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Direct Bioconversion of Sorghum Straw to Ethanol in a Single-step Process by *Candida* species

Blessing A. Adelabu[1,*] , Sarafadeen O. Kareem[1], Abideen I. Adeogun[2] and Kehinde O. Ademolu[3]

[1]*Department of Microbiology;* [2]*Department of Chemistry;* [3]*Department of Pure and Applied Zoology, Federal University of Agriculture Abeokuta, Nigeria*

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Abstract

The present study explores the potential of *Candida* species to convert sorghum straw biomass to ethanol. Two strains of *Candida* species (*C. tropicalis* and *C. shehatae*) were used to produce ethanol by distillation of fermented sorghum straw medium. These yeasts exhibited high amylolytic, cellulolytic and fermentative ability and were used for bioconversion of sorghum straw [2.5 - 15 % (w/v)] at pH (4.0 – 7.0). The yeasts were capable of producing ethanol from solutions containing 7.5 % sorghum straw. Ethanol production during optimization of growth parameters showed that *C. tropicalis* produced more ethanol (38.12 g/L) than *C. shehatae* (30.32 g/L), except optimization of incubation temperature where *C. shehatae* produced more ethanol (43.96 g/L) than *C. tropicalis* (35.10 g/L). The present study suggests cellulolytic yeasts, such as *C. tropicalis* and *C. shehatae*, for direct ethanol production from lignocellulosic material.

Keywords: Ethanol; Direct bioconversion; cellulolytic yeast; *Candida* species; Lignocellulosic.

1. Introduction

Bioethanol production is being considered an alternative source of energy due to the prediction that there will be exhaustion of fuel energy supply (Ariyajaroenwong *et al*., 2012). Bioethanol is mainly produced from sugar or starchy biomass (Agbogbo and Coward-Kelly, 2008) which poses a competition for the raw materials with food industry. In the last decade, attention started to shift to lignocellulosic feed stocks for ethanol production through multistage process including pretreatment, enzymatic hydrolysis, sugar fermentation and process design. Most of the processes developed toward industrial scale involve the addition of enzymes for cellulose and hemicellulose hydrolysis and use of specific yeast strains engineered to utilize pentose and hexose sugars during fermentation process (Bettiga *et al*., 2009). Both achieving effective biomass hydrolysis and complete sugar conversion are essential for an economical process (Kurian *et al*., 2010).

A process that aims at circumventing this multistage and cost prohibitive, such as critical cost-increasing item, is the direct microbial conversion or Consolidated Bioprocessing (CBP) is considered necessary (Lynd *et al*., 2002). In CBP, an organism or a mixed culture of organisms simultaneously produce hydrolytic enzyme and ferment the pentose and hexose sugars into ethanol or other valuable products without the addition of cellulolytic enzymes. This alternative process is envisaged to reduce energy consumption of the overall process of ethanol production (Lynd *et al*., 2002). *Pichia stipitis*, *Candida shehatae*, and *Pachysolan tannophilus* are known to use both pentose and hexose sugars (Agbogbo and Coward-Kelly, 2008). The advantage of the single-step bioconversion is that the process is carried out in one bioreactor where hydrolysis and fermentation take place at the same time. Microbial conversion of lignocellulosic materials to ethanol is performed by the action of xylose reductase (XR), xylitol dehydrogenase (XDH) and xylulokinase (XK) (Khan and Dwivedi, 2013). These metabolic capacity has been reported in several yeast species, such as, *Debaryomyces hansenii*, *Meyerzyma guillermondii* and *Candida parapsilosis* (Lourenco *et al*., 2014; Latif and Rajoka, 2002). *Candida* species are asporogenous diploid yeast, which can utilize a very large variety of carbon sources including many sugars, disaccharides, phenols, alkanes, alkane derivatives, and fatty acids (Sanchez *et al*., 2009).

Huge volumes of cellulosic materials, such as sorghum straw, are renewable resources being generated as waste from various agro allied industries (Das and Singh, 2004). These potential can be exploited as sustainable resource for production of many organic fuels and bioenergy. They can reduce greenhouse gas emissions, enhance energy security, improve the economy, dispose problematic solid wastes, and improve air quality (Das and Singh, 2004).

Bioconversion of corn straw into ethanol seems to be one of the solutions to the increasing demands of energy. Although Oyeleke and Jibrin (2009) had produced bioethanol from guinea corn and millet husk through acid

* Corresponding author. e-mail: bola.blessing@yahoo.com.

hydrolysis and fermentation with *Aspergillus niger* and *Zymomonas mobilis*. Wakil *et al*. (2013) also produced bioethanol from palm oil mill effluent using moulds and yeast, but literature has been silent on single-step production of bioethanol through consolidated bioprocesses. The present study presents here reports on the production of ethanol from sorghum straw by *Candida* species in a single step process.

2. Materials and Methods

2.1. Source of Microorganisms

Four *Candida* species (*C. tropicalis*, *C. shehatae*, *C. utilis* and *C. krusei*) were obtained from the Culture Collection Centre of the University of Agriculture, Abeokuta, Nigeria. The cultures were maintained on Yeast Extract Peptone Dextrose (YEPD) agar slant at 4 ^{o}C and sub-cultured twice a month.

2.2. Screening of Yeasts

2.2.1. Screening for Amylolytic Yeasts

Yeast isolates were qualitatively screened for using Gram iodine solution. Purified yeast isolates were grown on agar plates containing 1% starch agar which were inoculated with pure yeast isolates and were incubated at 30 ˚C for 3 days. The plates were flooded with grams iodine solution, colonies forming clear zones were selected for quantitative screening (Kareem *et al*., 2009). Quantitative screening was carried out using YEPD broth containing $MgSO_4.7H_2O$, 0.03 g; $FeSO_4.7H_2O$, 0.5 g; $MnSO_4.H_2O$, 0.16 g; $ZnSO_4.7H_2O$, 0.14 g. Culture media were inoculated with pure yeast isolates and incubated under shaking condition (150 rpm) at 30 ˚C for 3 days, amylase production was quantified using the method of Kareem *et al*. (2009).

2.2.2. Screening for Cellulolytic Yeast

Yeast isolates were screened for cellulose qualitatively using congo red test. Purified yeast isolates were grown on agar plates containing 1% carboxyl methyl cellulose (CMC). Plates were inoculated with pure yeast isolates and were incubated at 30 ˚C for 3 days and flooded with 1% Congo red solution for 30 min and de-stained with 1 M NaCl solution for 20 min (Saliu, 2012). Quantitative screening was carried out using modified YEPD which consist of 1% CMC, NH_4NO_3, 0.2 g; KH_2PO_4, 0.5 g; $CaCl_2.2H_2O$, 0.03 g; $MgSO_4.7H_2O$, 0.03 g; $FeSO_4.7H_2O$, 0.5 g; $MnSO_4.H_2O$, 0.16 g; $ZnSO_4.7H_2O$, 0.14 g; Tween-80, 0.1 g. Culture media were inoculated with pure yeast isolates and were incubated under shaking condition (150 rpm) at 30 ˚C for 3 days and cellulase production was quantified according to the method of Saliu (2012).

2.2.3. Screening for Ethanol Producing Yeast

Purified yeast isolate were screened for fermentative ability using YEPD broth prepared in test tubes containing inverted Durham tube (Wakil *et al*., 2013). Test tubes were inoculated and incubated at 30 ˚C for 3 days, isolates were selected based on the volume of gas in Durham tube during the incubation period (Brooks, 2008). Quantitative screening was carried out by distillation using 5% starch according to the method of Wakil *et al*. (2013).

2.3. Selection of Starters

Two *Candida* spp (*C. tropicalis* and *C. shehatae*) with best amylolytic, cellulolytic and ethanol producing abilities were selected from the four *Candida* species obtained.

2.4. Determination of Fermentative Parameters of Selected Yeasts

Enzymes released from selected yeast were used for hydrolysis of corn and sorghum straw (10 % w/v). Each product of hydrolysis was fermented by the yeasts. Using the method of Lazarova *et al*. (1987), fermentative parameters of selected yeasts were determined using 10 mL needle and syringe inverted into injection bottles. Carbon dioxide productivity, volumetric ethanol productivity, theoretical alcohol recovery, actual alcohol recovery and fermentation efficiency were determined.

2.5. Processing of Substrate

Sorghum straws were collected from a farm at Kishi in Oyo State, Nigeria. The straws were oven dried at 70 ^{o}C for 2 hours and grounded into powdered using an electric blender (Philips INO23) and was sieved using 40 mm mesh. 10 % of the straw was used for fermentation.

2.6. Ethanol Production

2.6.1. Fermentation of Sorghum Straw

Yeast strains were grown in a 1 L Erlenmeyer flask that contained 700 mL of basal medium containing: NH_4NO_3 1.2 g; KH_2PO_4 0.8 g; $CaCl_2.2H_2O$ 0.3 g; $MgSO_4.7H_2O$ 0.3 g; $FeSO_4.7H_2O$ 0.4 g; $MnSO_4.H_2O$ 1.5 g; $ZnSO_4.7H_2O$ 1.3 g; Tween-80 0.15 g; peptone 0.75 g, yeast extract 0.3 g; glucose 5 g and 10 % sorghum straw. The pH of the medium was adjusted to 5.5 prior to sterilization. The flask was inoculated with 5 % yeast suspension and incubated at 30 ºC for 96 hours (Hashem *et al*., 2013). Fermented corn straw was analyzed for ethanol production at 24, 48, 72 and 96 hour.

2.6.2. Fractional Distillation

Distillation of the fermented medium was carried out using 100 mL of each fermented medium which was dispensed into round-bottom flasks fixed to a distillation column enclosed in running tap water. A conical flask was fixed to the other end of the distillation column to collect the distillate. A heating mantle with temperature adjusted to 78 ˚C was used to heat the round bottom flask containing the fermented sample (Wakil *et al*., 2013).

2.6.3. Determination of Quantity of Ethanol Produced

The distillate collected over a slow heat at 78°C was measured using a measuring cylinder, and expressed as the quantity of ethanol produced in g/L by multiplying the volume of distillate collected at 78°C by the density of ethanol (0.8033 g/mL). Gram/L is equivalent to the yield of 100 g of dried substrate (Wakil *et al*., 2013).

2.7. Optimization the Fermentation Conditions of Ethanol Production

2.7.1. Effect of Substrate Concentration

Ethanol production was carried out at constant pH, incubation temperature and inoculum concentration using

various substrate concentrations (5%, 7.5%, 10% 12.5%) of sorghum straw. Samples were taken at 72 hours of incubation. Ethanol productions by yeast stains were determined as previously described.

2.7.2. Effect of Temperature

Ethanol was produced from the substrates in flasks inoculated with yeast cells. The flasks were incubated at different temperature (30 , 35 , 40 , 45 , 50 and 60 ^{o}C). Other growth conditions were constant. Ethanol productions by yeast stains were determined as previously described.

2.7.3. Effect of pH

Effect of pH on ethanol production, using the selected yeast strains, was studied by conducting experiments at different pH (4.0, 4.5, 5.0, 5.5 and 6.0) while all other parameters were kept constant. Ethanol productions by yeast stains were determined as previously described.

2.7.4. Effect of Inoculum Concentration

Effect of inoculum concentration on ethanol production by the selected yeast strains was carried out using YEPDA medium incorporated with sorghum straw. The medium was sterilized and inoculated with varying yeast suspension of 5, 7.5, 10, 12.5 and 15 %. Other growth conditions were constant. Ethanol productions by yeast stains were determined as previously described.

2.7.5. Statistical Analysis

All the experiments were performed in triplicates and the results were presented as mean ± standard deviation and were also analyzed by ANOVA using statistical software SPSS version 17. 0.

3. Results

3.1. Screening of Yeasts

All the yeast strains tested positive for amylase and cellulase production by showing clear zones on starch and carboxyl methyl cellulose (CMC) agar. *Candida tropicalis* produced the highest halo zone (39.0 mm) followed by *C. shehatae* (36.0 mm), while the least was observed in *C. utilis* (10.0 mm) (data not shown). Result of the quantitative screening showed that the highest amylase activity was produced by *C. tropicalis* (319.50 U/mL) while *C. utilis* had the least amylase activity (136.46 U/mL). Highest cellulase activity was produced by *C. tropicalis* (174.67 U/mL) followed by *C. shehatae* (161.38 U/mL) while the least cellulase activity was observed in *C. utilis* (100.18 U/mL) (Table 1). Screening for ethanol production showed that *C. tropicalis* had the best ethanol producing ability (31.96 g/L), followed by *C. shehatae* (26.13 g/L) while *C. krusei* produced the least (13.63 g/L) (Table 1).

Two yeasts (*C. tropicalis* and *C. shehatae*), which displayed the best amylolytic, cellulolytic and ethanol producing abilities, were selected for bioethanol production in submerged fermentation.

Table 1. Screening for amylase, cellulase and ethanol production in yeast isolates.

Yeast Isolates	Enzyme activity (U/mL)		Ethanol (g/L)
	Amylase	Cellulase	
C. shehatae	246.63±11.76	161.38±23.21	26.13±6.27
C. krusei	171.84±80.62	112.31±14.98	13.63±0.90
C. utilis	136.46±35.64	100.18±9.44	16.32±2.17
C. tropicalis	319.50±34.63	174.67±24.54	31.96±10.58

Each value is a mean of 3 readings ± standard deviation

3.2. Measurement of Fermentative Parameters of Yeasts on Hydrolyzed Sorghum Straw Medium

Fermentative parameters (carbon dioxide productivity and volumetric ethanol productivity) of the yeasts on hydrolyzed sorghum straw are presented in Figure 1. *Candida tropicalis* had the highest carbon dioxide production (3.93 L/L.h) while *C. shehatae* had (3.81 L/L.h) (Figure 1). Maximum volumetric ethanol production was achieved by *C. tropicalis* (9.43 g/L.h) while *C. shehatae* had (9.14 g/L.h) (Figure 1). Total alcohol recovery, actual alcohol recovery and fermentation efficiency of the yeasts were presented in Table 2. The yeasts had total alcohol recovery of 4.0 %. *Candida tropicalis* had maximum actual alcohol recovery and fermentation efficiency of 1.68 % and 42 %, respectively, while *C. shehatae* had actual alcohol recovery and fermentation efficiency of 1.55 % and 39 %, respectively (Table 2).

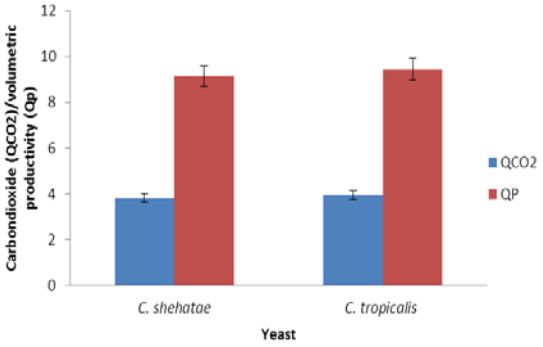

Figure 1. Carbon dioxide productivity and volumetric ethanol productivity of yeasts on hydrolysed sorghum straw medium

Note: QCO_2; Carbon dioxide productivity (L/L.h) ; Qp; Volumetric ethanol productivity (g/L.h)

Table 2. Fermentation parameters of yeasts on hydrolyzed sorghum straw medium.

Yeast	Total Alcohol Recovery (%)	Actual Alcohol Recovery (%)	Fermentation Efficiency (%)
C. shehatae	4.0±0.014	1.55±0.025	39±1.52
C. tropicalis	4.0±0.014	1.68±0.015	42±2.08

Each value is a mean of 3 readings ± standard deviation

3.3. Ethanol Production from Sorghum Straw

The result, presented in Figure 2, shows production of bioethanol from sorghum straw. Volume of ethanol increased with increased fermentation time with the two yeasts. The two yeasts produced ethanol throughout the fermentation period. Ethanol production by *C. tropicalis* was higher than that of *C. shehatae* (Figure 2). *Candida*

tropicalis produced maximum quantity of ethanol (16.25 g/L) at 72 hour of fermentation while *C. shehatae* produced 12.50 g/L at 72 hour. Further increase in fermentation time decreased ethanol production (Figure 2). Although the two yeasts had almost the same volume of ethanol at 24 hour of fermentation, rapid bioethanol production was observed in *C. tropicalis* after 48 hour (8.10 – 14.80).

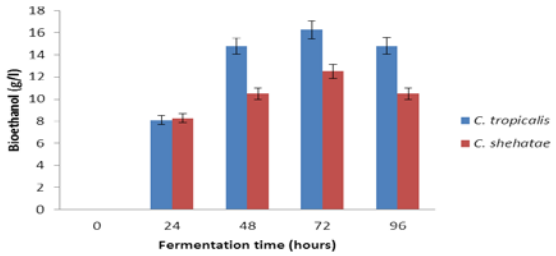

Figure 2. Ethanol production from sorghum straw

3.4. Optimization of Fermentation Conditions of Ethanol Production

3.4.1. Effect of Substrate Concentration on Ethanol Production

Ethanol productions at different substrate concentration of sorghum straw by the two yeasts are summarized in Figure 3. Ethanol production increased gradually with the use of 5 % to 7.5 % and thereafter declined. *Candida tropicalis* produced the highest volume of ethanol (28.65 g/L) awhile *C. shehatae* produced (22.08 g/L) (Figure 3). Bioethanol production with 15 % sorghum straw concentration with the two yeasts produced the least volume of ethanol.

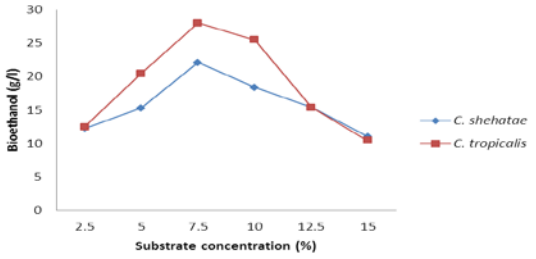

Figure 3. Effect of substrate concentration on ethanol production

3.4.2. Effect of pH on Bioethanol Production from Sorghum Straw

Ethanol production at different pH from sorghum straw by yeast strains are shown in Figure 4, with pH 5.5 having highest yield of ethanol. Ethanol production by direct conversion with *C. tropicalis* (35.81 g/L) was the highest among the yeast strains. On the other hand *C. shehatae* produced lowest volume of ethanol (17.0 g/L) during fermentation period. Fermentation at pH 7.0 produced the least volume of bioethanol (Figure 4).

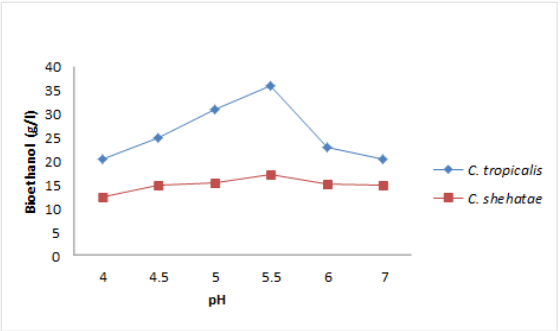

Figure 4. Effect of pH on bioethanol production from sorghum straw

3.4.3. Effect of Inoculum Concentration on Bioethanol Production

Result, presented in Figure 5, shows the effect of inoculum concentration on ethanol production from sorghum straw. Inoculum concentration of 7.5 % was observed as the optimum for ethanol production. Highest ethanol production was observed with *C. tropicalis* fermented sorghum straw (38.12 g/L) while *C. shehatae* produced least ethanol (30.32 g/L) during fermentation (Figure 5). Bioethanol production with 7.5 % inoculum concentration produced highest volume of ethanol, followed by 5 % inoculum concentration while 15 % produced the least bioethanol (Figure 5).

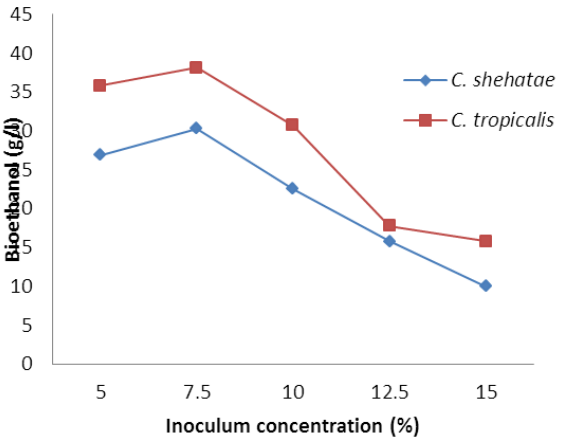

Figure 5. Effect of inoculum concentration on bioethanol production

3.4.4. Effect of Incubation Temperature on Bioethanol Production

Experimental data, presented in Figure 6, shows the effect of different incubation temperature ranging from 30 °C to 60 °C on bioethanol production by yeast strains grown in medium containing sorghum straw. The Figure indicates that the selected yeast strains were able to produce bioethanol from sorghum straw with all temperature. *Candida shehatae* produced the highest volume of ethanol during the fermentation (43.98 g/L) while *C. tropicalis* produced 35.1 g/L (Figure 6). Fermentation at 40 °C produced the highest volume of ethanol, followed by 35 °C while at 60 °C; *C. shehatae* produced 15.62 g/L and *C. tropicalis* produced 12.52 g/L which is the least volume of ethanol produced.

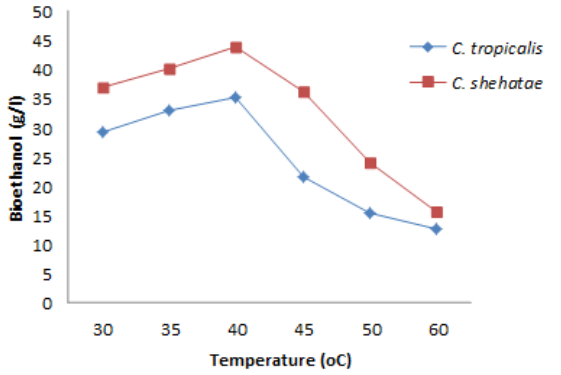

Figure 6. Effect of incubation temperature on bioethanol production

4. Discussion

Yeasts are industrially important unicellular microorganism due to their ability to hydrolyze polysaccharide to monomers and their fermentative role in biosynthesis of ethanol. They are found everywhere and thus can be easily isolated from the environment. *Candida* species are used in the present study for the production of ethanol. One factor that could have been responsible for the presence of *Candida* spp in the environment is the nature of the yeast. Yeast, especially *Candida* spp are known to adapt best in high temperature and low pH (4-5). These observations are in agreement with earlier studies (Bey *et al*., 2011; Boonmak *et al*., 2011). *Candida tropicalis* and *C. shehatae* used in the present study were identical to those earlier reported by Rai *et al*. (2012) who identified *Candida* spp as yeast used for saccharification of sugar cane baggase which shows that *Candida* spp are cellulolytic yeast. Idowu and Edema (2003) also identified *Candida* spp as cellulolytic yeasts that can digest food materials.

In this present study, all the yeast strains (*C. tropicalis*, *C. shehatae*, *C. utilis* and *C. krusei*) produced amylase, cellulase and ethanol with *C. tropicalis and C. shehatae* having the highest production, thus they were selected as very high ethanol producing yeast strains. These observations are in agreement with earlier studies by Limtong *et al*. (2012) who state that yeast species, such as *Candida shehatae*, *Pachysolen tannophilus*, *Scheffersomyces (Pichia) stipites*, had been reported to assimilate cellulose and ferment it to ethanol. *Candida shehatae* and *C. tropicalis* were introduced as new stains and are used to study the effect of fermentation conditions on their efficiency in ethanol production.

Yeasts growth is usually accompanied with fermentation. They have been referred to as being respiro-fermentative organisms (Aransiola, 2006). Actively growing yeasts are likely to be actively fermenting. Carbon dioxide production, volumetric ethanol productivity, theoretical alcohol recovery and fermentative efficiency are important parameters to be studied in ethanol producing yeasts (Nwachukwu *et al*., 2006). Analysis of fermentation parameters in the fermentation of hydrolysed corn and sorghum straw showed the difference in the fermentative parameters of the yeasts. *Candida tropicalis* fermented sorghum straw had the highest carbon dioxide productivity and fermentation efficiency; this may be due to the yeast to easily use up sugars present in the hydrolysed sorghum straw (Tahmina and Capareda, 2011).

Yeast may be confronted with different environmental factors that can cause the loss of yeast cell viability and decreased fermentation rates (Hashem *et al*., 2013). Fermentation period is an important factor in ethanol production. Results from the present work show that ethanol increased gradually with increasing in incubation time with each of the yeast used: *C. tropicalis* and *C. shehatae* and reached their maximum at 72 hours of fermentation and dramatically decreased with further extension of time with each of the yeast. These findings are in agreement with those of Kurian *et al*. (2010) who reported that highest ethanol production by yeasts at 72 hours. *Candida tropicalis* was found to be better than *C. shehatae*. This may be due to the fact this yeast has more cellulolytic enzyme (xylose isomerase) which is responsible for the breakdown of lignocellulolytic materials to glucose (Aristidou and Penttila, 2000). Latif and Rajoka (2002) confirmed *C. tropicalis* as the major yeast that has enzyme xylose reductase which is responsible for the bioconversion of lignocellulolysic materials. *Candida shehatae* had been also recorded as naturally occurring yeast that is xylose-fermenters (Khan and Dwivedi, 2013).

Production of ethanol was affected by sorghum straw concentration between 5 and 12.5 %. *Candida tropicalis* gave the highest yield. Production of ethanol decreased by increasing substrate concentration above 7.5 %, this could be due to decrease in sugar utilization which results in reduction of total ethanol production (Reddy and Reddy 2006). Increase in sorghum straw concentration could have also led to high concentration of complex sugars in the fermentation medium and this could have had a high inhibitory effect on yeast growth and their capability to produce ethanol (Wakil *et al*., 2013). This has been reported by Pratt-Marshall *et al*. (2003) who observed that the fermentation of high gravity worts has a negative effect on the yeast performance due to the elevated osmotic pressure. High substrate concentration leads to decrease ethanol production. This reduction could be due to increase in ethanol production at high sugar concentration which exerts high toxicity on yeast and the nutrients may be deficient at the final stage of the fermentation (Hashem *et al*., 2013). This is in agreement with the work of Kumar and Murthy (2011) who reported 6% xylose concentration for maximum ethanol productivity of *Pichia stipitis*, which is comparable with the present study.

Ethanol production varies with changes in physical parameters, such as temperature and pH of the production medium. The effect of initial pH of the fermentation media on ethanol production showed that the highest ethanol concentration was obtained by *C. tropicalis* in medium with initial pH 5.5. Any change in this parameter induces morphological changes in microbes (Bodade *et al*., 2010). Russell (2003) also recorded that yeast prefers an acidic pH and its optimum pH is 5.0-5.2 but brewing yeast can grow at the pH range of 3.5 to 6.0.

Inoculum concentration of 7.5 % produced the highest volume of ethanol. Although inoculum concentration is known to play a vital role in the production of microbial metabolites; however, higher concentration of cell did not

lead to improved ethanol yield. This may be attributed to substrate limitations or product inhibition and also supported by the finding of Mahoney (2003). The results of Kourkoutas *et al*. (2004) confirmed our results, where they observed maximum ethanol from *S*. *cerevisiae* at 10% inoculum size, whereas Anxo *et al*. (2008) observed the highest ethanol production by *S. cerevisiae* at 5.0% v/v inoculum size. Lower ethanol biosynthesis at lower inoculum size is probably due to the less cells which are insufficient to use the fermentation medium for enzyme maximal activity, while the decreased yield at higher inoculum size might probably due to nutritional imbalance caused by tremendous growth resulting in autolysis of cells (Shafei and Allam, 2010).

Fermentation temperature has a significant effect on ethanol production. *Candida shehatae* was observed to adapt and produced ethanol at high temperature than *C. tropicalis.* This may be due to the fact this yeast strain code for genes that help to tolerate high temperature. In industry, it is commonly believed that 20 -35 ^{o}C is the ideal range for fermentation and at higher temperatures, almost all fermentation would be problematic (Phisalaphong *et al*., 2006; Aldiguer *et al*., 2004). However, in the present study, when the temperature was increased to 40 ^{o}C, the yeast still produced high volume of ethanol. Using a higher fermentation temperature, similar to the optimal temperature for cellulolytic activity, it may be possible for direct microbial conversion process to improve the final efficiency (Yan *et al*., 2012). In addition, volume of ethanol was found to decline at temperature above 40 ^{o}C, the reason might be that fermentation at higher temperature might disrupt enzyme activity and membrane function (Aldiguer *et al*., 2004).

A recent finding shows that approximately 35 g/L of ethanol had been produced form agricultural waste (Cutzu and Bardi, 2017), while 38.12 g/L of ethanol was produced from the present study. Conversion of lignocellulosic material into ethanol still has economic, technical and environmental obstacles, thus different feedstocks and methods should be studied to make it more feasible. Bioethanol production method has to be efficient (high energy yields), cost effective (energy return on investment) and environmentally beneficial, in order to be feasible. Also single-step production of bioethanol is economically feasible; therefore, more research and technological development are needed. As a recommendation, governmental policies are important to promote bioethanol research and make its price competitive with other sources of energy. Moreover, there should be participation of all stake holders to enhance energy security.

5. Conclusion

From the present study, it is concluded that cellulolytic yeasts (*C. tropicalis* and *C. shehatae*) can produce ethanol directly from sorghum straw using a single-step approach. These yeasts produced appropriate hydrolytic enzymes thus no external enzymes were required. The direct conversion of sorghum straw to ethanol by *C. tropicalis* and *C. shehatae* is significant in single-step production of bioethanol

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