C. Degassed deionised distilled water at a flow rate of 0.6 ml/min was used as the mobile phase for the Aminex HPX-87C column. The Rezex ROA-organic acid column temperature was maintained at 65 °C and eluted with 0.005 N sulphuric acid at a flow rate of 0.6 ml/minute. For each of the saccharification mixtures, the initial glucose present was subtracted from the final glucose obtained at each sampling time. Peak detection and integration were done using LC Solutions software from Shimadzu (Kyoto, Japan). Peak heights were used for analysis of the results using Sigma plot Data analysis and graphing software.

2.7 Maintenance and propagation of yeast cultures

Saccharomyces cerevisiae WBSA 1386 was grown on malt agar broth (20g malt agar in 1 L distilled water) and aliquoted into 1 ml volumes in sterile Eppendorf tubes. The stock culture was kept at -20 °C. The stock culture was thawed when needed and used as inoculum for malt agar plate, slants and broth as required. The inoculated plates, slants or broth were incubated at 24 °C for 48 hours. The slants were kept as stock cultures at 4 °C.

Candida shehatae CSIR Y-0492 was prepared by inoculating the yeast on YM

broth (3 g yeast extract, 3 g malt extract and 3 g peptone in 1000 ml distilled water) at pH 4.5. Inoculated YM agar plates, slants or broth were incubated at 30 °C for 48 hours. YM slants were kept at 4 °C as stock cultures and subcultured on a monthly basis.

For the production of pre-inoculum for ethanol production, *C. shehatae* CSIR Y-0492 was grown in 100 ml of YM broth while *S. cerevisiae* WBSA 1386 was grown in 100 ml malt extract broth in 250 ml Erlenmeyer flasks. The *C. shehatae* CSIR Y-0492 culture was incubated at 30 °C for 24 hours while *S. cerevisiae* WBSA 1386 was incubated at 24 °C for 24 hours. A cell count was performed for each of the cultures after 24 hours on a Neubauer bright lined counting chamber (Germany) to determine the volume of inoculum required to give 1 x 10^6 cells/ml for the fermentation of the hydrolysate.

2.8 FERMENTATION OF ACID AND ALKALINE COMMON THATCH GRASS HYDROLYSATES FOR BIOETHANOL PRODUCTION

The fermentation medium consisted of either an alkaline or acid pre-treated hydrolysate with or without a nutrient supplement. The hydrolysate (100ml) was sterilised using a Millipore Sterifilm filtration system (Nihom Millipore Ltd, Yonezawa, Japan). The filtration unit was fitted with a 50 mm 0.2 µm sterile filter membrane. The nutrient supplement was aseptically added to the hydrolysate to give a final concentration of 1 g yeast extract, 0.5 g $(NH_4)_2HPO_4$, 0.025 g $MgSO_4.7H_2O$, 1.38 g NaH_2PO_4 in 1 L of the hydrolysate. The fermentation was carried out in 250 ml Erlenmeyer flasks fitted with rubber bung and a fermentation lock half filled with sterile distilled water. A pre-inoculum was used to give a final cell concentration of 1 x 10^6 yeast cells/ml. The fermentation broths were incubated at 24 °C for *S. cerevisiae* WBSA 1386 and at 30 °C for *C. shehatae* CSIR Y-0492. Aliquots of 1 ml were withdrawn at 12-hour intervals to determine the residual glucose and the ethanol quantities.

2.9 Quantification of ethanol

Ethanol was measured by capillary gas chromatography using Shimadzu (Kyoto, Japan) GC 2010 Plus apparatus equipped with Auto injector AOC 20i (Shimadzu) and Autosampler AOC 20S (Shimadzu), a flame ionization detector (FID) and a Zebron ZB wax Plus 30 M (Phenomenex, USA) column (30 m, 0.25 mm ID and film thickness of 0.25 µm). The column flow rate was maintained at 1.29 ml/min. The oven temperature was maintained at 40 °C for 1 minute then increased 140 °C and finally maintained at 250 °C for the duration of the analysis. The injection temperature was 200 °C and the injection volume was 1 µl. A split injection mode with a split ratio of 11.4 was applied. The detector was maintained at 250 °C. Nitrogen at a flow rate of 30 ml/min was used as the carrier gas. Absolute ethanol was used for preparation of the standards which were 0.1, 0.5 and 1 % (v/v). Peak detection and integration were done using GC Solutions software from Shimadzu (Kyoto, Japan). Peak areas were used for analysis of the results and ethanol peaks were identified based on elution time. Concentrations of the unknown were determined from peak areas of the standards.

Ethanol yield (Y_{ETOH}) was calculated as follows:

$$Ethanol\ yield = Ethanol\ produced \div glucose\ consumed \quad [3]$$

3.0 RESULTS

The *Hyperhhania* sp. (common thatch grass) that was used for the production of ethanol is shown in Figure 1.

Figure 1: Common thatch grass used for production of ethanol.

Proximate analysis of common thatch grass (CTG) was performed in order to ascertain the composition of CTG and is shown in Table 1

The maximum ethanol produced on an alkali pre-treated CTG hydrolysate 9 g/L after 60 hours of fermentation from *S. cerevisiae* WBSA 1386 is shown in Figure. 2. No nutritional supplement was added in the fermentation broth. The glucose consumption in the process was 96.3%. The same hydrolysate when fermented by *C. shehatae* CSIR Y-0492 produced a maximum of 9.8 g/L of ethanol after 60 hours with glucose consumption at maximum production at 97.6%.

Table 1: Proximate analysis of *Hyparrhenia sp.* Values are given as percentage dry weight

Component	% Dry weight
Ash	5.42
Nitrogen	0.5
Crude protein	3.13
Crude fibre	76.02
Neutral detergent fibre	80.32
Acid detergent fibre	56.51
Acid detergent lignin	10.24
Hemicellulose	23.81
Cellulose	46.27

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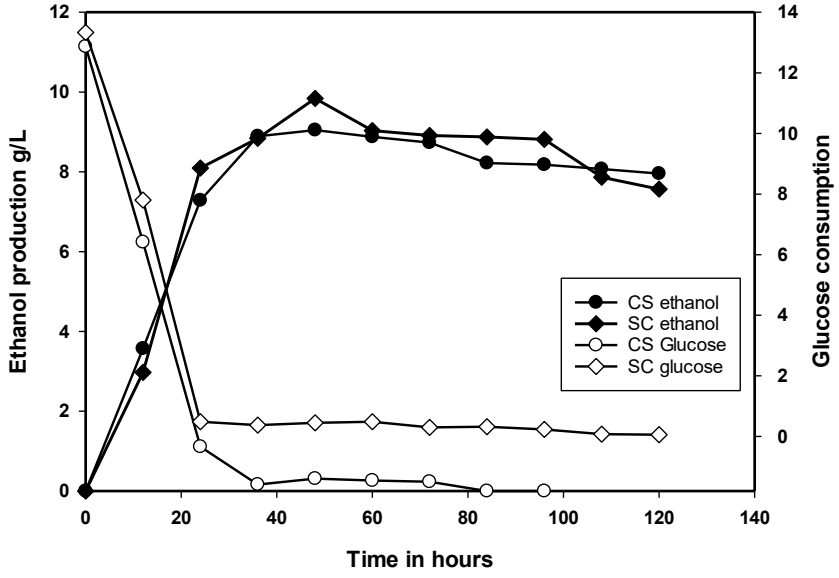

Figure 2: Ethanol production and glucose consumption during fermentation of alkaline non-supplemented pre-treated CTG hydrolysate by S*. cerevisiae* WBSA 1386 (SC) and *C. shehatae* CSIR Y-0492 (CS) over 120 hours at 30 °C*.

Fermentation of a hydrolysate obtained from saccharification of alkali pre-treated CTG by *S. cerevisiae* WBSA 1386 produced 8.8 g/L ethanol after 48 hours is shown in Figure 3. A nutritional supplement was added to the fermentation broth. There was a 97% utilisation of glucose after 48 hours of the fermentation process (Figure 3). Fermentation of the hydrolysate by *C. shehatae* CSIR Y-0492 produced 8.64 g/L ethanol after 48 hours. Glucose consumption at maximum ethanol production was 97%.

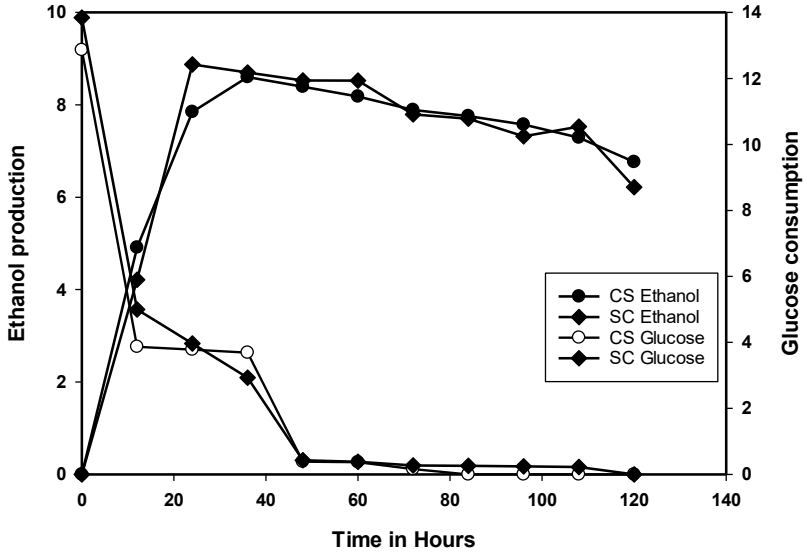

Figure 3: Ethanol production and glucose consumption fermentation of nutritionally supplemented alkaline pre-treated CTG hydrolysate by S*. cerevisiae* WBSA 1386 (SC) and *C. shehatae* CSIR Y-0492 (CS) over 120 hours at 30 °C*.

A maximum ethanol production of 8.39 g/L was obtained from fermentation of an acid pre-treated CTG hydrolysate by *S. cerevisiae* WBSA 1386 after 60 hours while C. *shehatae* CSIR Y-0492 produced 8.06 g/L (Figure 4). No nutritional supplement was added to the fermentation broth. Glucose consumption after 60 hours of fermentation was 98% for S. cerevisiae WBSA 1386 and 97.8% for C. *shehatae* CSIR Y-0492.

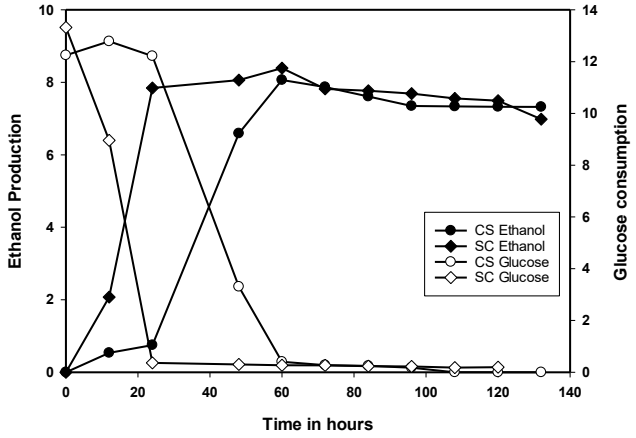

Figure 4: Ethanol production and glucose consumption during fermentation of acid non supplemented pre-treated CTG hydrolysate by *S. cerevisiae* WBSA 1386 and *C. shehatae* CSIR Y-0492 over 120 hours at 30 °C.

The enzymatic hydrolysate of acid pre-treated CTG had an ethanol yield of 8.7 g/L after 84 hours of the fermentation by *S. cerevisiae* WBSA 1386 while *C. shehatae* CSIR Y-0492 produced 8.8 g/L at 24 hours (Figure 5). The fermentation broth contained a nutritional supplement. Consumption of glucose at maximum production was 97.9% for *S. cerevisiae* WBSA 1386 whilst *C. shehatae* CSIR Y-0492 had 97.5%.

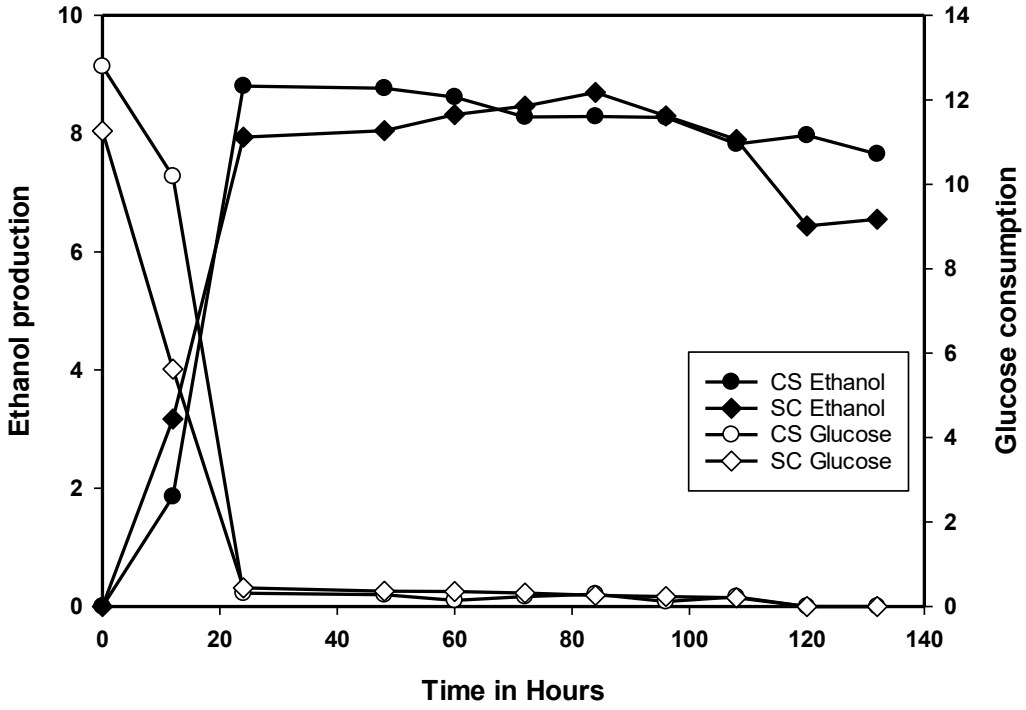

Figure 5: Ethanol production and glucose consumption during fermentation of nutritionally supplemented acid pre-treated CTG hydrolysate by S*. cerevisiae* WBSA 1386 SC) and *C. shehatae* CSIR Y-0492 (SC) over 120 hours at 30 °C

The yield factor for both *C. shehatae* CSIR Y-0492 and *S. cerevisiae* WBSA 1386 were calculated based on glucose as a substrate and ethanol as the product (Table 2) There were no significant differences in the yield factor obtained from the pre-treated CTG hydrolysate with or without nutrient supplementation for both *Candida Shehatae* CSIR Y0492 and Saccharomyces *cerevisiae* WBSA 1386 (Table 2) at 48 hours. There was a consistency in the yield factor for both organisms at 48 hours for all the hydrolysates

Table 2. Yield factor for *Candida shehatae* CSIR Y-0492 (CS) and based *Saccharomyces cerevisiae* WBSA 1386 (SC) on glucose as the substrate and ethanol as the product

Treatment	Time (h)	Yield factor CS	Yield factor SC
Alkali treated CTG, no supplement	48	0.7	0.8
Alkali treated CTG, supplemented	48	0.7	0.7
Acid treated CTG, no supplement	48	0.7	0.7
Acid treated CTG, supplemented	48	0.7	0.8

4.0 DISCUSSION

Fermentation of acid and alkali pre-treated CTG hydrolysates showed that the enzymatically produced hydrolysates contain sugars that can be fermented to ethanol (Figures 2-5). There were no significant differences in the yield factor obtained when *C. shehatae* WBSA 1386 was employed in the fermentation process compared to *S. cerevisiae* CSIR Y0492. *C. shehatae* WBSA 1386 was however expected to have yielded much higher amounts of ethanol compared to *S. cerevisiae* CSIR Y0492 because of its ability to utilize both glucose and xylose in the production of ethanol (Sanchez *et al.*, 2002; (Keshav *et al*., 2021). The xylose was expected to be available in the hydrolysate because of the use of xylanase in the saccharification process.

There was no difference in the ethanol yields as a result of nutrient supplementation in the fermentation medium. Nutrient supplementation was expected to increase the yield factor in both *C. shehatae* WBSA 1386 and *S. cerevisiae* CSIR Y0492. It is known that the medium components added to the hydrolysate influence the process kinetics and fermenting of lignocellulosic hydrolysates places more demands on the organism than when fermenting a laboratory formulated medium.

An addition of complex nutrients such as yeast extract and peptone has, therefore been found to be beneficial to the fermentation of lignocellulosic hydrolysates (Olsson and Hahn-Hägerdal, 1996). The negative effect of the pre-treatment methods was not observed in the current fermentation process though acid pre-treated hydrolysates are known to contain compounds that have an inhibitory effect on the fermentation process (Robak and Balcerek, 2020; Singh et al., 2020). Compounds formed or released during pre-treatment of lignocellulosic materials such as phenols, furans, carboxylic acids and inorganic salts have an inhibitory effect in downstream processes including enzymatic saccharification and fermentation process (Aguilar et al., 2002; McIntosh and Vancov, 2011). However, occurrence of the compounds is related to biomass composition and pre-treatment

severity and thus may have been absent or in low quantities in the current fermentation and hence the alkali and acid-treated hydrolysates had similar yield factors. There is also a possibility that the yeast strains that were used in the fermentation were resistant to the Inhibitory compounds.

The expected theoretical yield of ethanol when glucose is used as a substrate is two moles of ethanol for every mole of glucose used. This stems from the fact that theoretically 100g of glucose should produce 51.4 g of ethanol, 48.8 g of carbon dioxide assuming that all the glucose is used for ethanol production (Saiki et al., 1999). The yield actor expected thus becomes 0.5. The current results generally indicate more ethanol being produced from the current results generally indicate more ethanol being produced from the fermentation production process than the theoretical expectation. This could be as a result of the presence of some sugars such as sucrose and xylose (detected in the saccharified mixture) being utilised for the production of ethanol. Sucrose may be utilised by some fermentative organisms for production of ethanol.

5.0 CONCLUSION

From the experimental results obtained it can be concluded that pre-treated thatch grass that has undergone enzymatic saccharification can be utilized in ethanolic fermentations with yields above the theoretical yield of 0.5. Both *Saccharomyces cerevisiae* WBSA 1386 and *Candida shehatae* CSIR Y-0142 are good candidates for the fermentation process with ethanol yields averaging 8.3 g/L. The ethanolic fermentation can be performed without addition of nutrient supplements to the hydrolysate. The fermentation process showed no significant differences between hydrolysates obtained from acid or alkaline pre-treatments of the grass. This could be favourable in cutting down costs for industrial production of ethanol from thatch grass.

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