OPTIMIZATION OF BIOETHANOL PRODUCTION FROM SUGARCANE BAGASSE USING IMPROVED STRAINS OF *Saccharomyces cerevisiae*

ABSTRACT

Lignocellulosic biomass can be utilized to produce bioethanol, because they are abundantly available in nature. The cost of ethanol production from lignocellulosic materials is relatively high with low yield. But this can be solved by strain improvement processes. This study was aimed at optimizing bioethanol production from lignocellulosic residues using improved strains of *Saccharomyces cerevisiae* developed through random mutagenesis. Bagasse was hydrolysed with 1% NaOH and 1.0M H_2SO_4 respectively for five days. The hydrolysed bagasse was saccharified using *Aspergillus niger* isolated from soil samples. *S. cerevisiae* was isolated from different sources of local fermented wines. *Saccharomyces cerevisiae* isolated from locally produced wines; sorghum (*burukutu*) oil palm wine (*emu*) and raphia palm wine *(oguro*) with the highest ethanol production (5.0g/ml) were selected, and then treated with physical mutagen (ultraviolet light) and chemical mutagens (Acridine dye, Bromo acetaldehyde, dithiothreitol, Ketoconazole and Nitrous acid) respectively to develop mutant with high ethanol producing efficiency under varies operational parameters. Three mutant strains of *Saccharomyces cerevisiae*, SUV, SCD and SCK produced higher volume of ethanol (7.5 g/ml than wild type. SCD and SCK were able to grow at 25% ethanol concentration indicating that they had higher ethanol tolerance ability than the other strains. The optimum temperature and pH for ethanol production by all the strains were 35°C and 6.0 respectively. The improved strains of *Saccharomyces cerevisiae* developed through random mutation techniques produced more ethanol from the bagasse than the wild-type.

Key words: Bioethanol, mutants, biomass, pH, temperature, wild type.

INTRODUCTION

Abundant and sustainable energy resources could be considered as one of the basic needs of man. The increasing human population is constantly exerting more pressure on the world's natural resources, which include natural fossil fuels that are non-renewable (Abiodun, 2007). There are concerns regarding the use of fossil fuels due to its growing scarcity and its negative impact on our environmIent.

It was once suggested that current known crude oil reserves and the reserves yet undiscovered and concluded that would continue to decline worldwide before 2010. They also predicted that annual global oil production would decline from the current twenty-five billion barrels to

approximately five billion barrels in 2050, because the economy in Nigeria and many other nations depend on oil which the consequences could be severe (Campbell and Laherrere, 1998)

Therefore, there is a great interest in exploring alternative energy sources that are renewable, sustainable, and eco-friendly. Renewable energy is currently being derived from the wind, water and sun, but to a limited extent. These forms of natural resources are very attractive for the production of renewable energy, but the technologies needed to make them readily available for use are not yet common **(**Abila, 2010**)**. However, ethanol may provide an alternative to the current use of liquid fossil fuels and it could be used to sustain the present high energy consumption globally because it is cheaper, cost effective, and renewable and the raw materials to produce it is abundance (Ho *et al.,* 2014; Jambo *et al*., 2016; Pan and Xi, 2021).

Furthermore, ethanol serves as a major raw material for biomedical and pharmaceutical companies. The demand for ethanol has increased steadily over the last century as the world's population has grown and more countries have become industrialized (Ado *et al.,* 2009). There is a great interest in exploring alternative sources of producing ethanol. Lignocellulosic biomass can be utilized to produce ethanol, a promising alternative energy source for the limited crude oil because plants biomass waste raw materials are abundantly available in nature (Kim and Dale, 2004; Melekwe *et al*,. 2016; (Jambo *et al*., 2016; Budzianowski, 2017).

A lot of research had been done on conversion of lignocellulosic materials to ethanol in the last one decade (Singh *et al.,* 2010; Singh *et al.,* 2017; Zhao *et al*., 2012; Pan and Xi, 2021; Adegbehingbe *et al.,* 2021). The conversion includes two processes; hydrolysis of cellulose in the lignocellulosic materials to fermentable reducing sugars, and fermentation of the sugars to ethanol. The hydrolysis is usually catalyzed by cellulase enzymes while the fermentation is carried out by yeasts or bacteria (Adegbehingbe *et al.,* 2021).

The cost of ethanol production from lignocellulosic materials is relatively high based on current technologies, and the main challenges are the low yield and high cost of the hydrolysis process (Singh *et al.,* 2010; Achinas and Euverink, 2016). However, one of the primary goals of Industrial Microbiology research and development is the establishment of economically viable processes through increasing products yield and reduced operating cost in order to maximise profit. The most important means of achieving this has been by strain improvement, using a variety of techniques (Okafor, 2007; Rabbani, 2018).

Improvement of the productivity of industrial strains is an important field in Industrial Microbiology, because wild type strains isolated from the natural environment usually produce only a low level of products. The use of a more productive strain may possibly increase costs of production due to higher investment in extraction methods, culture media, more expensive fermentation operations and other unforeseen fermentation challenges (Okafor, 2007).

During the past years substantial progress has been made in the development of genetically-modified microorganisms to produce metabolites or substances that were not present in them naturally. Different methods have been used to improve microbial strains, some of these methods are; random mutagenesis, recombinant DNA technology, classical breeding and genetic crossing all these techniques are used to enhance properties of interest in an organism. However recombinant DNA method is very tedious, too expensive, and it involved many steps. However, random mutagenesis is very easy to carry out and less expensive. (Shinde *et al*, 2018).

Currently, *Saccharomyces cerevisiae* plays a crucial role in many industries such as the baking, brewing, bioethanol, wine and other alcoholic beverage industries (Rabbani, 2018). Therefore, *Saccharomyces cerevisiae* has become increasingly important in the production of bioethanol from the agricultural wastes biomass.

MATERIALS AND METHODS

Collection of Sugarcane bagasse

The sugarcane bagasse samples were got from the Main Market in Okitipupa, Ondo State, Nigeria (6.308^{o} N, 4.46^{o} E). They were collected into a clean polythene bag and transported to the Microbiology laboratory of Adekunle Ajasin University, Akungba-Akoko, Nigeria..

Pretreatment of Sugarcane bagasse sample

The bagasses were dried and blended, then sieved using a sieve with mesh size of 0.2 mm. The bagasses were chemically pretreated in two ways; the alkaline pretreatment and the acid pretreatment. Alkaline pretreatment was carried out by adding 30 g of the bagasse into 150 ml of solution containing 1% NaOH inside 250 ml beaker. The suspensions were left for five days at room temperature to produce a homogenous solution after which the mixture was filtered using Whatman number 1 filter paper. The filtrate was taken and stored inside a reagent bottle for further use. The same procedure was used for acid pretreatment using 0.5 M H_2SO_4 (Ballesteres *et al.*, 2008; Robak and Balcerek, 2018).

Enzymatic saccharification of pretreated lignocellulosic material (bagasse)

Aspergillus niger isolated fom a garden soil in Okitipupa was used for the saccharification process (Abu *et al*., 2005). Pretreated bagasse samples were saccharified enzymatically to get fermentable sugars by inoculating *A. niger* directly into 30ml five sets of the mixture of samples (pretreated bagasse) in the test tubes and then incubated at 28^{o}C for 72 hours. The test tubes were shaken at 4 hours intervals to produce homogenous solution after which the mixtures were filtered using Whatman filtered paper number 1. To test for reducing sugar, 1 ml of Benedict reagent was added to 5 mls of each sample placed in a boiling water bath and allowed to stand for 5 minutes. Positive result gives rise to a brick-red colour (Ado *et al*., 2009).

Glucose concentration was determined using infrared spectrophotometer (SMARTDROP) and read at 650nm (Oyeleke and Jibrin, 2009). Glucose concentration was then calculated using the formula: (Test / Standard absorbance) x Standard concentration

Isolation of ethanol tolerant *Saccharomyces cerevisiae*:

The *Saccharomyces cerevisiae* that was used for this project was isolated from *burukutu* and palm wine samples from raffia palm (*Raphia raphia*) tree and oil palm (*Elaensis guineensis*) obtained from sellers in Okitipupa, Ondo, Nigeria. The wines stored for 5 days were centrifuged for 5 min at low speed 400 rpm. The wines were serially up to 10^{-5}. About 0.5 ml each of dilution was poured into yeast malt agar (YMA) plates supplemented with 0.25 mg per ml chloramphenicol to inhibit the growth of bacteria (Abu *et al*., 2005) and was incubated at 25°C for 48 hours. The representative colonies were isolated and purified by further streaking on PDA (Oyeleke and Manga, 2008) and stored at 4°C for further use.

Morphological characterization of the Yeast:

A thin smear of the isolate was prepared to by emulsifying a loopful of the isolate on a clean slide with a drop of water, then air dried, stained with lactophenol cotton blue and then observed with a light microscope under X10 and X40 objective lenses (Oyeleke and Manga, 2008).

The yeasts were later subcultured on Yeast Malt Agar (YMA) and Potato Dextrose Agar (PDA) to observe their morphological characteristics such as the presence of pseudohyphae, hyphae and ascospore formation (Adetuwo, 2020; Sawalha, 2014)**.** The microscopic and cultural features of isolates were compared with the yeast database (https://theyeasts.org).

Biochemical characterization:

An inoculum culture from each test organism on PDA plates cultuvated for two days at 25°C was inoculated on YNB carbohydrate broth medium. The carbohydrates tested were glucose, sucrose, maltose, xylose, galactose, lactose, raffinose, melibiose, mannitol and trehalose (Oyeleke and Manga, 2008; Sawalha, 2014). Nitrate assimilation test was also determined according to (Sawalha, 2014) using lysine and nitrate as nitrogen souces. The biochemical features observed were noted and compared with yeasts database for the yeast species identification **(**https://theyeasts.org).

Screening of *Saccharomyces cerevisiae* isolates for bioethanol production

Saccharomyces cerevisiae Isola**t**ed from three different sources of wine were screened for their ability to produce bioethanol from hydrolysed bagasse were determined. Isolates that produced highest ethanol was used for further study.

Strain Improvement by UV radiation:

Saccharomyces cerevisiae culture was serially diluted appropriately. 1ml of the culture was streaked on solidified PDA plates. The plates were exposed to UV light at a distance of 50 cm for various time intervals (5, 10, 15, 20, 25 and 30 mins). The treated Petri plates were covered with dark nylon and incubated at 30°C for 3 days. Different colonies from the yeast plates were inoculated into 30 ml of hydrolysed bagasse (hydrolyzates) media and incubated for 5 days. Liquid samples separated from the suspension by centrifugation (8000 rpm) for 20 minutes. Ethanol content was analyzed from the supernatant (Singh and Sharma, 2015).

Strain Improvement by chemical mutagens method*:*

Liquid media were prepared and autoclaved for 20 mins at 121°C in test tubes. Different mutagenic chemicals (Acridine dye, Bromo acetaldehyde, Dithiothreitol, Ketoconazole, and Nitrous acid) with different concentrations (5, 10, 15, and 20 µM) were added into 30 ml of hydrolysed bagasse in sterilized test tubes. The pure yeast culture of *S cerevisiae* was inoculated in test tubes and incubated at 30°C for five days. After 72 hours samples were withdrawn and ethanol content was determined using Infrared Spectrophotometer **(**SMARTDROP**)**. The test tubes that contained the sample was inserted into infrared spectrophotometer and read at 650nm (Singh and Sharma, 2015). The absorption portion of each sample was determined and then recorded.

Mutants Selection:

After the treatment of culture with physical and chemical mutagens, mutant strains that showed higher ethanol production potential as comparison to wild type were selected for further study (effect of physical parameters, and the growth and product kinetics).

Effect of physical parameters on ethanol production of the wild, and the mutant strains *of Saccharomyces cerevisiae*

The effects of ethanol concentration, temperature and pH were investigated on both the wild type, and the mutant strains developed through random mutagenesis.

(i) Effect of Ethanol concentration on growth rate of wild-type and mutants strains of *Saccharomyces cerevisiae*

Ethanol tolerance of wild and mutants *S. cerevisiae* were analyzed. The broth media were prepared with different concentrations of ethanol (5%, 10%, 15%, 20%, 25% and 30%) and sterilized for fifteen minutes at 121°C, and inoculated with the wild-type and mutant strains of *Saccharomyces cerevisiae* respectively. The Cultures were incubated at 30^0C for five days. Cell growth was determined by infrared spectrophotometer. The test tubes that contained the sample was inserted into infrared spectrophotometer and read at 600 nm (Singh and Sharma, 2015). The absorption portion of each sample was determined and then recorded by taking O.D at 600 nm against the media as blank (Liu *et al*., 2011; Singh and Sharma, 2015).

(ii) Effect of pH on ethanol production of the wild, and the mutant strains of *Saccharomyces cerevisiae*.

The broth media were prepared (30 ml of hydrolysed bagasse) and sterilized for 15 minutes at 121°C. The pH of the media was varied from 5.0 to 7.5 at 12 hours interval using H_2SO_4 and NaOH respectively, and inoculated with the wild-type and mutants strains of *S. cerevisiae*. The cultures were incubated at 30°C for three days. Ethanol concentration was determined by infrared spectrophotometer The test tubes that contained the sample was inserted into infrared spectrophotometer and read at 650nm (Singh and Sharma, 2015). The absorption portion of each sample was determined and then recorded. By taking O.D at 650 nm against the media as blank (Singh and Sharma, 2015).

(iii) Effect of temperature on ethanol production of the wild, and the mutant strains of *Saccharomyces cerevisiae*

The broth media were prepared (30 ml of hydrolysed bagasse) and sterilized for 15 minutes at 121 °C. It was then inoculated with the wild-type and the mutant strains of *S. cerevisiae*. The cultures were incubated at temperatures between 20 °C to 45 °C (20, 25, 30, 35, 40 and 45°C) for three days. Ethanol concentration was determined by infrared spectrophotometer at 650nm. The absorption of each portion of the sample was determined and then recorded by taking O.D at 650nm against the media as blank (Singh and Sharma, 2015).

Extraction of Ethanol from the broth by Distillation Method

Three grams of calcium oxide powder was added to 150 ml of distillate before distillation was carried out with a distillation apparatus set up for each of the fermented broth. The fermented broth was transferred into round bottom flask and placed on a heating vessel fixed to a distillation column enclosed in running tap water. Another flask was fixed to the other end of the distillation column to collect distillate at 78ºC which is the standard temperature for ethanol production (Oyeleke and Jibrin, 2009; Ababio,2018).

Statistical Analysis.

Data were entered using SPSS version 20.01v computer software for analysis. Data was analysed using both descriptive and inferential statistics. For the descriptive statistics, frequency tabulations, mean, straight line graphs, and bar charts were generated. The main outcome was concentration of ethanol produced by wild-type and the mutant strains of Saccharomyces cerevisiae. The strength of association was determined using odds ratio and $p<0.05$ values at 95% level of confidence. The statistical significance difference between the concentrations of ethanol produced by wild-type, and the mutant strains was tested by using two-way ANOVA ($p<0.05$) (Ogbeibu, 2015).

RESULTS

Table 1 shows the results of the pretreatment of the bagasse samples. It was observed that concentration of reducing sugar obtained from bagasse treated with acid increased from 0 g to 0.423 g, while that of alkaline treated sample increased from 0 g to 0.313 g.

Table 2 reveals the characteristics of the *Aspergillus niger* used for the saccharification of bagasse. It was noted that *A. niger* has black mycelium with septate hyphae, long and smooth conidiophores, with a large and round head on potato dextrose.

Figure 1 shows yeast count of the wines samples. It was observed that more yeast, $9.41x10^{-4}$ cfu /ml was isolated from raffia palmwine, followed by oil palmwine, $5.8x10^{-4}$ cfu/ml and the burukutu wine, $3.51x10^{-4}$ cfu/ml.

Table 3 and 4 show the cultural and biochemical characteristics of the yeasts species isolated from the fermented wines samples**.** Colonies of yeasts had unique earthy smells. Other characteristics were color ranging from cream to white, Shape is oval, and occurring singly. All the isolates had raised elevation Microscopic observation of the isolates showed the cells to be ovoid to circular shape cells, the size ranged from 2 to 6 µm. No filament was observed in any of the isolates when samples were taken from colonies growing on a potato dextrose agar. All the isolates had budding cells. Spores were not observed under the cultural condition in this study. The colonies of yeast strain that possessed morphological features which corresponded to *S. cerevisiae* species were confirmed by its ability to metabolised glucose, raffinose, maltose and nitrate.

Table 1: Concentration of reducing sugar obtained per gram of sugarcane bagasse hydrolysed by acid and alkaline

Pretreatment	**Before hydrolysis**	**After hydrolysis**
Acid	0	0.423
Alkaline	0	0.313

Table 2: Morphology and characteristics of *Aspergillus niger* used for hydrolysis

Features	**Observation**
Colour of mycelium	Black
Septate hyphae	Present

Conidiosphores	Long and smooth

Figure 2 shows the Screening result of bioethanol produced from *Saccharomyces cerevisiae* isolated for from fermented wines samples before exposing them into physical and chemical mutagens. *Saccharomyces cerevisiae* isolated from Raphia palm wine (*oguro*) produced more ethanol per volume of innoculum, 5.2g/ml, followed by Oil palm wine (*emu*) and sorghum wine (*burukutu*) isolates wth the values of 4.2g/mi and 4.0g/ml respectively. Hence, *Saccharomyces cerevisiae* isolated from Raphia palm wine (*oguro*) were then selected for this study, because it has highest ethanol production capacity.

In strain improvement by UV radiation, only two mutants SUV_5 and SUV_{10} produced more ethanol, 7.50g/ml and 6.0g/ml respectively than the wild-type as shown in figure 3. All other isolated culture exhibited decrease in ethanol production. However, S. cerevisiae exposed to UV radiation for 25 minutes does not produce significant ethanol.

In strain Improvement by chemical mutagens shows in figure 4. Mutants obtained from acridine dye, bromo acetaldehyde and nitrous acid treatments exhibited decreased in ethanol amounts of 4.0g/ml, 4.2g/ml and 4.8g/ml respectively in comparison to wild-type 5.0g/ml. Dithiothreitol and ketoconazole mutants showed higher ethanol production compared to the wild-type. The mutant strains SCD and SCK produced, 11.2g/ml and 9.5g/ml ethanol respectively at 10 µM. But higher concentration of mutagen (>10 µM) were less effective to increase ethanol production.

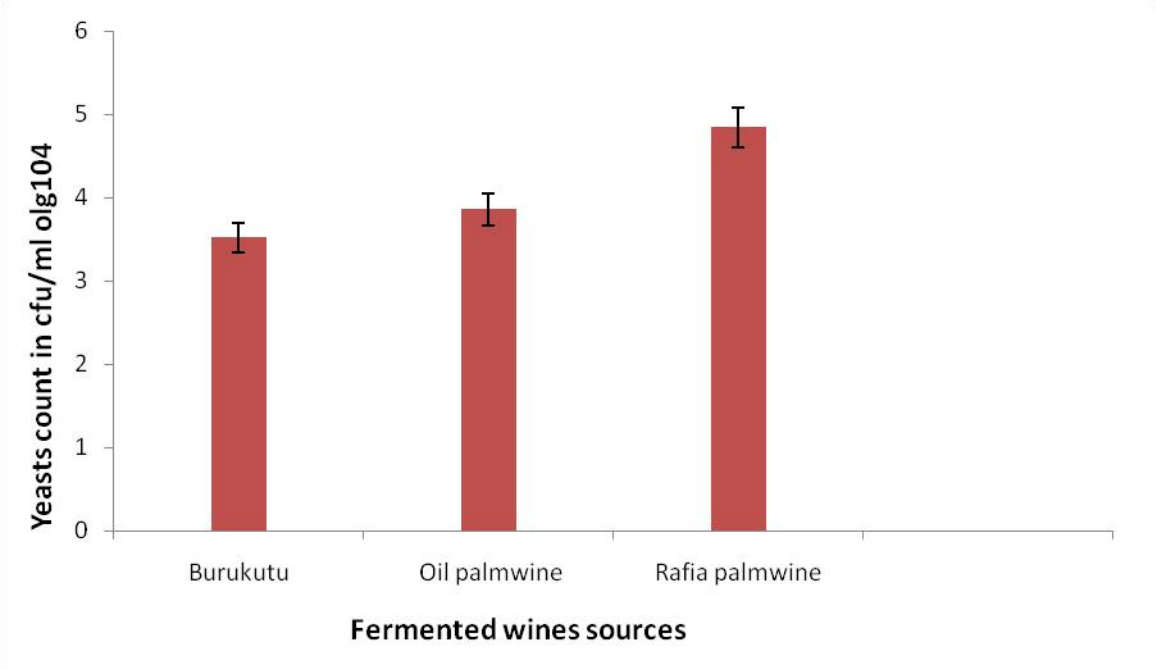

Figure 1: Yeasts counts of the different fermented wine samples

Table 3: Morphological characteristics of yeast isolates from the wines samples

Fungi isolates	Description of isolates cultural and morphology features observed on YMA and PDA media
***Candida* species**	Colonies are whitish-cream in color, smooth, glabrous and yeast-like in appearance. Presence of spherical to sub-spherical blastoconidia.
Hanseniaspora avarum	Colonies are white. Smooth and slightly raised at the centre.
Saccharomyces carlsbengensis	Colonies occur singly or in pair, have flat, moist, dull appearance. Globorus and yeast-like mycelium
Saccharomyces globosus	Colonies have convex shape, whitish appearance.
Saccharomyces cerevisiae	Colonies extent quickly and developed within two days. They have flat, moist, glittering, and cream in color. Blastoconidia are present.

Table 4: Biochemical characteristics of the yeasts isolated from fermented wines samples

Yeasts	GL	SU	MA	XY	LA	RA	TE	MN	CE	ER	LY	UR	NO_3
***Candida* species**	+	+	+	+	-	-	-	-	-	+	-	+	-
H. avarum	+	+	+	+	+	-	-	-	+	-	+	+	-
S. carlsbengensis	+	+	+	+	+	+	+	+	+	-	-	-	-
S. globosus	+	+	+	+	+	+	+	+	-	+	+	-	-
S. cerevisiae	+	+	+	+	+	+	+	+	+	-	+	-	+

KEYS: positive (+) means it utilizes the substrate, while negative (-) means it does not utilize the substrat. GL=Glucose, SU=Sucrose, MA=Maltose, XY=Xylose, LA=Lactose, RA=raffinose,

MN=Mannose, TE= Trehalose, Cellobiose, LY=Lysine, ER=Ethylamine, UR= Urease, NO_3^- =Nitrate,

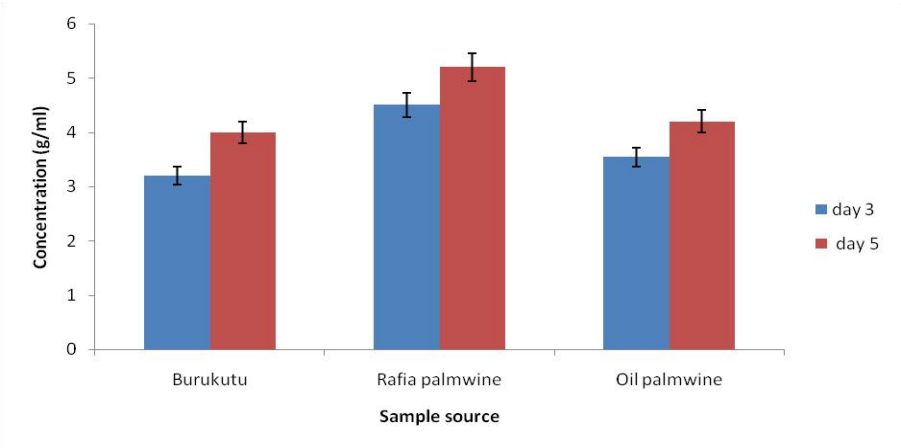

Figure 2: Ethanol Produced by *Saccharomyces cerevisiae* isolates from different wine sources

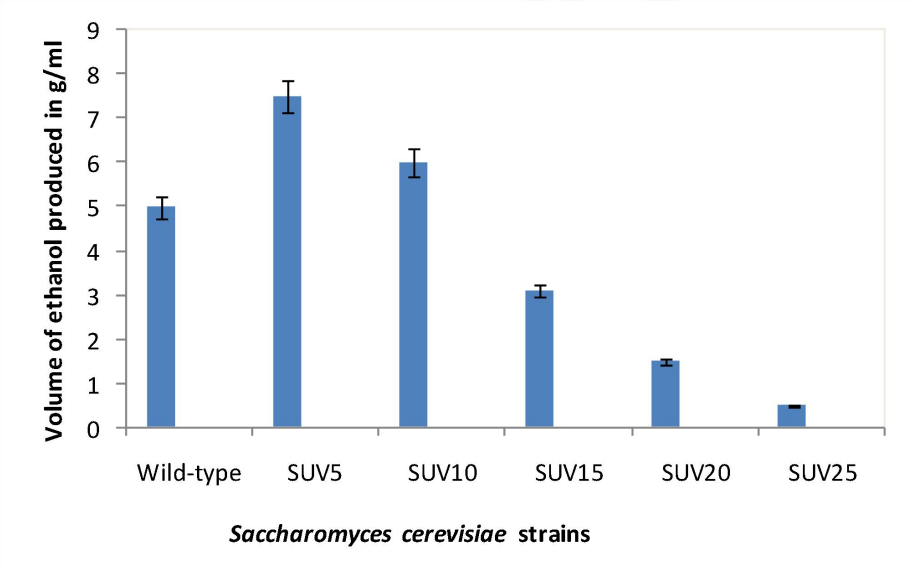

Figure 3: Volume of ethanol produced in g/ml by *Saccharomyces cerevisiae* wild-type and the UV strains

KEYS: SUV means *S. cerevisiae* ultra-violet strains. The number indicates time of exposed to UV radiation in minutes.

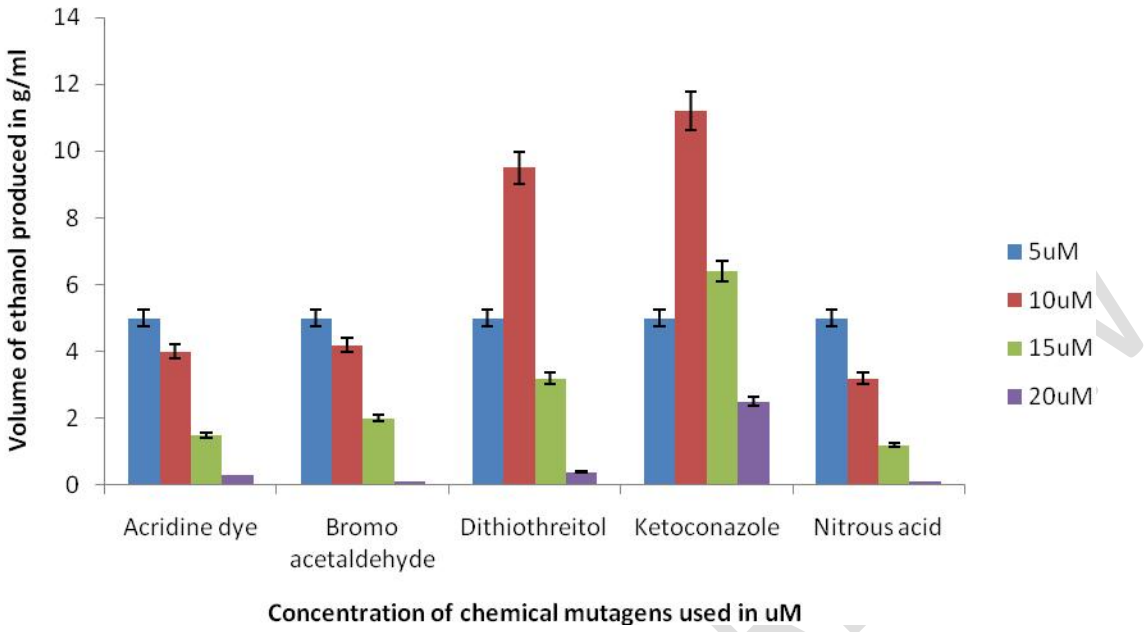

Figure 4: Volume of ethanol produced by *S. cerevisiae* strains developed through chemical mutagens.

Figure 5 shows the effect of ethanol concentration on the growth rate of yeast strains. At 5% ethanol concentration, all the S. cerevisiae strains exhibited maximum growth**.** The maximum ethanol tolerance were observed on SCK mutant,3.9cell/ml, at 25% ethanol concentration, the growth rate of all the yeast strains declined. The wild-type showed not growth at 25% ethanol concentration, and at 30% ethanol concentration, SUV mutant showed no growth.

The effect of temperature on ethanol production by S. cerevisiae strains were shown on figure 6 below. The wild-type and mutants strains showed maximum ethanol production at 35^0C. The highest ethanol production was observed in SCK (11.2g/ml). The ethanol production by all *Saccharomyces cerevisiae* strains decreased after 35℃. Lowest ethanol production was observed in wild-type (2.0g/ml) at 45℃.

Figure 7 shows the effect of pH on ethanol production by S. cerevisiae strains. The optimum pH for the ethanol production by all the yeast strains was pH 6.0. The volume of ethanol produced at this pH by mutant SCK and SCD were 11.2 g/ml and 9.5g/ml respectively, At pH below and above 6.0, ethanol production were decreased form all yeast strains.

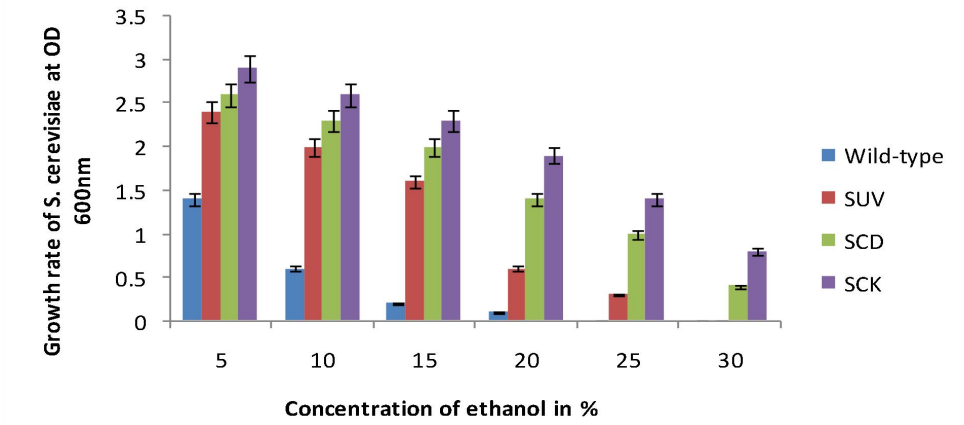

Figure 5: Effect of % ethanol concentration on growth of *Saccharomyces cerevisiae* at OD 600nm

KEYS: SUV means *S. cerevisiae ultra-violet* strain SCD means *S. cerevisiae dithiothreitol* strain SCK means *S. cerevisiae ketoconazole* strain

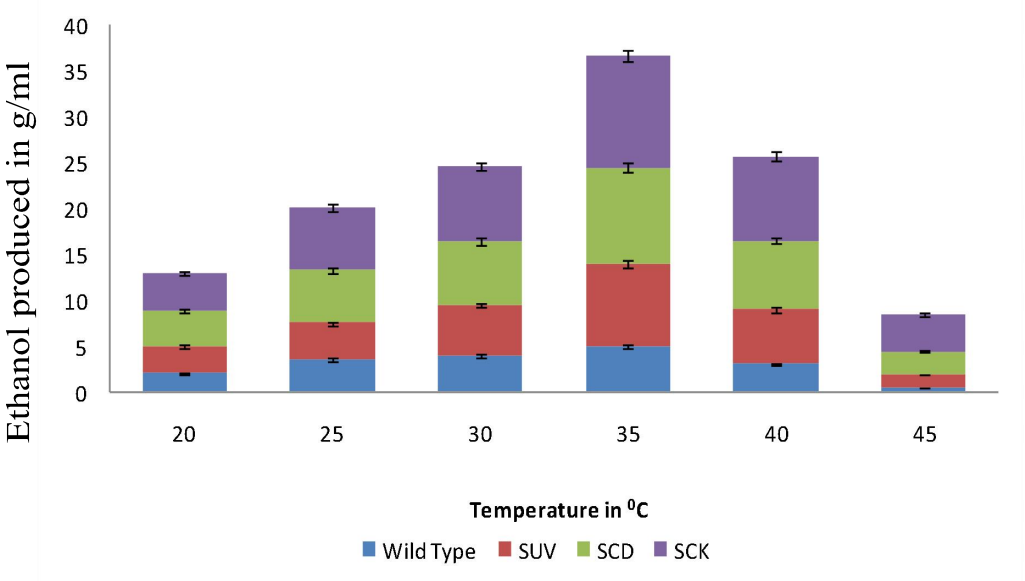

Figure 6: Effect of temperature on ethanol produced by the wild-type and the three selected mutants of *Saccharomyces cerevisiae*

KEYS:

SUV means *S. cerevisiae ultra-violet* strain SCD means *S. cerevisiae dithiothreitol* strain

SCK means *S. cerevisiae ketoconazole* strain

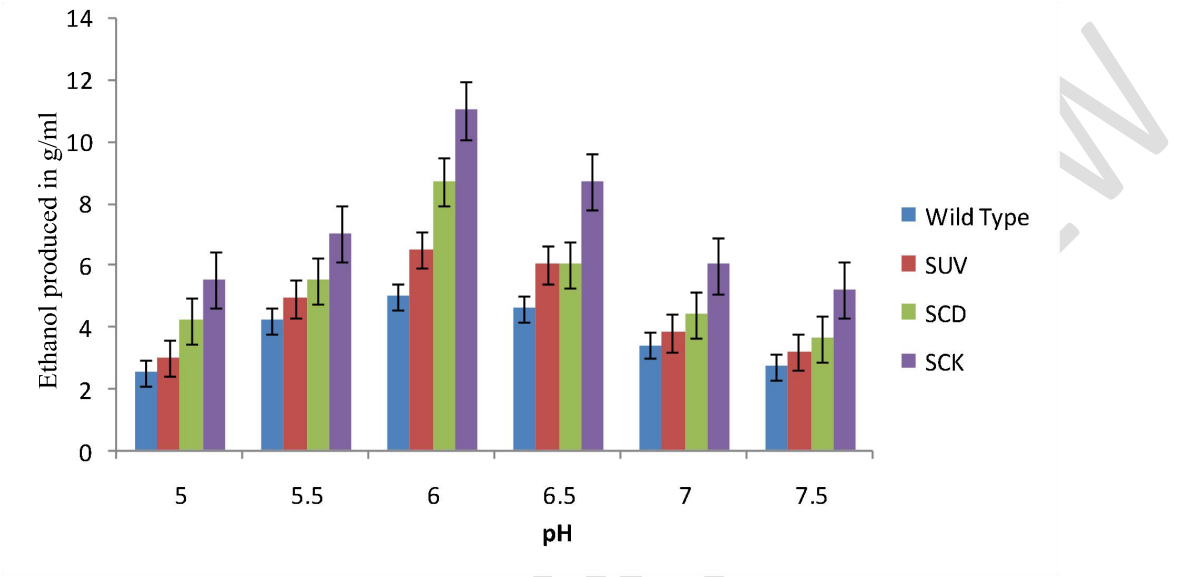

Figure 7: Effect of pH on ethanol produced by the wild-type and the three selected mutants of *Saccharomyces cerevisiae*.

KEYS:

SUV means *S. cerevisiae ultra-violet* strain SCD means *S. cerevisiae dithiothreitol* strain

SCK means *S. cerevisiae ketoconazole* strain

DISCUSSION

Bagasse is a potential source for low-cost ethanol production. It was discovered in this study that sugarcane bagasse pretreated with dilute tetraoxosulphate (vi) acid producd more glucose than the one pretreated with Sodium hydroxide solution. Similar results were reported by researchers while worked on pretreated bagasse with acid and alkaline respectively (Oyeleke and Jibrin, 2009; Alvira *et al*., 2016; Robak and Balcerek, 2018; Pan and Xi, 2021). The pretreatment process breakdown lignin and hemicellulose, and at the same time reduces cellulose crystallinity, and increases the porosity of the bagasse. Thus, pretreatment process using dilute significantly improved the hydrolysis of bagasse in this study.

Saccharification of pretreated bagasse to fermentable sugar was carried out by *Aspergillus niger*. The fungus produced cellulase enzymes that catalysed the conversion of cellulose in bagasse into fermentable sugars (Okafor, 2007; Sarris and Papanikoaou, 2016). The fermentable sugars produced were utilized by Saccharomyces cerevisiae for ethanol production. *Saccharomyces cerevisiae* is able to produced ethanol due to the presence of pyruvate decarboxylase and alcohol dehydrogenase which are key enzymes in ethanol formation, as reported by Gunasekaran and Chandra (2007).

The wild type *Saccharomyces cerevisiae* strains used were isolated from five days old fermented wines samples. The idea behind the used of five days old fermented wines is to get ethanol tolerant strain of *Saccharomyces cerevisiae.* Selection of natural strain from the environment can practically enhanced the ability of microorganisms to produce important metabolites. Selection of *S. cerevisiae* ethanol tolerant strain can enhance the ethanol production from bagasse because it would increase ethanol tolerance of the yeast. Ashenafi (2008) Opined that in wine fermentation, yeasts were repeatedly taken from the best vats until yeasts of suitable properties were obtained, as to increase ethanol adaptation mechanism in *S. cerevisiae*. The setback in screening for natural strains is that, selection of natural wild-type is not only slow but its course is largely outside the control of the biotechnologist, an intolerable condition in the highly competitive world of modern biotechnology industry. Strain improvement is therefore mostly the best option has proved in this study, and collaborated by many researchers (Crook and Alper, 2012; Singh and Sharma, 2015).

Different mutants of *Saccharomyces cerevisiae* with varies ethanol production abilities were generated when *Saccharomyces cerevisiae* were exposed to ultraviolet radiation. Two of the mutants, SUV_5 and SUV_{10} produced more ethanol than the wild-type (7.5g/ml and 6.0g/ml respectively as compared to wild-type 5.0g/ml). *Saccharomyces cerevisiae* exposure to UV radiation for twenty-five minutes shows no significant ethanol production. Similar results were reported by Singh and Sharma (2015) when they exposed S. cerevisiae to UV radiation for twenty-five minutes. Therefore, exposing S. cerevisiae culture to longer periods of UV radiation generates mutants that carry multiple mutations, of which many may be deleterious, leading to a large fraction of inferior or even unviable cells. Consequently, the optimal time is the one that gives the largest proportion of beneficial mutants out of all cells that manage to survive; as observed in mutants SUV_5 and SUV_{10}. Hashimoto *et al.* (2005) and Hockberger (2011) demonstrated the influence of the UV radiation to induce mitosis gene conversion by using diploid strain of *S. cerevisiae*. They reported that UV radiation induced multiple strains of *S. cerevisiae* that were exhibited various ethanol production capacity. This study showed that exposure time of 5-10 minutes of *Saccharomyces cerevisiae* cultures to UV radiation will increase ethanol production from sugarcane bagasse by seventy-five percent..

In another way, Chemical mutation was performed with five chemical mutagens. It was noted that all chemicals used in this study have different mode of action to cause mutations. The

chemical mutagens used in this study produced different effects in *Saccharomyces cerevisiae* because two of the chemicals, dithiothreitol and ketoconazole increased ethanol production whereas acridine dye, bromo acetaldehyde, and nitrous acid cause decreased in ethanol produced by *Saccharomyces cerevisiae,* similar result was reported by Singh and Sharma (2015) when they treated yeast cells with chemical mutagens. *Saccharomyces cerevisiae* isolates used in this study were sensitive to mutagenic dosage, higher concentration of mutagens (>10µM) were less effective to increase ethanol production. From the result of this study, it can be concluded that higher concentration of chemical mutagens may result in decreasing ethanol production in Saccharomyces cerevisiae, while tolerable dose (less than 10µM) favours higher yield of ethanol.

Saccharomyces cerevisiae mutants have high ethanol tolerance than the wild-type in this study. This result supported Kumari and Pramanik (2012) findings while investigated effect of ethanol concentration on yeasts mutant strains. They opined that mutagenesis in Saccharomyces cerevisiae improved tolerance of *S. cerevisiae* to ethanol. Therefore in this study, with increases in ethanol concentration above 20% the growth of *Saccharomyces cerevisiae* in wild-type stopped, while the mutant strains SCD and SCK still grow fairly. Ethanol is an inhibitor of yeast growth at relatively low concentrations, inhibit cell division, decreasing cell volume and specific growth rate, while high ethanol concentrations reduce cell vitality and increase cell death (Stanley *et al.,* 2021). Ethanol also affects cell metabolism and macromolecular biosynthesis by inducing the production of heat shock-like proteins, lowering the rate of RNA and protein accumulation, enhancing the frequency of point mutations, altering metabolism, denaturing intracellular proteins and glycolytic enzymes and reducing their activity (Udom *et al.,* 2007; Stanley *et al*., 2021).

The optimum temperature for ethanol production for wild-type and the mutant strains, SUV, SCD and SCK in this study was 35^0C. Similar temperature was reported by many researchers (Liu *et al.,* 2012; Khattak *et al*., 2014; Singh and Sharma, 2015) while investigating effect of Temperature on ethanol production in yeast. Temperature plays major role in the ethanol production from the yeast, temperatures below or above 35°C affected ethanol production in this study. Although the thermotolerant mutant SCD and SCK still produced appreciable amount of ethanol at 45°C, still below optimum production capacity.

All strains (wild-type and mutants), showed maximum ethanol production at pH 6.0. Thus, increasing or decreasing in pH, will either cause increase or decrease in the concentration of the ethanol produced. *Saccharomyces cerevisiae* strains grow better at optimum pH and temperature. The optimum pH range for ethanol production from this study is 6.0-6.5. The enzymes involved in catalysing fermentable sugar into ethanol are able to function optimally at lower pH, because they are more active at acidic medium (Okafor, 2007; Buijs *et al.,* 2013).

Conclusion

In this research, the use of improved strains *Saccharomyces cerevisiae* gives a better yield as there was significance difference between ethanol produced by wild-type and, the improved strains. The result of this study can be of a better application in the large production of biofuel from bagasse which is renewable and highly abundant. It saves costs by recycling wastes, and it also helps to alleviate environmental problem such as an excessive release of greenhouse gases from combustion of non-renewable fossil fuel. Pretreatment can enhance the release of fermentable sugar for bioethanol production from lignocellulosic biomass. Also mutagenesis techniques such as Ultra-violet radiation and some chemical mutagens can be used to improve *Saccharomyces cerevisiae* in bioethanol production. Furthermore, the optimum temperature and pH for bioethanol production in S. cerevisiae is 35℃ and 6.0 respectively.

Findings in this research strongly proved that optimization of bioethanol production from bagasse by using mutant strains of *Saccharomyces cerevisiae* not only reinforced ethanol overproduction, but also diminished the cost of the production process.

Recommendations

The result of this research work can be applied in the large-scale production of bioethanol from bagasse. This study recommended that using random mutation technique could be resulted in a better and faster for having overproducer strains in industrial microbiology and biotechnology.

References

Ababio, O.Y. (2018). New Senior Secondary School Chemistry. Africana Publishers PLC. Onisha, Nigeria, Pp 350-400.

Abila, N. (2010). Biofuels adaption in Nigeria: A preliminary review of feedstock and fuel production potential. Department of Industrial Management, University of Vaasa, Finland, Pp1-11.

Abiodun, O. (2007). Biofuel opportunity and development of renewable energies markets in Africa: A paper presented during the biofuel market Africa 2007 conference, Cape Town South Africa, Pp10-15.

Achinas, S. and Euverink, G.J.W. (2016). Consolidated briefing of biochemical ethanol production from lignocellulosic biomass. *Electron Journal of Biotechnology*, 23: 44-57.

Adegbehingbe, K.T., Faparusi, F, and Adeleke, B.S. (2021). Bioethanol production from Cassava peels inoculated with *Saccharomyces cerevisiae* and *Zymomonas mobilis. Journal of Advances in Microbiology* 21(9): 58-67.

Adetuwo, O.J. (2020). Evaluation of the microbiological quality and safety of pupuru and garri on sale at Okitipupa main market in Okitipupa Local Government Area, Ondo, Nigeria. *American Journal of Food Sciences and Nutrition,* 2(1): 32-44.

Ado, S.D., Kachalla, G.U., Tijjani, M.B., and Aliyu, M.S. (2009). Ethanol production from corn cobs by co-cultures of *Saccharomyces cerevisiae* and *Aspergillus niger*. *Bayero Journal of Pure and Applied Sciences*, 12(2): 99-101.

Almeida, P., Barbosa, R., Zalar, P., Imanishi, Y., Shimizu, K., and Turchetti, B. (2015). A population genomics insight into the Mediterranean origins of wine yeast domestication. *Journal* of *Molecular Ecology,* 24 5412–5427.

Alvira, P., Negro, M.J., Ballesteros, I., González, A. and Ballesteros, M. (2016). Steam explosion for wheat straw pretreatment for sugars production. *Bioethanol,* 2(1):66-75.

Ashenafi, M. (2008). A review on the microbiology of indigenous fermented foods and beverages of Ethiopia. *Ethiopian Journal of Biological Science,* 5: 189-245

Ballesteros, I., Ballesteros, M. and Manzanares, P. (2008). Dilute Sulfuric acid pretreatment of Carson for ethanol production. *Journal of Biochemical engineering,* 42: 84-91.

Budzianowski, M.W. (2017). High-value, low-volume bioproducts coupled to bioenergies with potential to enhance business development of sustainable bio- refineries. *Renewable Sustainable Energy Review*, 70:799-804.

Buijs, N.A., Siewers, V., Nielsen, J. (2013). Advanced biofuel production by the yeast *saccharomyces cerevisiae*. *Current Opinion in Chemistry and Biology*. 17(3):480-488.

Campbell, C.J., and Laherrere, J.H., (1998). The end of cheap oil. *American Journal of*

Crook, N. and Alper, H.S. (2012). Classical strain improvement. In: Patnaik R, editor. Engineering Complex Phenotypes in Industrial Strains. Hoboken, N.J: John Wiley & Sons, Inc; 2012. pp. 1-33.

Gunasekaran, P. and Chandra, K.R. (2007). Ethanol fermentation technology: M. mobilis. Madura Kamary University, Madurai, India, Pp1-22.

Hardison, R. (2021). Mutations and mutagens. The Pennsylvania State University Press, Pennsylvania, USA, Pp1-60.

Hashimoto, S., Ogura, M., Aritomi, K., Hoshida, H., Nishizawa, Y. and Akada, R. (2005). Isolation of auxotrophic mutants of diploid industrial yeast strains after UV mutagenesis.

Hockberger, P.E. (2011). A history of ultraviolet photobiology for humans, animals and microorganisms. *Photochemistry and Photobiology*, 76(6): 561-579.

Ho, D.P., Ngo, H.H., Guo, W. (2014). A mini review on renewable sources for biofuel. *Bioresources Technology*, 169: 742-749.

Jambo, S.A., Abdulla, R., Mohd Azhar, S.H., Marbawi, H., Gansau, J.A., and Ravindra, P. (2016). A review on third generation bioethanol feedstock. *Renewable Sustainable Energy Review* 65:756–69.

Khattak,W. A., Ul-Islam,M., Ullah, M. W., Yu, B., Khan S., and Park, J. K. (2014). Yeast cell-free enzyme system for bio-ethanol production at elevated temperatures. *Process Biochemistry,* (3):357–364.

Kim, S. and Dale, B.E. (2004). Global potential bioethanol production from wasted crops and crops residues. *Biomass and Bioenergy,* 26: 361-375.

Kumari, R. and Pramanik, K. (2012). Improvement of multiple stress tolerance in yeast strain by sequential mutagenesis for enhanced bioethanol production. *Journal of Bioscience and Bioengineering*, 114:622–629.

Liu, J., Ding, W., Zhang, G., and Wang, J. (2011). Improving ethanol fermentation performance of Saccharomyces cerevisiae in very high-gravity fermentation through chemical mutagenesis and meiotic recombination. *Applied Microbiology and Biotechnology*, 91:1239–1246.

Melekwe, E.I., Lateef, S.A., Rowland, G. and Ekpeyong, E. (2016). Bioethanol production potentials of corn cob, waste, office paper and leaf of *Thaumatococcus daniellii*. *Bayero Journal of Applied Science Technology*, 17(4):1–10.

Ogbeibu, A.E. (2015). Biostatistics: A practical approach to research and data handling (3rd edition). Mindex Publishing, Benin-City, Nigeria. pp 17-128

Okafor, N. (2007). Modern Industrial Microbiology and Biotechnology. Science Publishers, Enfield, USA. pp 126-148

Oyeleke, S.B., and Jibrin, N.M. (2009). Production of bioethanol from guinea corn husk and millet husk. *African Journal of Microbiology,* 3(4):147-152.

Oyeleke, S.B., and Manga, S.B. (2008). Essential of laboratory practical in microbiology (1st Edition),Tobest, 2009 Minna Nigeria, pp36-69.

Pan, W. and Xi, L. (2021). Advances in 2nd generation of bioethanol production. A volune in Woodhead Publishing Series in Energy. Pp1-7.

Rabbani, G. (2018). Involvement of mutagens in the production of bioethanol by Saccharomyces cerevisiae: A Review. *Journal of Environmental and Analytical Chemistry,* 5: (248) 1-4.

Robak, K, and Balcerek, M. (2018). Review of second generation bioethanol production from residual biomass. *Food Technology and Biotechnology,* 56(2):174-187.

Sarris, D. and Papanikolaou, S. (2016). Biotechnological production of ethanol: Biochemistry, processes and technologies. *Journal of Engineering and Life Science,* 16(4):307–329.

Sawalha, H. (2014). Laboratory manual of mycology and plant pathogenic fungi. Al-Jamea'yah Bookshop Press, Nablus, Palestine, Pp1-66.

Shinde, S.A., Chavhan, S.A., Sapkal, S.B., and Shrikhade, V.N. (2018). Recombinant DNA technology and its application: Review. *International Journal of MediPharm Research,* 04(2): 77-80.

Singh, A., Pant, D., Korres ,N.E, *et al*. (2010). Key issues in life cycle assessment of ethanol production from lignocellulosic biomass: challenges and perspectives. *Bioresources Technology,* 101:5003–5012.

Singh, J. and Sharma, R. (2015). Kinetic and modeling of ethanol production by wild and mutant *S. cerevisiae MTCC 170*. *European Journal of Experimental Biology,* 5(4): 1-6.

Singh, S, Chakravarty I, and Kundu, S. (2017). Mathematical modelling of bioethanol production from algal starch hydrolysate by *Saccharomyces cerevisiae*. *Journal of Cell and Molecular Biology,* 63: 83-87.

Stanley, D., Bandara, A., Fraser, S., Chambers, P.J. and Stanley, G.A. (2021). The ethanol stress response and ethanol tolerance of Saccharomyces cerevisiae. *Journal of Applied Microbiology* 109(1): 13-24.

Udom, N., Chansongkrow, P., Charoensawan, P. and Auesukaree, C. (2019). Coordination of the Cell Wall Integrity and High-Osmolarity Glycerol Pathways in Response to Ethanol Stress in Saccharomyces cerevisiae. *Applied and Environmental Microbiology*, (85)15: 21-56.

Zhang, W. and Geng, A. (2012). Improved ethanol production by a xylose-fermenting recombinant yeast strain constructed through a modified genome shuffling method. *Biotechnology and Biofuels*, 5:46-56.

Zhao, X., Zhang, L., and Liu, D. (2012). Biomass recalcitrance. Part I: the chemical compositions and physical structures affecting the enzymatic hydrolysis of lignocellulose. *Journal of Biofuel, Bioproduction and Biorefining*, 6:465–482.