Consolidated Bioprocessing of Ethanol from Corn Straw by *Saccharomyces Diastaticus* and *Wikerhamomyces Chambardii*

Blessing Adebola Adelabu[1,*], Sarafadeen Olateju Kareem[1], Floura Oluwafemi[1] and Abideen Idowu Adeogun[2]

Abstract

Consolidated bioprocessing (CBP), which integrates enzyme production, saccharification and fermentation into a one-step process, is a promising strategy for cost-effective ethanol production from starchy biomass. Two yeast strains namely; *Saccharomyces diastaticus* and *Wickerhamomyces chambardii* were selected for direct bioethanol production from corn straw medium for 72 h. These strains were able to utilize cellulose directly and highest bioethanol production was recorded at 48 hour of fermentation. Maximum ethanol production was achieved by *S. diastaticus* at 35°C, pH 5.0 in the medium containing 7.5% (w/v) during fermentation. The yeast isolates were able to tolerate wide ranges of temperature, pH and substrate concentration for higher ethanol production. This study presents the potential of *S. diastaticus* and *W. chambardii* in cellulose-based ethanol production by consolidated bioprocessing. This strategy will eliminate multistage process of ethanol production which will lead to reduction of the overall production cost.

Keywords: Consolidated bioprocessing, Cellulosic ethanol, Yeast, *W. chambardii*, *S. diastaticus*

1. Introduction

Bioethanol production is receiving attention as an alternative source of energy due to the predictable exhaustion of fuel energy supply (Ariyajaroenwong *et al.,* 2012). Bioethanol is commercially produced on a moderate scale from sugar cane, corn, and other starchy biomass sources (Brooks, 2008). However, this first generation bio-ethanol has been blamed for causing food insecurity. Therefore, attention is being shifted from edible to inedible biomass or lignocellulosic biomass for sustainable ethanol production (Wakil *et al.,* 2013).

Microbial diversity from various habitats such as soil, river water, hypersaline lakes, and insects offers vast opportunities for exploration, as these habitats are the source of useful biomolecules which include enzymes, fatty acids, pigments, antibiotics, *etc*. (Butinar *et al*., 2005; Sláviková and Vadkertiová 2003). Moreover, the development of biotechnology has raised much interest in using cellulase producing microorganisms to convert lignocellulosic biomass from agro-industrial wastes to glucose that can be used in applications such as production of bioethanol (Shah *et al*., 2006).

[1] Department of Microbiolgy, Federal University of Agriculture Abeokuta, Nigeria

[2] Department of Chemistry, Federal University of Agriculture Abeokuta, Nigeria

* Corresponding authors, e-mail: abuaisha2k3@yahoo.com, bola.blessing@yahoo.com

Lignocelluloses are major components of biomasses which come from different industries, forestry, agriculture and municipalities. The biodegradation of this lignocellulosic biomass is limited by several factors like crystallinity of cellulose, available surface area, and lignin content (Valcheva *et al*., 2016). Among these lignocellulosic biomasses, agricultural wastes are more important as this can be converted into products that are of commercial interest such as ethanol, glucose, and single cell protein. Industrial production of ethanol from lignocellulosisic hydrolysates requires the use of microorganisms capable of utilizing the different types of sugar present in it (Balat *et al*., 2008; Bettiga *et al*., 2009). Yeasts have higher ethanol tolerance than bacteria. It is easier to harvest and recycle yeast cells than bacteria cells from the fermentation broth and yeast fermentation is resistant to contaminant from bacteria and viruses. Many types of yeast are known to be potential source of extracellular enzymes to produce ethanol and they are *Pichia stipitis*, *Candida shehatae* and *Pachysolan tannophilus* (Saravanakumar *et al*., 2013). These yeasts possess enzymes such as xylose reductase (XR), xylitol dehydrogenase (XDH) and xylulokinase (XK) for direct bioconversion of lignocellulosic materials to ethanol (Khan and Dwivedi, 2013).

There are few studies (Osho 2005; Brooks, 2008) showing the growth of yeast and ethanol production in concentrated D-glucose solutions. Kurian *et al*. (2010) had also produced ethanol from hemicellulose hydrolysate of sweet sorghum straw, but few studies have addressed single-step conversion of lignocellulosic substrates to ethanol. In this present study, corn straw was used as substrate to investigate optimum fermentation conditions for direct production of ethanol by two different yeasts; *Saccharomyces diastaticus* and *Wickerhamomyces chambardii*.

2. Materials and Methods

2.1 Collection and processing of samples

Corn straw was collected from a farm site at Osiele in Abeokuta, Ogun State, Nigeria. The samples were dried and ground to a powder form using an electric blender (Philips INO23) and was sieved using 40 mm mesh.

2.2 Isolation and characterization of yeast

Soil from 500 m depth of compost piles was collected from the herbarium of Federal University of Agriculture, Abeokuta. The sample was serially diluted and dilutions of 10^{-3} and 10^{-5} were plated on Yeast Extract Peptone Dextrose (YEPD) agar. The plates were incubated at 30°C for 72 h (Wakil *et al*., 2013). After incubation, yeast colonies that grew on the agar medium were characterized based on size, shape and colour. Colonies from different agar plates were subcultured on standard solid medium by the streak plate technique. The agar

plates were incubated at 30°C for 48 h. The subsequent pure cultures were maintained on agar slant for further characterization and identification. Purified yeast isolates were subjected to standard test and classification schemes as described by Barnett (2003). The tests include those for colony and cell morphology and fermentation tests.

2.3 Screening of yeasts

2.3.1 Screening of yeasts for amylase production

These were screened for qualitatively using Gram iodine solution. Purified yeast isolates were grown on agar plates containing 1% starch agar. Plates were inoculated with pure yeast isolates and were incubated at 30°C for 3 days and 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 medium were inoculated with pure yeast isolates and were incubated under shaking condition (150 rpm) at 30°C for 3 days, amylase production was quantified using the method of Kareem *et al*. (2009)

2.3.2 Screening of yeasts for cellulase production

These were screened for cellulose qualitatively using congo red test. Purified yeast isolates were grown on agar plates containing 1% Carboxyl Methly Cellulose (CMC). Plates were inoculated with pure yeast isolates and were incubated at 30°C for 3 days and were flooded with 1% Congo red solution for 30 minutes and then de-stained with 1M 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).

2.3.3 Screening of yeasts for ethanol production

These 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 and isolates were selected based on the volume of gas in Durham tube during the incubation period. Quantitative screening was carried out by distillation using 5% starch according to the method of Wakil *et al*., 2013.

2.4 Selection of Starters

Two yeasts with best amylolytic, cellulolytic and ethanol producing abilities were subjected to optimization studies and sent for molecular identification. The selected isolates were used for cellulase and ethanol production in submerged fermentation.

2.5 Determination of fermentative parameters of selected yeasts

Enzymes released from selected yeasts were used for hydrolysis of corn straw (10% w/v). 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.

2.6 Genetic analysis

2.6.1 Molecular identification

DNA was extracted from this culture by using Zymo-Fungal/Bacteria DNA extraction kit (Zymo Reaearch, USA). This was carried out according to manufacturer's instructions. The purity and concentration of the extracted DNA was evaluated using a NANODROP (ND 1000) Spectrophotometer (Thermo Scientific, USA) (Sripiromrak, 2006). The primers of ITS1 (5´TCCGTAGGTGAACCTGCGG3´) and ITS4 (5´TCCTCCG CTTATTGATATGC3´) were used for amplification. Nucleotide sequences were analyzed by Macrogen (Macrogen, Inc., Korea). All the sequences were compared for their similarity with reference yeast strains by a BLAST search. The amplified product was separated on a 1.5% agarose gel.

2.6.2 Identification of Gene Sequences

The obtained sequences of genomic DNA were aligned by submitting them to the non-reductant nucleotide database at Genebank using the BLAST program in order to determine the identity of the isolates (www.ncbi.nlm.nih.gov). Genetic distances were estimated by using the Maximum parsimony with bootstrap method employed by PHYLIP (Sripiromrak, 2006). The tree was displayed with the TREEVIEW program

2.7 Inoculum Preparation

A loopful of culture from 48 h old YEPDA plate was inoculated into sterile 100 ml of 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 in 250 ml Erlenmeyer flasks. Inoculum was grown aerobically at 30°C on a rotary shaker at 150 rpm for 48 h (Saliu, 2012).

2.8 Fermentation of corn straw

Yeast strains was 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% corn 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

30ºC for 96 h (Hashem *et al*., 2013). Fermented corn straw was analyzed for ethanol production at 24, 48, 72 and 96 h.

2.8.1 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 the temperature adjusted to 78°C was used to heat the round-bottom flask containing the fermented sample (Wakil *et al*., 2013).

2.8.2 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). g/L is equivalent to the yield of 100 g of dried substrate (Wakil *et al*., 2013).

2.9 Cell Mass Determination

Dry cell mass of the yeast were estimated by centrifuging 50 ml of the fermenting medium in a pre-dried and pre weighed centrifuge tube. Centrifugation was carried out at 5,000 rpm for 10 min. The cell mass was dried and calculated by reweighing the tube (Rai *et al*., 2012).

2.10 Optimization the fermentation conditions of ethanol production

2.10.1 Effect of pH

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

2.10.2 Effect of inoculum concentration

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

2.10.3 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 corn straw. Samples were taken at 72 h of incubation. Ethanol productions by yeast stains were determined as previously described

2.10.4 Effect of temperature

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

2.11 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 and Discussion

3.1 Isolation of yeast

A total of twelve (12) yeasts were isolated. All the isolates grew well at 10% NaCl combined with 5% glucose while only two grew at 50% glucose. Sugar fermentation test showed that all the isolates ferment glucose and fructose, two isolates ferment maltose, arabinose and raffinose. Two isolates do not ferment sorbose. Physiological and biochemical tests identified the yeasts as *Saccharomyces cerevisiae, Debaryomyces hansenii, Pchia chambardii, Trichosporon beemeri Trichosporon aquatile. Saccharomyces cerevisiae* were the predominant yeast isolated, they have been reported to be ubiquitous and utilize a wide range of nutrients due to its ability to secrete a large number of digestive enzymes (Saliu, 2012). Abah *et al*., (2010) identified *Saccharomyces spp* as cellulolytic yeast isolated from rotten iris potato.

3.2 Screening for enzymes and ethanol producing yeast

All the yeast tested positive for amylase production by showing clear zones on starch agar. The yeast isolates produced zones of clearance from 8.0 to 21.0 mm in diameter. *Wikerhamomyces chambardii* had the highest halo zones (21.0 mm) followed by *D. hansenii* (20.0 mm) while *T. aquatile* had the lowest (8.0 mm) (Data not shown). Quantitative screening showed that *Wikerhamomyces chambardii* had the highest amylase activity (551.54 U/ml), followed by *S. diastaticus* (527.21 U/ml) while the lowest was recorded for *T. aquatile* (100.78 U/ml) (Table 1). All isolates also tested positive for cellulase production by showing clear zones on CMC agar. *Wikerhamomyces chambardii* produced the highest halo zone (35.0 mm), *Wickerhamomyces* spp formally known as *Pichia* spp had been described by Virginia de Garcia *et al*, (2010) as novel yeast from natural environments, which are related to *Candida* spp. Result of the quantitative screening shows that the highest cellulase activity was produced by *W. chambardii* (174.67 U/ml) followed by *S. diastaticus* (161.38 U/ml) while the least cellulase

activity was observed in *T. aquatile* (72.35 U/ml) (Table 1). Among the isolates, eight showed fermentative ability, *S. diastaticus* showing the best fermentative ability, this could be due to presence of enzymes in the yeast. Amutha and Gunasekaran, (2000) had described *S. diastaticus* as amylolytic and cellulolytic yeast that can produce ethanol (Data not shown). Quantitative screening for ethanol production showed that *S. diaststicus* had the best ethanol producing ability (31.96 g/L), followed by *W. chambardii* (26.13 g/L), this yeast had just been recently added to group of yeasts that produce ethanol from lignocellulosic materials (Kurtzman, 2011). Two yeast which displayed the best amylolytic, cellulolytic and ethanol producing abilities were physiologically identified as *S. cerevisiae* and *P. chambardii* which were molecularly identified as *Saccharomyces diastaticus* and *Wikerhamomyces chambardii* 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	
S. cerevisiae	171.63±20.28^{bc}	100.38±9.67^{b}	23.59±2.37^{b}
D. hansenii	411.84±18.36^{ef}	142.50±18.03^{d}	14.40±0.96^{ab}
W. chambardii	551.54±32.54^{f}	174.67±23.21^{f}	26.13±6.27^{c}
T. beemeri	319.50±22.21^{de}	135.66±10.34^{c}	13.62±2.87^{a}
S. cerevisiae	158.78±20.62^{b}	94.86±6.23^{ab}	22.53±3.44^{b}
W. chambardii	376.06±34.63^{e}	139.21±20.67^{c}	Nill
S. cerevisiae	264.51±33.81^{d}	125.78±22.54^{bc}	Nill
S. diastaticus	527.21±25.80^{f}	161.22±20.21^{e}	31.96±10.58^{d}
T. aquatile	100.78±4.48^{a}	72.35±7.61^{a}	Nill
D. hansenii	229.65±33.32^{c}	104.81±9.44^{b}	Nill

Note: Each value is a mean of triplicates ± standard deviation.

3.3 Molecular identification

Polymerase Chain Reaction (PCR)-based method is described as effective in the differentiation of yeast strains of different species. Differentiation of *Saccharomyces cerevisiae* and *S. diaststicus* by PCR was a particular interest. Biochemical characterization identified all *Saccharomyces* species as *S. cerevisiae* but PCR identified particular specie as *S. diastaticus*. This is an important finding since traditional taxonomic methods do not allow the differentiation of all *Saccharomyces* species. This agrees with work of Sripiromrak (2006) where yeast species were identified using PCR. *Pichia chambardii* identified by biochemical test as yeast

from compost soil was identified as *W. chambardii* by PCR method. PCR method was not developed to replace existing techniques in yeast taxonomy and systematics, but as a preliminary tool in microbiology and biotechnology because it will not lead to false positive results (Chanchaichaovivat *et al*., 2007). The amplified DNA Fragment of approximate size 500 bp was generated for the two yeasts. The distribution of organisms identified in the library of DNA yeast isolates based on the analysis by sequence of the PCR product in the non-redundant nucleotide database from National Centre for Biotechnology Information (www.ncbi.nhi.gov) (NCBI) was presented Table 2. The two yeasts with NCBI Accession number X02649.4 and EF550482.1 were identified as *Saccharomyces diastaticus* and *Wickerhamomyces chambardii* respectively (Table 2). Their maximum percentage identity was 100 and 99% respectively (Figure 1).

Table 2 Identity of yeast strains by sequences of genomic DNA

Isolate	Accession No NCBI	Name of organisms	Identity
1	X02649.4	*Saccharomyces diastaticus*	100%
2	EF550482.1	*Wickerhamomyces chambardii*	99%

Note: NCBI = National Centre for Biotechnology Information

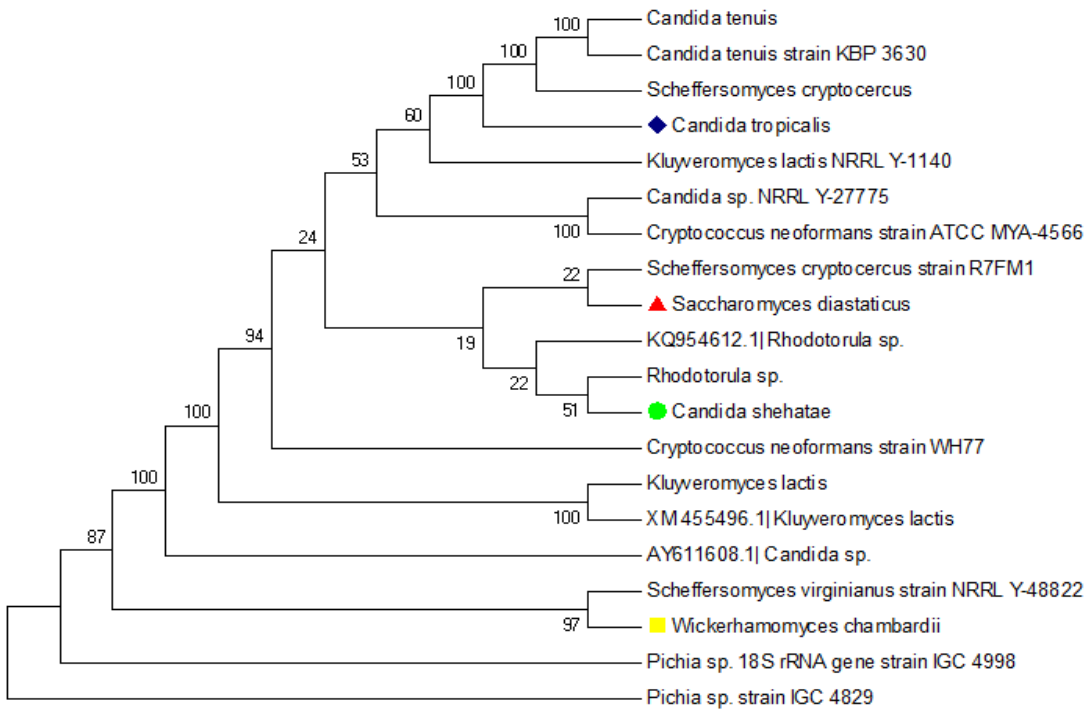

Figure 1 Phylogenetic relationship of yeasts.

(Branch lengths are proportional to the numbers of nucleotide and amino acid changes and the numerals given on the branches are the frequencies with which a given branch appeared in 100 bootstrap replications. Reference sequences were retrieved from GenBank under the accession numbers in parentheses.)

3.4 Measurement of fermentative parameters of yeasts on hydrolyzed corn straw medium

Fermentative parameters (carbon dioxide productivity and volumetric ethanol productivity) of the yeasts on hydrolyzed corn straw are presented in Figure 1. *Saccharomyces diaststicus* had the highest carbon dioxide production (4.09 L/L.h), this may be due to the ability of the yeast to easily use up sugars present in the hydrolysed corn straw, while *W. chambardii* had (3.98 L/L.h). Yeasts have been referred to as being respire-fermentative organisms, thus actively growing yeast are likely to be actively fermenting (Aransiola *et al*., 2006). Maximum volumetric ethanol production was achieved by *S. diastaticus* (9.82 g/L.h) while *W. chambardii* had (9.55 g/L.h) (Figure 2). Total alcohol recovery, actual alcohol recovery and fermentation efficiency of the yeasts were presented in Table 3. The yeasts had total alcohol recovery of 4.60%. *Saccharomyces diastaticus* had maximum actual alcohol recovery and fermentation efficiency of 2.45% and 53.00% respectively while *W. chambardii* had actual alcohol recovery and fermentation efficiency of 2.19% and 47.70% respectively. Carbon dioxide production, volumetric ethanol productivity, theoretical alcohol recovery and fermentative efficiency are reported to be important parameters to be studied in ethanol producing yeasts (Sarris and Papanikolaou, 2016).

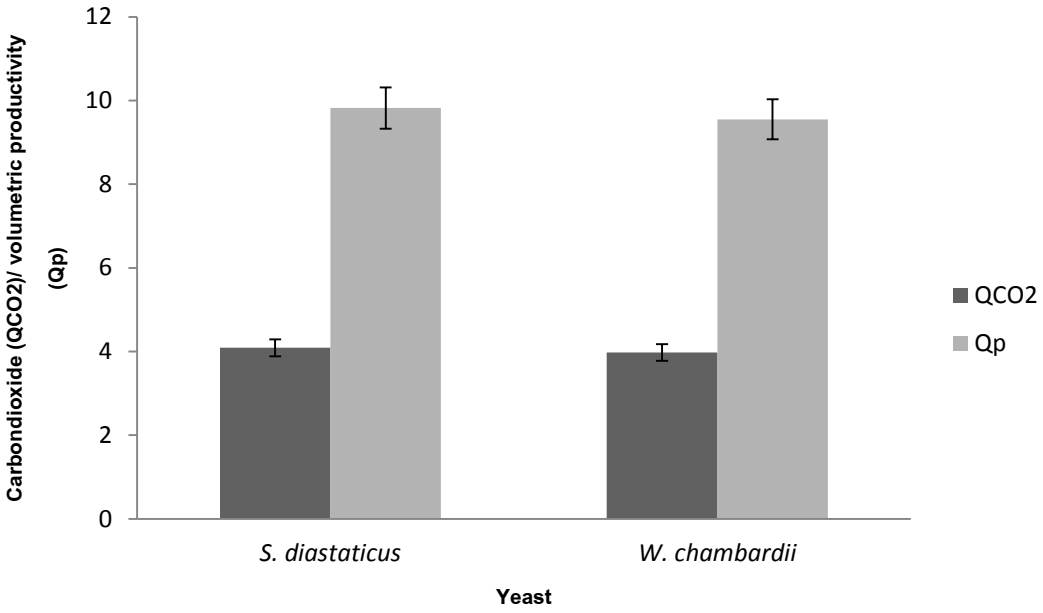

Figure 2 Carbon dioxide productivity and volumetric ethanol productivity of yeasts on hydrolysed corn straw medium
(QCO_2; Carbon dioxide productivity (L/L.h), Qp; Volumetric ethanol productivity (g/L.h))

Table 3 Fermentation parameters of yeasts on hydrolyzed corn straw medium

Yeast	Total Alcohol Recovery (%)	Actual Alcohol Recovery (%)	Fermentation Efficiency (%)
S. diastaticus	4.60±0.030[a]	2.45±0.020[b]	53.00±10.17[b]
W. chambardii	4.60±0.030[a]	2.19±0.017[a]	47.00±2.48[a]

Note: Each value is a mean of triplicates ± standard deviation.

3.5 Bioethanol production from corn straw

The result presented in Figure 3 shows production of bioethanol from corn straw. Ethanol production increased with increase in fermentation time with each of the yeast, such increase may be due to the gradual breaking down of complex sugars to simple sugar during fermentation (Oyeleke and Jibrin, 2009). The two yeasts had highest bioethanol production at 72 h of fermentation and decreased with further increase in incubation period (Figure 3). Bioethanol production with *W. chambardii* was higher than that produced by *S. diastaticus* during the first 48 h of the incubation period. *Wickerhamomyces chambardii* and *Saccharomyces* spp. has been described as yeast that secret xylulokinase (XK) which is responsible for cellulose assimilation, hence fermentation of the product to ethanol (Couto and Sanromán, 2006). *Saccharomyces diastaticus* produced more bioethanol during the incubation period and was found to be better than *W. chambardii*. Maximum bioethanol production was achieved by *S. diastaticus* (27.50 g/L) while *W. chambardii* had its highest bioethanol production of 22.70 g/L (Figure 3). Although *W. chambardii* produced higher bioethanol at the beginning of fermentation; rapid bioethanol production was observed in *S. diastaticusi* after 48 h (15.57–27.50 g/L). Mazmanci (2011) reported similar value in the fermentation of fruits of *Washingtonia robusta* by *Saccharomyces* spp where bioethanol production from fermentation of sweet sorghum juice by *S. cerevisiae* increased throughout 50 h of fermentation during ten-day fermentation.

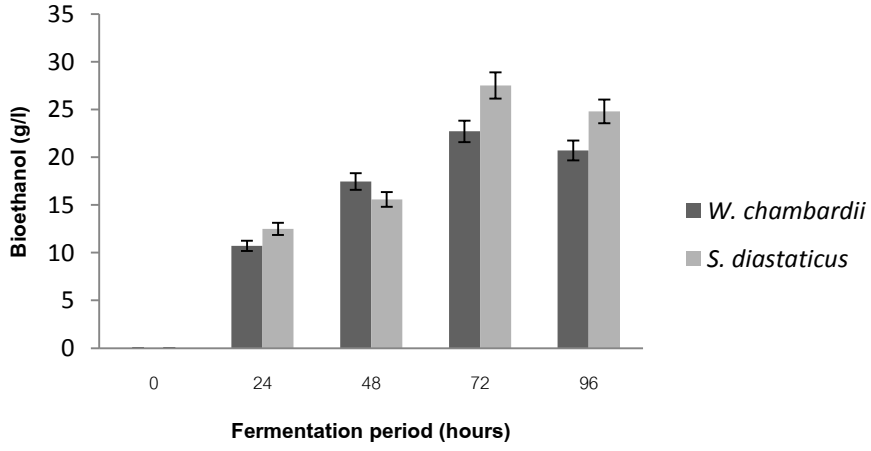

Figure 3 Bioethanol productions from corn straw

3.6 Cell mass production from corn straw

The biomass produced during the incubation period showed that yeast strains grew and produced cell mass during direct microbial conversion of corn straw to bioethanol. Cell mass increased at the beginning of incubation period till 72 h of fermentation with the two yeasts, while further increase in incubation days decreased cell mass production (Figure 4). Highest microbial cell mass was observed by *W. chambardii* (0.08 g/mL), while *S. diastaticus* had 0.06 g/mL as its highest microbial cell mass, this could have been due to presence of elements such as Mg, Zn, Mn, NH_4 and K present in the fermentation medium (Kongkiattikajorn and Sornvoraweat 2011). Although high growth rates result in oxygen depletion but the neutral reaction products allowed growth to be sustained in the anaerobic environment (Rai *et al*., 2012).

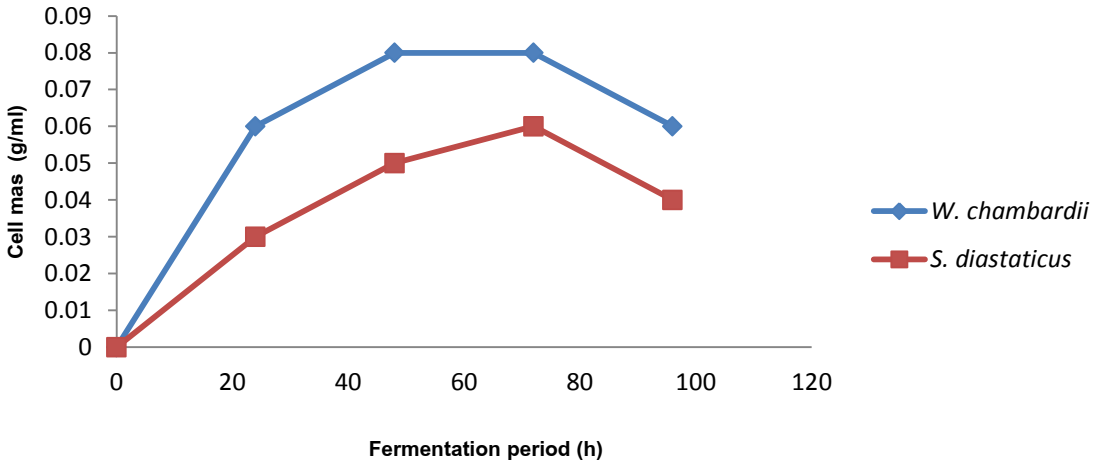

Figure 4 Cell mass production from corn straw

3.7 Optimization of fermentation conditions of ethanol production

3.7.1 Effect of hydrogen ion concentration (pH)

Figure 5 showed the effect of different initial pH values on bioethanol production by *W. chambardii* and *S. diastaticus* at 30°C. This result clearly showed that optimum ethanol production by *S. diastaticus* and *W. chambardii* was obtained at pH 5.0 and 4.5 respectively (Figure 5). This showed that *W. chambardii* could have possessed gene that code for low pH tolerance. Highest volume of ethanol produced by *S. diastaticus* was 24.10 g/L while *W. chambardii* had its highest bioethanol yield of 18.19 g/L. This must have been to the morphological changes in microbes and in enzyme secretion (Bodade *et al*., 2010). Although *S. diastaticus* produced the highest volume of ethanol, the result clearly showed that with *W. chambardii*, rapid bioethanol yield was observed when pH was increase from 4.0 to 4.5, but little increased in bioethanol production was observed with *S. diastaticus,* this could have resulted from a change in pH which affects the ionization of essential active site of amino acid

residues that are involved in substrate binding and catalysis (Bobade *et al*., 2010). In agreement with the results obtained in this research, Limtong *et al*, (2007) examined the ethanol production by *K. marxianus* DMKU 3-1042 in sugar cane juice medium and found that the highest ethanol concentration was obtained at pH 5.0.

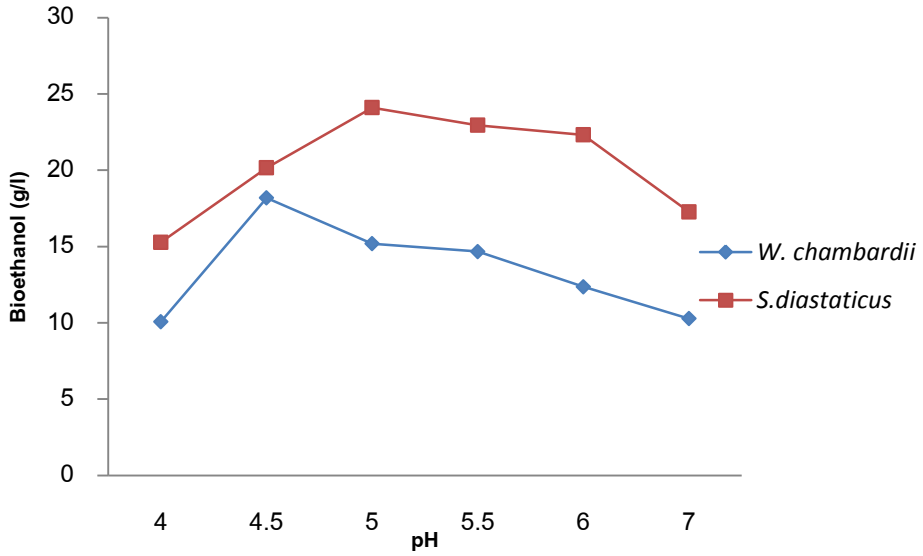

Figure 5 Effect of pH on bioethanol production from corn straw

3.7.2 Effect of inoculum concentration on ethanol production

Effect of inoculum concentration on ethanol production is shown in Figure 6. Result of bioethanol production from different concentration of inoculum (Figure 6) showed that the yeasts were able to produce bioethanol at different inoculum concentration ranging from 5% to 15%. The use of 7.5% inoculum produced highest volume of ethanol (41.82 g/L), followed by 5% inoculum (38.86 g/L) while 15% inoculum lead decrease in ethanol yield, this may be attributed to substrate limitations or product inhibition (Murado *et al*., 2008). *Saccharomyces diastaticus* had maximum bioethanol production of 41.82 g/L, while *W. chambardii* had 35.10 g/L as its maximum volume of ethanol. Lower ethanol biosynthesis at lower inoculum size is probably due to 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).

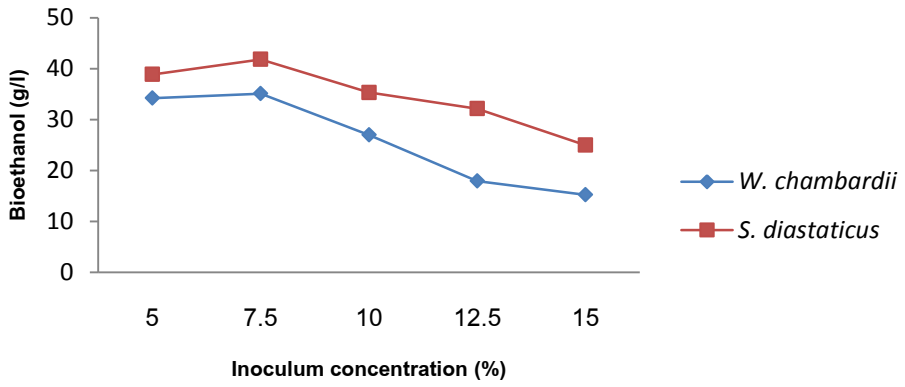

Figure 6 Effect of inoculum concentration on bioethanol production from corn straw

3.7.3 Effect of substrate concentration

The yeasts were capable of producing bioethanol from solution containing up to 15% of corn straw, with 7.5% having the optimum ethanol production (Figure 7). Increasing the substrate concentration beyond 7.5% resulted in decreased the bioethanol concentration in the medium, this could have been due to high concentration of complex sugars in the fermentation medium which could have had high inhibitory effect on yeast growth and their ability to produce ethanol Iqbal *et al*., (2010). *Saccharomyces diastaticus* produced more bioethanol (34.18 g/L) than *W. chambardii* (26.39 g/L) (Figure 6). Lowest bioethanol production was observed with 15% corn straw. Rai *et al*., 2012 reported that high sugar concentration may exert high toxicity on yeast and the nutrient may be deficient at the final stage of fermentation.

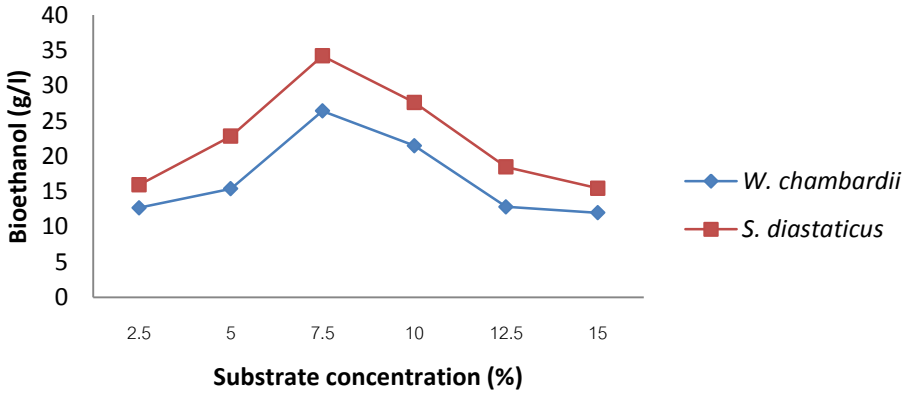

Figure 7 Effect of substrate concentration on ethanol production

3.7.4 Effect of incubation temperature

Effect of different incubation temperature (30–60°C) on bioethanol production by the two yeast strains grown in medium containing corn straw was evaluated. Figure 8 showed that *S. diastaticus* had its optimum bioethanol production at 35°C while *W. chambardii* had its optimum production at 40°C. Variation in optimum temperature by the yeasts could be due to genetic composition of the yeasts (Khan and Dwivedi, 2013). Further increase in temperature resulted in decrease in bioethanol production; this could be that fermentation at higher temperature might disrupt enzyme activity and membrane function

Saccharomyces diastaticus produced the maximum bioethanol yield of 45.87 g/L while *W. chambardii* produced maximum ethanol yield of 41.12 g/L (Figure 8). *Wikerhamomyces chambardii* was observed to be better bioethanol producer at high temperature (40–60°C). *Wikerhamomyces chambardii* produced more ethanol than *S. diastaticus* in all temperatures used except 30 and 35°C. This shows that using a higher tolerant fermentation temperature, similar to the optimal temperature for cellulolytic activity, it may be possible for consolidated bioprocessing to improve the final efficiency (Yan *et al*., 2012).

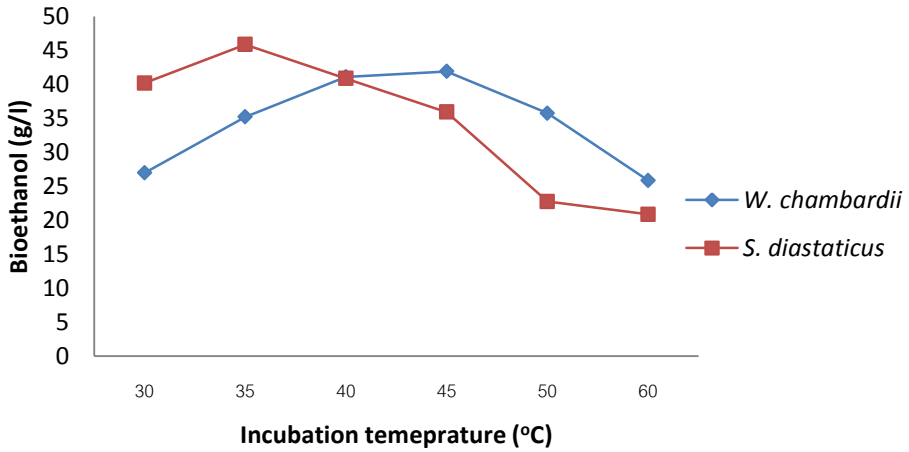

Figure 8 Effect of incubation temperature on bioethanol production from corn straw

4. Conclusion

Result in this study showed that yeast strains can directly produce ethanol through consolidated bioprocessing using natural yeasts including *S. diastaticus* an *W. chambardii*. No external enzymes were required since the appropriate hydrolytic enzymes were provided by the yeast strains. The single-step conversion of lignocellulosic materials to ethanol by natural yeasts represents a significant step toward practical bioethanol production by CPB.

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