PRETREATMENT OF LIGNOCELLULOSIC SUBSTRATES BY *PLEUROTUS* AND *LENTINUS* SPECIES FOR PRODUCTION OF BIOETHANOL USING *SACCHAROMYCES CEREVISIAE*

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CERTIFICATION

This is to certify that this research work was carried out by Samuel Adedayo FASIKU (160667) under my supervision in the Department of Microbiology, University of Ibadan, Ibadan, Nigeria.

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DEDICATION

This research work is dedicated to God who saw me through this programme.

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ABSTRACT

Generation of bioethanol from food crops is a well-established industrial process. However, this poses serious challenge to global food security. Lignocellulosic substrates are possible alternatives for production of bioethanol, but there is need for their pretreatment, which may be expensive. Biological pretreatment is recognised as a low cost technique. However, there is paucity of information in literature for bioethanol generation from biologically pretreated lignocellulosic wastes. Therefore, this study was designed to convert lignocellulosic substrates waste for the production of bioethanol.

Laboratory grown mushroom strains of *Pleurotus ostreatus* (PO) and *Lentinus squarrosulus* (LS) were screened for production of cellulase, xylanase and lignase using solid agar. Yeasts were isolated from palm wine and screened for ethanol production through gas evolution. The selected yeasts were genotypically and phenotypically characterised. Lignocellulosic substrates (groundnut shell, maize cob, maize stalk, sugarcane bagasse and rice straw) were degraded with PO and LS singly and in consortium (POLS) for 70 days, during which residual cellulose, hemicellulose, lignin and the reducing sugar content were determined at 7 days intervals using standard methods. The best substrate of the lot was equally pretreated with NaOH prior to degradation by the better mushroom and its sugar profile determined using HPLC. This was fermented with selected yeasts for bioethanol production. The effects of pH, temperature, sugar concentration, nitrogen sources, inoculum load and incubation period for optimum bioethanol production were determined. Data obtained were analysed using descriptive statistics.

Pleurotus ostreatus and LS hydrolysed lignocellulose with hydrolytic zones (mm) of 35, 41 (cellulase); 35, 52 (xylanase); and 18, 31 (lignase), respectively. Sixty-four yeasts were obtained out of which two *Saccharomyces cerevisiae* (SA01 and SA02), had better carbon dioxide height (2 mm/hour). Highest maize stalk degradation of cellulose (5.60 %), hemicellulose (33.40 %), lignin (18.42 %) and highest reducing sugar (16.89 mg/g) were recorded in PO-degraded maize stalk, POLS-degraded maize stalk, POLS-degraded maize stalk and PO-degraded maize stalk at 42, 28, 7 and 21 days of degradation, respectively. The reducing sugar of alkaline PO-pretreated maize stalk was higher than that of PO-pretreated maize stalk. The sugar profile of the alkaline PO-pretreated maize stalk included (mg/100g) glucose (850.60), xylose (837.04), fructose (754.29), arabinose (502.76), ribose (2.066×10^{-4}) and rhamnose (3.552×10^{-5}). Higher ethanol (1.97 g/L) was recorded at pH 5.5 by both SA01 and SA02. At 30 °C, SA01 produced higher ethanol content (2.76 g/L) compared to SA02 (2.37 g/L). Supplementation with 2% glucose gave ethanol yield of 3.95 g/L by SA02. Corn steep liquor improved ethanol yield of SA01 (14.20 g/L) and SA02 (13.41 g/L) with 1% of 1.0 MacFarland standard inoculum load. The highest ethanol content (14.99 g/L) was produced by SA01 at pH 5.5, 30°C, 2% glucose supplementation, corn steep liquor and 1% of 1.0 MacFarland standard inoculum load after 72 hours of fermentation.

Bioethanol was successfully obtained through fermentation of lignocellulosic substrates, with the maize stalk found to be the best substrate. *Pleurotusostreatus* and *Saccharomyces cerevisiae* could be employed in the conversion of lignocellulosic substrates into ethanol.

Keywords: Lignocellulose, *Pleurotus ostreatus*, *Saccharomyces cerevisiae*, Corn steep liquor, Bioethanol

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CHAPTER ONE

INTRODUCTION

A fuel that is derived from renewable biomass is referred to as biofuel. Biodiesel and bioethanol are the most common forms of biofuel. Other examples are Fischer-Tropsch Diesel, Biogas, Bio-hydrogen, Bio-methanol, Bio-dimethyl-ether, Butanol,Dimethylformamide, Pyrolytic bio-oil and Hydro Thermal Upgrading Diesel(Nikolic *et al*., 2016).Biofuel has four generations which are first, second, third and fourth. Edible crops like corn, sweet sorghum, wheat, sugar beet, cassava and sugarcane are used to generate first generation of biofuel (Muktham *et al*., 2016).Non-edible agricultural wastes are utilized to form second generation biofuel.Substrates engaged for producing second generation biofuel are non-edible agricultural wastes (lignocellulose)which contain chieflylignin, hemicellulose and cellulose. Sugar cane bagasse, maize straw, rice straw, maize cobs, groundnut shells are some of the substrates that are utilized for producing second generation biofuel. Third generation biofuel has to do with growing of algal culture for biomass production and the biomass will then be converted to biofuel. Fourth generation biofuel involves capturing and storing of carbon dioxide for continuous production of biofuel (Lu *et al*., 2011; Eva-Mari, 2016).

The fermentable sugars in lignocellulose are not readily available for fermentation because of the structure of lignocellulose. Lignocellulose needs to be pretreated so that it releases fermentable sugars in it. There are varying kinds of pretreatment which are biological, chemical, physical and physicochemical. Milling, irradiation, pyrolysis, pulsed-electric field and mechanical extrusion are examples of physical pretreatment method(Taherzadeh and Karimi, 2008; Kumar and Sharma, 2017). Wet oxidation, sulphite pretreatment, carbon dioxide explosion, ammonia-based pretreatment, liquid hot water and autohydrolysisare different kinds of physicochemical pretreatment method (Taherzadeh and Karimi, 2008; Kumar and Sharma, 2017).Alkali pretreatment, dilute acid

pretreatment, ozonolysis, organosolv and ionic liquids are chemical pretreatment methods. Biological pretreatment

requiresmicroorganisms or their metabolites to break lignocellulosic biomass to simple sugars. Soft rot,brown rot and white rot fungi are involved in breaking lignocellulose to simple sugars. White rot fungal enzymesdegrade lignocellulose(Isroi *et al*., 2011). It has been revealed that combining these pretreated methods gives more yield than using just one (Isroi *et al*., 2011).

Many organisms are engaged in the conversion of fermentable sugars to bioethanol. Filamentous fungi, bacteria and yeasts have been studied to be involved in the fermentation of sugar to bioethanol. *Rhizopus*, *Rhizomucor* and M*ucor* are some of the reported filamentous fungi involved in converting sugar to ethanol (Millati *et al*., 2005).Some of the bacteria that have been engaged in the production of ethanol are *Lactobacillus pentoaceticus*, *Klebsiella oxytoca*, *Clostridium thermocellum*, *Clostridium acetobutilicum*, *Zymomonas mobilis* and *Escherichia coli* (Gamage *et al*., 2010). *Saccharomyces cerevisiae*, *Schizosaccharomyces*,*Pichia*, *Candida*, *Kluyveromyces* and *Pachysolen* are some of the yeasts that have been used to convert simple sugar to bioethanol (Mussato*et al*., 2012).

Various factors have effect on the fermentation of sugar to ethanol like temperature, pH, nitrogen source, inoculum size, incubation time and sugar content (Nadeem *et al*., 2015). Different hydrolysis and fermentation strategies are Direct microbial conversion (DMC), Simultaneous saccharification and cofermentation (SSCF), Simultaneous saccharification and fermentation (SSF) and Separate hydrolysis and fermentation (SHF) (Balat, 2011).Another name for direct microbial conversion is Consolidated Bioprocessing (CBP) that combines enzymes production, hydrolysis and fermentation of liberated sugar to ethanol (Balat, 2011). In SSCF, enzymes that are able to hydrolyze biomass and different microorganisms that have ability to ferment hexose and pentose sugar are introduced in a single set up. In SSF, hydrolysis of pretreated lignocellulose and fermentation of released sugar to ethanol are combined together in a single step. Enzymatic hydrolysis is first performed and followed by fermentation differently in SHF (Balat, 2011).

Distillation is used to recover ethanol from fermentation medium. The concentration of ethanol has effect on monetarycost of distillation. The lower the content of ethanol, the higher the amount to be spent on distillation (Onuki *et al*., 2008; Saini *et al*., 2015; Farias

et al., 2017).Ethanol produced from lignocellulose is performedvia pretreatment, hydrolysis, fermentation and distillation.

Transportation sector depends entirely on fossil fuel as source of energy worldwide and industries also depends on this same source of energy. Transportation sector is accountable for 19% global release of carbon dioxide and 70 % carbon monoxide emissions globally (Balat, 2011). About 8 kg of carbon dioxide are emitted from a gallon of gasoline (Balat and Balat, 2009; Balat, 2011). The numbers of cars on the road is increasing yearly which means that the quantity of emitted carbon dioxide is also increasing. These will have effect on the global climate and stability of ecosystem. Fossil fuel is non-renewable which might soon be exhausted. Increase in the use of this fuel is having negative impact on the global oil reserves (Balat, 2011).

Lignocellulosic biomass is about fifty percent of the global biomass with estimated annual production of ten to fifty billions ton (Mood *et al*., 2013; Srivastava *et al*., 2014).Bioethanol production from lignocelluloses is another option of energy which is environmental friendly, renewable, biodegradable and reduces dependence on imported oil (Balat, 2011).

1.1 Statement of Problem

Almost all the energy requirements are satisfied by non-renewable source of energy, leading to depletionof resources, environmental degenerationand public well-being problems; the cost of using sugar and starch crops for ethanol production is calculated as 40-70% of the total production cost and these foodstuffs will no longer be available for consumers at cheap prices; these have inspired the search for alternative sources of liquid fuels, mainly those derived from renewable resources, which will help to solve these problems.

1.2 Justification

There is an estimated annual global production of 10–50 billion tons of lignocellulose which is about 50 % of the world biomass yield; utilization of this renewable feedstock (lignocellulosic biomass) for production of ethanol will lower dependence on food stuff

for producing bioethanol and lower the cost of large-scale ethanol production which will eventually lead to decrease of greenhouse effect.

1.3 Aim and Objectives

1.3.1 General Objective

This research work aimed at converting lignocellulosic wastes to bioethanol.

1.3.2 Specific Objectives

- Isolation and screening of higher fungi and yeast for their ability to degrade lignocellulosic wastes and produce ethanol respectively.
- Phenotypic and molecular characterization of screened yeast.
- Determination of products of degradation.
- Production and optimization of ethanol from degraded substrates by fermentation.

CHAPTER TWO

LITERATURE REVIEW

2.1 Biofuel

Biofuels are fuels derived from renewable biomass. Bioethanol and biodiesel are the two very important examples of biofuel. There are several benefits of biofuels. It has high impacts on economic, environment and energy security (Balat, 2011). The economic impacts of biofuel are sustainability, diversity offuel, agricultural development, decrease in the dependency on imported petroleum product, increase in income taxes, increase of investments in plant and equipment, increase in numbers of rural manufacturing jobs and international competitiveness (Balat, 2011).Higher combustionefficiency, carbon sequestration, reduction in greenhouse gas and air pollution, biodegradability and improve in land and water use are some of the environmental impacts of biofuels. Renewability, supply reliability, reduction of use of fossil fuels ascertain energy security of biofuel (Balat, 2011).

Bioethanol blended with different concentration of petrol are used for biofuel for transport. E-diesel is ethanol mixed with diesel fueland it is used for transport. It is used in the production of cosmetics, perfumes, shampoo, soaps, aftershave lotions, and mouthwash (Sharma and Sharma, 2018).

2.2 Generations of Biofuel

Generations of biofuel are classified based on the raw materials that are used for generating biofuel. Biofuel has four generations which are first, second, third and fourth.

2.2.1 First Generation Biofuel

This is the biofuel that is derived from eatable food crop. First generation biofuel can also be classified based on the raw materials from which bioethanol are produced as starch-containing feedstocksand sucrose-containing feedstocks(Muktham *et al*.,2016). Cassava, wheat and cornareexamples of starch-containing feedstockwhile sucrose-containing feedstockaresweet sorghum, sugar beet and sugarcane.First generation biofuel are derived from traditional food crops likecorn, sugar cane, sorghum, cassava and wheat. Use of traditional edible food crops for producing bioethanol disturbs production offoods and feeds and have negative effect on food security.

Despite high yield of bioethanol from first generation biofuel, there are several restraints to bioethanolproduction. It has immediate influence on production of food with regards toavailability, soil usage, quality andprice. Utilization of edible food for bioethanol production has negative impact on food industry. Balan *et al*. (2013) reported that reduction in greenhouse gas emission is limited in first generation biofuel.The price of biofuel production is often more than thecost of producing fossil fuel (Nikolic *et al*., 2016).

2.2.1.1 Sucrose-Containing Feedstocksfor Bioethanol Production

Muktham *et al*. (2016) reported that sweet sorghum, sugar beet and sugarcane are the leading sucrose-containing feedstocks for producing bioethanolwith feedstock yields of 50-62, 54-111 and 62-74 tonnes peracrerespectively. These feedstocks are largely exploited in France, Brazil, Germany and India. Glycerol formation and foaming are the main parameters that have effect on ethanol production cost from feedstock containing sucrose. There is need to do more research on sucrose-containing feedstock for ethanol production so as to have better yields of ethanol and reduction in foaming and glycerol production (Mukthame*t al*.. 2016).

2.2.1.2 Starch-Containing Feedstocks for Bioethanol Production

The main starch-containing feedstock utilized for production of bioethanol in America, Europe and Tropical countries are cassava, corn and wheat (Muktham *et al*., 2016). Starch is one of the polymers of glucose. Gluco-amylase and α-amylase can breakdown starch to

glucose. Starch-containing feedstock can be transformed to fermentable sugar throughmilling, liquefaction and saccharification with enzymes. Variety and quality of starch-containing feedstock has effect on the yields of ethanol. Quality of corn based onendosperm hardness, kernel composition,presence of mycotoxins and planting location had effect on bioethanol yields with yield varying from 3–23% because of quality of grain (Singh, 2012).

2.2.2 Second Generation Biofuel

This is made from non-edible agricultural waste recognized as lignocellulose. Lignocellulose consists of lignin, hemicellulose, cellulose, a small fraction of extractive,minerals and salt (Muktham *et al*., 2016). The carbohydrate polymers of lignocellulose are hemicelluloseandcellulose. Cellulose is made up of glucose subunit with a crystalline property. Hemicelluloses are made up of pentose sugar with anunshaped structure. Therigid and highly crystalline portion of lignocellulose is lignin. Hemicellulose and cellulose commonlyare two-third of lignocellulose dry mass whichchange with the kind of lignocellulose feedstock. Lignocellulose are converted to bioethanol through pretreatment of substrates, enzymatic hydrolysis of pretreated substrates, fermentation of released sugar and downstream process (Muktham *et al*., 2016).

2.2.3 Third Generation Biofuel

It involves photosynthetic capture of carbon dioxide in algal cultures for production of biomass and subsequent extraction of biofuel from algal cells (Aro, 2016). The production of biofuel in this third generation biofuel is not economical, because production of biodiesel occurs under extreme conditions which has adverse effect on the growth of algae biomass (Aro, 2016). Metabolic engineering of algae so as to produce large quantity of lipids without altering the growth of algae negatively will be a breakthrough in biodiesel production from algae biomass (Aro, 2016).

2.2.4 Fourth Generation Biofuel

Carbon dioxide are captured and stored for continuous production of biofuel in fourth generation biofuel. In fourth generation biofuel, fermentation and/or processing of biomass feedstock are avoided because end product is secreted out of the cells (Lu *et al*.,

2011). Eva-Mari (2016) reported that fourth generation biofuel are produced in three ways: First is production through designer photosynthetic microbesfor production of photobiological solar fuels while the second is combination of photovoltaics and microbial fuel production also known as electrobiofuels. The last way fourth generation biofuel can be produced is by synthetic cell factories tail engineered to form biofuels and high-value chemicals.

2.3 Lignocellulose

Biomass of lignocellulose is the largestethanol renewable resource on earth. Some of the examples of lignocellulosic substrates that are utitilized for production of second generation bioethanol are maize straw, rice straw, groundnut shell, maize cob, sugarcane bagasse. These substrates are wastes that are released into the environment annually after harvesting. Converting these wastes to bioethanol is turning waste to wealth.

2.3.1 Sources of Lignocellulose

2.3.1.1 Rice Straw

The vegetative segment of rice plant is referred to as rice straw. It is cut during or after grain harvest which could be consumed with fire and abandoned on the farmland until the following season of planting. It is a by-product and one of the surplus lignocellulosic waste on earth. According to Belal (2013), rice straw can be used to produce two hundred and five billion litres of bioethanol per annum in the universe which is aroundfive percent of total consumption of fuel.

2.3.1.2 Maize Straw

Maize straw is a lignocellulose containing lignin, hemicellulose and cellulose. This ismade up of plant residues that are abandoned in farmland after harvest of maize. It is also referred to as corn straw, corn stover or maize stover. Nigeria is the highest producer of maize in Africa (IITA, 2018; Ogbeh, 2018) and large quantity of this maize straw is released to the environment annually. Conversion of this waste to bioethanol will reduce waste in the environment and will also create job for some individuals.

2.3.1.3 Sugar Cane Bagasse

The substance that is left over after extracting juice of sugar cane is referred to as sugar cane bagasse. This waste is used to produce biogas and ethanol. Sugar cane bagasse is pretreated before the simple sugar in it is liberated to be fermented to ethanol (Bharathiraja *et al*. 2014).

2.3.1.4 Maize Cob

One of the by-products from maize crop is maize cob. It is the fibrous rachis in the centre of female inflorescences. Maize cobs are without the grains. Maize cobs are highly fibrous products with industrial and agricultural applications. It has been observed to be one of the promising source of sustainable biofuel production (Zakpaa *et al*., 2009).

2.3.1.5 Groundnut Shell

Pod is the complete seed of groundnut which contains one to five kernels (Sada *et al*., 2013). Groundnut shell is a residue that is left after separation of pod of groundnut which is also the outer layer. Nigeria is one of the main producers of groundnut and larger quantity of groundnut shell is found as agricultural wastes (Sada *et al*., 2013). Utilization of groundnut shell for producing ethanol will promote waste to wealth.

2.3.2 Composition of Lignocellulose

Lignin, hemicellulose and cellulose are the major constituents of lignocellulose. Small fractions of extractives, fat and minerals might also be observed in lignocellulose.

2.3.2.1 Cellulose

Cellulose is seen in plants' cell walls,specifically in stems, stalks, truncks and woody parts of plant. Cellulose is a tough, fibrous and not soluble in water which constitutes much of the mass of wood. It is a linear, unbranched homopolysaccharide which is made up of ten thousand to fifteen thousand D-glucose which are connected through β(1→4) glucosidic bonds (Nelson and Cox, 2004). Cellulose cannot be used as fuel source by many animals because they lack enzymes that will hydrolyze β(1→4) bonds (Nelson and Cox, 2004). Lignocellulosic materials contain high content of cellulose. If this cellulose could by fully degraded to simple sugars at low cost, lignocellulose would be promising substrates for ethanol production.

2.3.2.2 Hemicellulose

Hemicellulose reported to be second most surplus plant cell-wall polymer is composed of largelymannan and xylan (Rocha-Meneses *et al*., 2017). Hemicellulose contains different sugars. Pentoses and hexoses are the two classes of monosaccharide in hemicellulose. The pentoses in hemicellulose are D-xylose and L-arabinose while D-glucose, mannose and galactose are the hexoses present in hemicellulose (Cardona and Sanchez, 2007). Five carbon sugar likearabinose and xylose are from xylan, arabinan, xyloglucanand arabinogalactan which are part of polysaccharide of plants' cell wall (Battaglia *et al*., 2011). Xylan is the largest part of hemicellulose (Balat *et al*., 2008).

2.3.2.3 Lignin

It is a rigid biopolymer part of lignocellulose and aromatic.It is the third most surpluspart of lignocellulose which is a three-dimensional polymer of 4-propenyl-2-methoxy phenol,4-propenyl phenol and 4-propenyl-2.5 dimethoxylphenol (Rocha-Meneses *et al*., 2017). It has a covalent bond with hemicellulosic xylans which results in high level of compactness and rigid nature of lignocellulose (Hendriks and Zeeman, 2009).Lignin binds all components of lignocellulose which makes it more hard to break and not soluble in water. It is the major hindrance to hydrolysis of lignocellulose by enzymes (Agbor *et al*., 2011).

2.4 Pretreatment of Lignocellulose

Pretreatment methods could be physical, physico-chemical, chemical, biological or combination. Physical can either be mechanical or non-mechanical. Autohydrolysis, steam explosion, sulphur IV oxide-added steam explosion and ammonia explosion are physico-chemical pretreatment methods. Chemical pretreatment could be acid hydrolysis, alkaline hydrolysis or organosolv process (Kumar and Sharma, 2017).

2.4.1 Physical Methods

2.4.1.1 Milling

This is one of the physical methods employed in pretreating lignocellulose. Milling alters the inherent ultrastructure of lignocelluloses and reduces the cellulosecrystallinity. Chipping could decrease biomass size to ten to thirty millimeters while milling and grinding could reduce its size to almost 0.2mm (Kumar and Sharma, 2017).Further reduction of biomass particles below 0.4mm do not have significant value on rate and output of hydrolysis(Kumar and Sharma, 2017).

There are different milling process, some like dissolver, fibrillator and colloid mill are ideal for only wet materials like wet paper from paper pulps or domestic waste separation. Other milling processes such as extruder, cryogenic mill, hammer milland roller mill are commonly utilized for milling dry biomass (Taherzadeh and Karimi, 2008). Vibratory milling is reported to perform better than traditional milling process in decreasingthe crystallinity of cellulose and improving digestibility of aspen and spruce chips (Kumar and Sharma, 2017).Another milling process is wet disk milling which requires low energy consumption and this attribute makes it a popular mechanical pretreatment method. Disk milling had better output than hammer milling because it aids hydrolysis of cellulose through fibers production (Zhua *et al*., 2009).

Milling has good effect on susceptibility of lignocellulose to enzymatic hydrolysis by reducing the degree of crystallinity and the size of the biomass (Taherzadeh and Karimi, 2008). Zeng *et al*. (2007) observed that non-pretreated corn stover with sizes 35-75µm was 150% more productive than bigger particles (425-710 µm) of corn stover. Taherzadeh and Karimi(2008) reported that better digestion of smaller particles for production of biogas however, size reduction combined with other methods of pretreatment would be more efficient.

2.4.1.2 Irradiation

Another physical method of pretreatment is irradiation.Treatment with irradiation can be through microwaves, electron beam and gamma rays which enhance hydrolysis of lignocelluloses by enzymes (Taherzadeh and Karimi, 2008). Combining radiation and other pretreatment methods like acid treatment will aid enzymatic hydrolysis and cellulose is enzymatically degraded to simple sugar by irradiation (Taherzadeh and Karimi, 2008).

Microwave irradiation is universallyutilized for lignocellulose pretreatment due to the following. It has ability to degrade cellulose structure, it has low inhibitors generation, great heating capacity within a brief period, low required energy and it is easy to operate(Kumar and Sharma, 2017).

Sonication is a feasible pretreatment option which is comparably a new approach that is used for physical pretreatment of lignocellulose(Kumar and Sharma, 2017). Ultrasound waves have chemical and physical effect on the structure of lignocellulose (Kumar and Sharma, 2017). Small cavitation bubbles are formed when lignocellulose is treated with ultrasound which break the hemicellulose and cellulose part. This will boostaccess of cellulose-degrading enzymes to the material for efficient disintegration of cellulose into simple sugars like glucose and others(Kumar and Sharma, 2017). Ultrasound is employed for pretreating substrates in production of biogas (Taherzadeh and Karimi, 2008). Pretreatment with ultrasound could be influenced by many factors. Some of these factors are solvent used, reactor type and itsgeometry, ultrasonic duration andfrequency, biomass characteristics, reactor configurationand kinetics (Bussemaker and Zhang, 2013).

2.4.1.3 Pyrolysis

Pyrolysis is defined as a thermal disintegrationtechnique where lignocellulose was exposed to and treated with high temperature of about 500-800 °C with no agent of oxidation (Kumar and Sharma, 2017). This method has been employed in the pretreatment of lignocellulose for production of bio oil but little report is available on the production of reducing sugar using pyrolysis (Kumar and Sharma, 2017). It can be grouped into two – slow and fast pyrolysis – subject to the heating rate. Reaction parameters, biomass characteristics and type of pyrolysis affect the amount of each end product. Pyrolysis is more effective whenexecutedat lower temperature in the presence of oxygen (Kumar *et al*., 2009).

2.4.1.4 Pulsed-Electric Field

Biomass are subjected to rapidexplosion of high voltage within 5.0-20.0KV/cm for brief period – nanoseconds (ns) to milliseconds (ms)in this pretreatment method (Kumar and Sharma, 2017). Requirement of low energy because the treatment is executed at room

conditions and brief period of pulse time are advantages derived from pulsed-electric field pretreatment method (Kumar and Sharma, 2017). Methane production was increased by 80% and two fold when Salerno *et al*. (2009) applied pulse-electric field treatment onpig manures and wastes activated sludge respectively.

2.4.1.5 Mechanical Extrusion

This is the usualtraditional way of pretreating biomass. The biomass are exposed to heating above three hundred degree Celsiusunder shear stirring(Kumar and Sharma, 2017). The crystalline and amorphous cellulose in the biomass residues is disintegrated because of the unitedimpacts of the shearing force which is developed by the turning screw blades and high temperatures which are retained in the cylindrical container (Kumar and Sharma, 2017). This pretreatment methodneeds huge amount of energy that makes the method a cost demanding techniquewhich is difficult toimprove for both industrial and commercial goals (Zhu and Pan, 2010). The barrel temperature, screw speed, compression ratio and the kind of screw layouthave effect on pretreatment ofbiomass (Zheng and Rehmann, 2014). Mechanical extrusion pretreatment have significant effect on disintegration of holocellulose from lignocellulose feedstocks however it performs better in reducing sugar yields when used with other treatment methods.

2.4.2 Physico-Chemical Pretreatment

Pretreatments which involve both physical and chemical techniques are known as physico-chemical pretreatment.

2.4.2.1 Steam Explosion (Autohydrolysis)

Substantial attention has been received with steaming with or without explosion for pretreating lignocellulosic biomass for biogas and ethanol production (Taherzadeh and Karimi, 2008). It combineschemical and mechanical impacts. Lignocellulose is exposed to high pressure of about 0.70 to 4.80 Mpa with saturated steam at high temperatures of one hundred and sixty to two hundred and sixty Celsius for short period (seconds – minutes)thatresults in hydrolysis of biomass andrelease of hemicellulose (Kumar and Sharma, 2017). Steam enters into the biomass, expands the fiber resulting in incomplete

hydrolysis and improvingaccessibility of enzymes. Hemicellulose is removed with this pretreatment which then improves enzymatic digestion.Hemicellulose is disintegrated to xylose and glucose by acetic acid that was produced fromacetyl group of hemicellulose during pretreatment with steam explosion (Kumar and Sharma, 2017). Temperature, size of biomass, residence time and moisture content are the determinantswhich haveinfluenceon steam pretreatment (Rabemanolontsoa and Saka, 2016). Environmental friendly, no recycling cost, low energy requirement and limited uses of chemical are advantages of autohydrolysis(Kumar and Sharma, 2017).

2.4.2.2 Liquid Hot Water

This pretreatment techniqueis also referred to as hot compressed water. Elevated temperature of water within 170 to 230 °C is utilized with pressure of about 5 MPa rather than usingsteam for pretreatment of biomass. This process removes lignin, hydrolyses hemicelluloseand exposes cellulose toenzymatic hydrolysis(Kumar and Sharma, 2017).Inhibitors are not produced at elevated temperature (Yang and Wyman, 2004). Aquasolv, aqueous fractionation, hydrothermolysis and solvolysis are various namesused by different researchers to term liquid hot water (Agbor *et al*. 2011).This pretreatment can be carried out by three ways relying upon the orientation of flow of the biomass and water into the reactor. The first way is co-current pretreatment where heat is applied to water and slurry of the biomass until it attains the prescribed temperature and maintains condition for the specific period before it cools. The second way is counter current pretreatment where heated water is injected against biomass under a controlled condition. The third way is flow through pretreatment in which the biomass performs similar to stationary bed and heated water flows over the biomass which carries the disintegrated biomass away through the flow (Kumar and Sharma, 2017).

2.4.2.3 Ammonia-Based Pretreatment

Soaking aqueous ammonia (SAA), Ammonia recycle percolation (ARP) and Ammonia fiber explosion (AFEX) are different techniques that employ liquid ammonia for pretreating lignocellulose(Kumar and Sharma, 2017). ARP is executed at elevated temperature whereas AFEX is executed at ambient temperature (Agbor *et al*., 2011). SAA is a type of AFEX where biomass is treated with aqueous ammonia within 30-60 °C in a

batch reactor reducing the liquid through-put during pretreatment(Kim and Lee, 2005). Liquid ammonia and lignocellulosic biomass are mixed in ratio 1:1 and heated in a closed system at 60 to 90°C and pressure above 3MPa for thirty to sixty minutes in AFEX (Kumar and Sharma, 2017). One of the major differences between ammonia-based and steam explosion is the utilization of ammonia in ammonia-based in place of water in steam explosion during pretreatment process (Rabemanolontsoa and Saka, 2016).

2.4.2.4 Carbon Dioxide (CO_2)Explosion

Supercritical carbon dioxidegas is used to carry out pretreatment of biomass wherecarbon dioxide gas behaves like a solvent (Kumar and Sharma, 2017). The gas is transferred through a pressurized vessel that containsthe lignocellulose (Kim and Hong, 2001). The released pressurized gas disrupts the lignocellulose and increases its surface area (Zheng *et al*., 1995). This technique of pretreatment is not appropriate for lignocellulose that do not contain moisture content because the hydrolytic yield is directly proportional to the moisture content (Kim and Hong, 2001). Some of the advantages of carbondioxide explosion are low temperature requirement, environmental acceptability, easy recovery after extraction, non-flammability, availability at relatively low costand non-toxicity (Kumar and Sharma, 2017).

2.4.2.5 Sulphite Pretreatment

This is another pretreatment method which break down lignocellulose and it is popular and efficient (Xu *et al*., 2016). It is executed in two steps. In the first step, the lignocellulose is treated with either magnesium or calcium sulphite to take out the lignin and hemicellulose parts. The size of the pretreated lignocellulosedecreased significantly with the use of mechanical disk miller in the second step (Kumar and Sharma, 2017). Sulphite pretreatment has capacity to process various lignocelluose and has effective scalability for industrial and commercial productionsthrough modifying thecurrentmills for biofuels production. Specific issues like huge price of getting back pretreatment chemicals, demand of large volume of water utilized for washing afterpretreatment and sugar degradation need to be looked into in order to make sulphite pretreatment a low cost pretreatment technology (Bajpai, 2016).

2.4.2.6 Wet Oxidation

Wet oxidation is a physicochemical technique of biomass pretreatment in whichoxygen/air with hydrogen peroxide or water is used to treat lignocellulose at elevated temperature greater than one hundred and twenty degree Celsius for thirty minutes (Varga *et al*., 2003). Pressure, oxygen, reaction period and temperature are the determinants of wet oxidation effectiveness. Water acts in similar way as acid which then catalyzes hydrolytic reaction attemperature beyondone hundred and seventy degree Celsius. During wet oxidation, cellulose is the least affected but lignin is oxidized while hemicellulose issplitto pentose monomers (Kumar and Sharma, 2017). Combustible characteristic of pure oxygen and high price of hydrogen peroxide will make this pretreatment techniquerarely reach industrial and commercial scale for treating lignocellulose.

2.4.3 Chemical Pretreatment

2.4.3.1 Dilute Acid

This pretreatment method is the most commonly used conventional method of breaking down lignocellulose. Large amount of inhibitory product likealdehydes, phenolic acids, 5-hydroxymethylfurfural and furfurals observed during pretreatment makes it less attractive (Kumar and Sharma, 2017).Still, it is the most universally pretreatment method that is used in the industries. The toxic and corrosive effect of acid require the reactor to be made of materials that will be able to withstand the corrosive effect of acid. Acid pretreatment method can be differentiated into two based on the type of end application. The first islow temperature (less than 120 °C) for period of thirty to ninety minuteswhile the second is high temperature at over 180 °C in one to five minutes(Kumar and Sharma, 2017).In some cases, there might not be need for enzymatic hydrolysis after acid pretreatment because dilute acid alone hydrolyses lignocellulose to fermentable sugars. There is need for excessive washing of the residual acid before fermentation. There are variouskinds of reactors that have been built for pretreatment with dilute acid like counter current reactors, flow-through, batch, shrinking-bed, plug flow and percolation (Kumar and Sharma, 2017). Many acid have been utilized for pretreatingbiomass such as sulfuric acid, maleic acid and oxalic.

2.4.3.2 Mild Alkali

Hydroxyl derivatives of ammonium, calcium, potasium and sodiumsalts are the usualfrequently utilizedalkali reagents for pretreating lignocellulosic biomass (Kumar and Sharma, 2017). The most efficient among hydroxyl derivatives method of pretreatment is sodium hydroxide (Kumar and Wyman, 2009). Degradation of glycosides and esters's side chainsare done by alkaline reagents which led to hemicellulose solvation, cellulose decrystallization, cellulose swelling and structural modification of lignin (Cheng *et al*., 2010; McIntosh and Vancov, 2010; Ibrahim *et al*., 2011; Sills and Gossett, 2011). Pretreatment with alkali could be executed at room temperature and pressure (Kumar and Sharma, 2017). Acid pretreatment solubilizes hemicellulose and cellulose than alkali pretreatment. The conditions of pretreating with mild alkali are less harsh than other methods of pretreatment specifically acid pretreatment.Disruption of lignin structure, reduction in the degree of crystallinity and polymerization and increase in the surface areas of cellulose boost the solubility of the biomass (Taherzadeh and Karimi, 2008). Pretreatment with akali has been utilized for pretreatinglignocellulosefor ethanol (Nadeem *et al*., 2015) and biogas (Taherzadeh and Karimi, 2008)production. During alkali pretreatment, various uronic acid and acetyl which reduce accessibility of hemicellulose and cellulose are eliminated(Chang and Holtzapple, 2000).

2.4.3.3 Organosolv

This is a technique of removing lignin with different degree of simultaneous solubilization. Aqueous or organic solventwith or without acid, alkali or salt catalysts is utilized in breaking lignin content and solubilizing hemicellulose of biomass (Bajpai, 2016).Biomass and catalyst are the determinants of the temperature utilized for organosolv. The temperature could be as high as 200 °C (Kumar and Sharma, 2017). Organosolv is used to extract lignin from biomass leaving behind hemicellulose syrup of 5-carbon and 6-carbon sugars and fraction ofcellulose. Agbor *et al*. (2011) reported that elimination of lignin from lignocellulosic biomass makes fibers open for enzymatic hydrolysisresulting inbetter conversion of lignocellulosic biomass to reducing sugar.Various determinants such as catalystused, reaction time, temperature and solvent concentration have effect on physical features of pretreatedlignocellulosic biomass like

crystallinity, degree of cellulose polymerization, fiber length and others (Kumar and Sharma, 2017).

2.4.3.4 Ozonolysis

Ozonolysis involves treatment of lignocellulose with ozone. This pretreatment method is primarily utilized for decreasing the percentage of lignin in lignocellulose. It primarily breaks lignin but have negligible impact on cellulose and hemicellulose contents of lignocellulose (Kumar *et al*., 2009).Ozonolysis is executed at ambient temperature and pressure. No toxic inhibitors is produced during ozonolysis which makes it environmental friendly and has no effecton post pretreatment techniquessuch as hydrolysis byenzymes and yeast fermentation (Quesada *et al*., 1999). High volume of ozone is needed for ozonolysis and this makes it costly and less attractive for industrial and commercial use (Kumar and Sharma, 2017).Concentrations of ozone, particle sizes and moisture contents are the majorfactors that affect ozonolyis pretreatment (Taherzadeh and Karimi, 2008).

2.4.3.5 Ionic Liquids

It is a chemical pretreatment method that has attracted great considerationin past ten years for pretreating biomass(Kumar and Sharma, 2017). A new kind of solvents known as ionic liquid consisting of ions (anions and cations) have high polarities, high thermal stabilities, low melting points (less than 100 °C), and negligible vapor pressure (Zavrel *et al*., 2009; Behera *et al*., 2014). Imidazolium is the most usually utilized ionic liquids(Kumar and Sharma, 2017). Considerable quantity of cellulose can be dissolved at reasonable moderate conditions and practicability of recovering one hundred percent of ionic liquids used make its use attractive(Taherzadeh and Karimi, 2008). Biodegradability, low viscosity, low hydrophobicity, low toxicity, vastchoice of combination of cation and anion, low volatility with potential minimal environmental impact, high reactionrates,non-flammable property, thermal stability and enhanced electrochemical stability are the advantages ionic liquids have over regular volatile organic solvents (Taherzadeh and Karimi, 2008).

2.4.4 Biological Pretreatment

This pretreatment technique is known as low-energy, competent and environmental safe technique when compared with traditional physical and chemical pretreatment techniques (Kumar and Sharma, 2017). Biological pretreatment are carried out by microbes like brown, soft and white rot fungi which are capable of breakinghemicellulose, lignin and little amount of cellulose. It has been reported that white rot fungi are part of the most efficient in biological pretreatment of lignocellulose (Sun and Cheng, 2002). Degradation of lignin by white rot fungi is due to laccases, peroxidases produced by these set of fungi (Kumar *et al*., 2009). *Phanerochaete chrysoporium*, *Pleurotus ostreatus*, *Pycnoporus cinnarbariunus*, *Cyathus stercolerus*, *Ceriporiopsis subvermispora* and *Ceriporia lacerate* are white rot fungi species that are utilized in pretreating lignocellulose (Kumar and Sharma, 2017). Some of the advantages of this pretreatment are mild environmental conditions, low energy requirement and no chemical requirement. The rate at which biological pretreatment degrades lignocellulose is too slow (Sun and Cheng, 2002).

Biological pretreatment is not used for lignin removal alone, it can be used to remove other specific components like antimicrobial substances (Taherzadeh and Karimi, 2008).Microorganisms and their enzymes are employed to disintergrate lignin and change lignocellulose structures in biological pretreatment. White rot fungi produce various enzymes that are connected to removal of ligninolytic enzymes like laccase, versatile peroxidase, manganese peroxidases and lignin peroxidases (Wong, 2009). With these enzymes produced by white rot fungi, these organisms can be used in producing biogas, bioethanol and enzymes.

2.4.4.1 White-Rot Fungi

The three major classes of fungi that are engaged in the biodegradation of wood are soft-rot, brown-rot and white-rotfungi (Isroi *et al*., 2011).Soft-rot fungi are Ascomycetes while brown-rot and white-rot fungi are Basidiomycetes (Hatakka, 2001; Isroi *et al*., 2011).A white fibrous appearance is left on wood degraded by white-rot fungi.

There are two types of white-rot fungi which are selective white-rot fungi and non-selective white rot fungi (Hatakka and Hammel, 2010). Selective white rot fungi degrade

lignin in woody plant cell walls relatively to a higher extent than cellulose. Lignin and hemicellulose components of lignocellulose are selectively degraded by selective white rot fungi while the cellulose part is essentially unaffected. Selectivity of white-rot fungi relies upontemperature, cultivation time, wood species and many other determinants (Hatakka and Hammel, 2010). Non-selective white-rot fungi degrade all components of the wood relatively equally.In non-selective degradation, white rot fungi degrade approximated equal amount of all fractions of lignocellulose (Isroi *et al*., 2011).

Different enzymes like lignases (for breaking lignin), cellulases (for breaking cellulose) and xylanases with other hemicellulases (for breaking hemicellulose) are produced by white rot fungi. (Isroi *et al*., 2011).

2.4.4.2 Combined Biological Pretreatment

Longer period of pretreatment than physical/chemical pretreatment and loss of polysaccharides are the principalsetbacks of biological pretreatment of lignocelluloses. Biological pretreatment could be combined together with other pretreatment method (chemical/physical) in order to reduce loss of polysaccharides, duration pretreatment and to have better yield of fermentable sugar (Isroi *et al*., 2011). Combining another pretreatment method with biological method of pretreatment is efficient than single pretreatment method (Kumar and Sharma, 2017).

2.5 Hydrolysis

This is the breaking of hemicellulosicand cellulosic parts of lignocelluloseto simple sugars prior to fermentation to bioethanol. The two most usually used methods of hydrolysis are chemical and enzymatic hydrolyses. There are some other hydrolyses in which no chemical or enzymes is involved. Cellulose are hydrolysed to glucose while xylose, galactose, mannose, glucose, acetic acid are released during hydrolysis of hemicellulose. Lignin is hydrolysed to phonolics (Garima *et al*., 2015).Three enzymes needed in hydrolysing lignocellulose are cellulases, hemicellulases and ligninases.

2.5.1 Cellulases

Cellulases that are engaged in converting cellulose to fermentable sugar are of three types which are endoglucanases, exoglucanases, and β-glucosidase. Endoglucanases are also known as carboxymethyl cellulases and they attacked at randomnumerous internal sectionsin amorphous parts of cellulose fibre. Exoglucanases are also referred to as cellobiohydrolases and they are the major part of cellulase which hydrolyse crystalline cellulose. β-glucosidase liberate glucose from cellobiose (El-Naggar *et al*., 2014). Synergistic activities occur when the three types of cellulase act together on cellulolytic materials (Fariq, 2016).Factors that could influence cellulase production included initial pH, inoculum size, nitrogen source, carbon source, incubation temperature and agitation (Fariq, 2016).

Cellulase are used in textile industry, foods and animal feeds, paper and pulp industry, biofuel, wine and brewery industry (Sirohi *et al*., 2018). Cellulase are utilized in producing faded look and softness in bio-stoning of denim garments (Sirohi *et al*., 2018). They help releasing indigo dye utilizedin coloring fabric when they act on cellulose fiber and produce faded view of denim. They are used in detergents and are important in extracting olive oil, producing purees, inextracting and clarifying fruit juices fromvegetables. Malting in beer production and winery is improved by the addition of cellulase. Cellulase is used to boost drainage and run-ability of paper mills, and biochemical pulping for adjustment of the coarse mechanical pulp and hand sheet strength de-inking of recycled fibers (Sirohi *et al*., 2018).

Use of lignocellulosic wastes for producing biofuel is the most vital current application of cellulase. Cellulase converts the cellulosic substrates to simple and fermentable sugars that could later be fermented to bioethanol and other products. Utilization of pure enzyme in converting biomass to fermentable sugar and ethanol is not economical because of huge cost of commercial cellulase. Cellulase isused in treating phytobezoars disease whichis responsible for concretion of indigestible fruit fibers and vegetable in gastrointestinal tract which could result in surgical intrusion (Fariq, 2016).

Genera of bacteria that are well-known for production of cellulase are *Bacillus*, *Cellulomonas*, *Cytophaga*, *Acetivibrio*, *Ruminococcus Cellvibrio*, *Microbispora*, *Pseudomonas*, *Acidothermus*, *Clostridium*, *Rhodothermus*, *Streptomyces* and

Thermomonospora (Fariq, 2016; Singh *et al*., 2016; Obeng *et al*., 2017). Fungi genera with cellulolytic abilities are *Phenerochaete*, *Trichoderma*,*TalaromycesPenicillium*, *Neorospora*, *Melanocarpus*, *Humicola*, *Fusarium*and *Aspergillus*(Fariq, 2016; Singh *et al*., 2016).Juturu and Wu (2014) explained that cellulases are usuallyextracellular with aggregated structures fastened to the bacteria cells. *Trichoderma reesei*, *Aspergillus niger*, *Aspergillus fumigates* are well recognized filamentous fungi that produced cellulase (Magrey *et al*., 2018).

2.5.2 Hemicellulases

Hemicellulases liberate simple sugar from hemicellulose. Hemicellulolytic enzymes include α-galactosidases, endo-arabinases, galactanases, exo-1,4-β-D-xylosidases, endo-1,4-β-D-xylanases, α-glucuronidases, α-L-arabinofuranosidases, β-mannosidases and endo-1, 4-β-D-mannanases (Jorgensen *et al*., 2003; El-Naggar *et al*., 2014).

Xylan, the most abundantpart of hemicellulose can be converted to fermentable sugar (Malhotra and Chapadgaonkar, 2018). Many xylanolytic enzymes perform synergistically in hydrolyzing xylan to simple sugarcompletely. Xylanases are formed by varieties of organisms like bacteria, yeasts, molds, insect, crustaceans, snails, marine algae, seeds and protozoans (Kumar *et al*., 2017). Xylanases are commercially important because of their involvement in the degradation of lignocellulose and agricultural wastes to simple sugar and other high-value products (Malhotra and Chapadgaonkar, 2018).

Production of xylanase by the following organisms has been reported *Bacillus subtilis* B230 (Oakley *et al*., 2003), *Bacillus circulans* (Pithadiya *et al*., 2016), *Bacillus pumilus* SY30A (Bakri *et al*., 2016), *Trichoderma* species (Pandey *et al*., 2014; Gomez-Garcia *et al*., 2018; Oyedeji *et al*., 2018),*Pleurotus ostreatus*, *Coprinus comatus* and*Agaricus brunnescens* Peck (Huang *et al*., 2019), *Neurospora intermedia* (Shahryari *et al*., 2019), *Penicillium chrysogenum* (Terrone *et al*., 2018), *Streptomyces turgidiscabies* C56 (Maehara *et al*., 2018), *Aspergillus* species (Cunha *et al*., 2018; Oyedeji *et al*., 2018) and *Fusarium* species (Shankar *et al*., 2018).

Xylanases are applied in pulp and paper industry, lignocellulose degradation, baking industry, fruit juice clarification and animal feed industry (Malhotra and Chapadgaonkar,

2018). It is involved in the depolymerizing carbohydrate polymer to give free sugars and other value added product. Water insoluble hemicelluloses are converted to water soluble forms by xylanase. The use of xylanase in most baking industries could reduce utilization of chemical additives like bromate in baking industries (Kulkarni *et al*., 1999; Malhotra and Chapadgaonkar, 2018). Xylanase can be used to separate wheat starch and gluten which will result in efficient coagulation of gluten. Presence of polysaccharides likelignin, hemicellulose, cellulose, pectin, and starch in extracted fruit juice make fruit juice turbid and viscous. Use of xylanase and other enzymes improve organoleptic characteristics of fruit juices (Kumar *et al*., 2014; Malhotra and Chapadgaonkar, 2018).

Xylanase catalyzes the hydrolysis of α 1, 4 glycosidic linkage of xylosidase, forming free aglycone non sugars and a hemiacetal sugar by replacing it with hydrogen bonds (Selvarajan and Veena, 2017). Xylanase can be groupeddifferently depending on their crystal structure, kinetic properties, isoelectric point and molecular weight(Selvarajan and Veena, 2017). Exoxylanase and endoxylanase are the two major types of xylanase activities. Endoxylanases shows preference for internal xylan bonds while exoxylanases show priority for side groups at xylan chains terminals (Selvarajan and Veena, 2017).

2.5.3 Ligninases

Ligninases are enzymes that degrade lignin. Laccases (phenol oxidases),versatile peroxidases,lignin peroxidasesand manganese peroxidases are major examples of lignin degrading enzymes (Isroi *et al*., 2011). Lignin is hard to degrade when compared with hemicellulose and cellulose. Lignin degrading enzymes play major role in degrading lignin (El-Naggar *et al*., 2014). These enzymes that degrade lignin are synthesized by microorganisms especially white rot fungi (Isroi *et al*., 2011).Incubation time, inoculum type and size, pH, nitrogen sources, carbon sources,incubation temperature and agitation are major factors that could affect production of lignolytic enzymes (Kumar and Chandra, 2018; Vandana *et al*., 2018).

Laccases are oxidases that contain blue copper. They catalyse oxidations of phenolic compounds and aromatic amine of lignin. Laccase is produced by most white rot fungi (Wong, 2009; Isroi *et al*., 2011). Yang *et al*. (2017) considered*Trametes versicolor*and *Pleurotus ostreatus*as the model organisms in basic and applied laccase studies. Other

well-recognized laccase-producing organisms are *Cryptococcus*, *Agaricus*, *Coriolopsis*, *Cerrena*, *Fomes*, *Cyathus*, *Ganoderma*, *Panus*, *Pycnoporus*, *Phlebia*, *Rigidoporus*, *Schizophyllum*, *Polyporus* (Baldrian, 2006; Arora and Sharma, 2010, Forootanfar and Faramarzi, 2015; Yang *et al*., 2017). Non-phenolic sub-structure of lignin is oxidized by laccase using mediator of low molecular weight.Phenoxyl radicals' formation is catalyzed by laccase (Isroi *et al*., 2011). Occurrence of laccase is well spread in nature.

The porphyrin ring of lignin peroxidases contains iron with lower electron thantraditional peroxidases and this makes lignin peroxidases strong oxidant (Isroi *et al*., 2011). *Trametes cervina*, *T. versicolor*, *P. chrysosporium*and*Bjerkandera* species are some white rot fungi with ability of producing lignin peroxidase (Isroi *et al*., 2011; Miki *et al*. 2011; Vandana *et al*., 2018).

Manganese peroxidase catalyses Mn-dependent reaction and are more widespread than lignin peroxidase (Wong, 2009; Isroi *et al*., 2011). *P. chrysosporium*, *Pleurotus ostreatus*, *Trametes* species, *Klebsiella* species, *Enterobacter* speciesand *Salmonella* species produced manganese peroxidase (Isroi *et al*., 2011; Gaur *et al*., 2018; Kumar and Chandra, 2018). Manganese peroxidase contains 357 amino acid residues, of heme (one molecule),calcium ions (two structures), 478 solvent molecules, one substrate of manganese (II) ions with three residues of sugar (Isroi *et al*., 2011).

Versatile perosidases oxidise aromatic compounds (phenolic and non-phenolic) and manganese (II) ions. Versatile peroxidase are found in *Pleurotus* and *Bjerkandera* species. The catalytic mechanism of versatile peroxidase are similar to lignin peroxidase and oxidize Mn^{2+} to Mn^{3+}. Veratrylglycerol β-guaiacyl ether, a nonphenolic model of lignin is degraded by versatile perosidase (Isroi *et al*., 2011).

2.6 Morphology and Physiology of Fungi

Morphology is the science of the form of things (Kossen, 2000). It starts with classification of objects based on their forms and the different forms of filamentous fungi are hyphal element, flock and pellet (Kossen, 2000). Hyphal element is made up of a single hypha (single thread), usually with a number of branches, branches of branches

from a single spore. A loosely packed, temporary agglomerate of hyphal elements is called a flock and a pellet is a dense of hyphae.

Fungal physiology is known as the nutrition, metabolism, growth, reproduction and death of fungal cells (Walker and White, 2018). It also refers to the way fungi relate with their biotic and abiotic environment. Fungal physiology is affected by several environmental factors such as carbon and nitrogen source, presence of oxygen, temperature, incubation time, pH and others (Walker and White, 2018). Fungal physiology has significant impact on human health, industrial processes and the environment. Fungi participate in the cycling of carbon as primary decomposers of organic matters. Fungal metabolism is responsible for bioremediation of heavy metals and various recalcitrant chemicals in the environment and is in detoxification of organic pollutants (Walker and White, 2018). Morphology and physiology of fungi have significant effect on their production efficiencies (Kossen, 2000; Walker and White, 2018).

Filamentous fungi are used for production in several industrial processes (Veiter *et al*., 2018). Many scientist initially thought that these organisms could only grow on solid surface but later got to know that they can grow in submerge media. Filamentous fungi grow in their natural environment in long, branched form referred to as hyphae which is ideal for the survival of these organisms. The hyphae form is a nuisance in submerged culture because it gives the medium high viscousity and interfares with oxygen, carbondioxide and nutrients available in the medium which results in low productivity (Kossen, 2000).

Fungi are taxonomically grouped as oomycetes (e.g *Pythium oligandrum*), chytridomycetes (e.g. *Batrachochytrium dendrobatisdis*), zygomycetes (e.g. *Dimargaris arida*), basidomycetes (e.g. *Agaricus bisporus*), ascomycetes (e.g. *Saccharomyces cerevisiae*) and deuteromycetes (e.g. *Aspergillus niger*). The elemental fungal cells requirements are carbon, hydrogen, oxygen, nitrogen, phosphorus, potassium, magnesium, sulphur, calcium, copper, iron, manganese, zinc, nickel and molybdenum in different concentrations (Walker and White, 2018).Fungi derived their energy from breaking down large organic molecules to simple molecules. Most fungi breakdown large molecules using extracellar enzymes produced by the organism.Hydrodynamic conditions

generated during cell culture in fermenters have effect on morphology and metabolism of cells (Serrano-Carreon *et al*., 2015). Mushroom develops from pinhead or nodule referred to as primordium. And thier fruiting bodies live for a short period of time. While some are edible, others are toxic.

Yeasts have different cell shape like ellipsoid, dimorphic, hyphal, pseudohyphal, flask-shaped, miscellaneous, ogival, apiculate and cylindrical (Walker and White, 2018). They are unicellular that reproduce asexually by budding or fission. *Saccharomyces cerevisiae*, commonly referred to as baker's or brewer's yeast is generally ellipsoid in shape with diameter in the range of 5 to 10 micro meter (Walker and White, 2018). Yeasts have great diversity in cell shape and mode of reproduction. There is diversity in the morphology of yeast when grown on agar in terms of colour, texture, and geometry (contours and peripheries) of giant colonies. Many yeasts are pigmented on agar. *Saccharomyces cerevisiae* is cream while*Geotrichum candidum* is white and*Aureobasidium pollulans* is black. *Rhaffia rhodozyma* is pink while*Rhodotorula rubra* is red and*Cryptococcus laurentii* is yellow in colour

2.7 Fermentation

Different fermentation processes (cofermentation and consolidated bioprocessing,simultaneous saccharification,simultaneous saccharification and fermentation and,separate hydrolysis and fermentation) are involved in the conversion of lignocellulose to ethanol (Phwan *et al*., 2018). Microorganism convert sugar in the hydrolysate to ethanol. Several factors influence production of bioethanol. Some of these factors are incubation time, pH, temperature, inoculum size, sugar content, nitrogen source and source of substrates (Nadeem *et al*., 2015). Temperature influences microbial growth. Cells of *Saccharomyces cerevisiae* have optimal temperature of 30 °C for ethanol production (Nadeem *et al*., 2015). The initial fermentation pH is very important during ethanol production. Optimum pH for producing ethanol is from 4.0 to 5.5. Staniszewski *et al*. (2007) reported that when pH is less than 4.0, a longer fermentation time is needed however it did not significantly affectethanol production but a pH of more than 5.0 led to reduction of ethanol content that was produced. Nadeem *et al*. (2015) obtained optimum production of ethanol at pH of 5.5. Increase in inoculum size within specific range

decreases fermentation period as the cell grow very quickly and convert the sugar to ethanol (Zabed *et al*., 2014). Increasing the sugar concentration to specific level results in increase in ethanol content (Azhar *et al*., 2017).Many microorganisms have been used for production of ethanol which can be categorized into four groups as bacteria, yeast, filamentous fungiand mushroom (Gamage *et al*., 2010; Mori *et al*., 2019).

2.7.1 Acoholic Fermentation

Alcoholic fermentation begins with the breakdown of a molecule of glucose to two molecules of pyruvate in the cytoplasm through glycolysis. Glycolysis is the metabolic pathway through which glucose is breakdown to pyruvate. Conversion of glucose to pyruvate occur through any of Embden-Meyerhof, Etner-Doudoroff and pentose phosphate pathways (Willey *et al*., 2017). These three pathways are referred to as glycolytic pathway or glycolysis and they all produced pyruvate. The most common of all these pathways involved in breakdown of glucose to pyruvate is Embden-Meyerhof pathway. Embden-Meyerhof pathway has two phases which are 6-carbon and 3-carbon phases. In 6-carbon phase, glucose is phosphorylated twice by adenosine triphosphate to give fructose 1, 6-bisphophate. Organism sow energy in form of ATP in this phase. The 3-carbon phase starts when fructose 1, 6-bisphosphate is broken down to dihydroxyacetone phosphate and glyceraldehyde 3-phosphate. Dihydroxyacetone is immediately converted to glyceraldehyde 3-phosphate. Glyceraldehyde is converted to pyruvate through five-step process.The yields of NADH and ATP in Embden-Meyerhof pathway can be calculated. Two ATP and two NADH are generated from conversion of one molecule of glucose to two molecules of pyruvate (Willey *et al*., 2017).

$$\text{Glucose} + 2\text{ADP} + 2\text{Pi} + 2\text{NAD} \xrightarrow{\text{(Glycolysis)}} 2\ \text{Pyruvate} + 2\ \text{ATP} + 2\text{NADH} + 2\text{H}^{+}$$

This is followed by conversion of pyruvate to acetaldehyde by pyruvate decarboxylase. The activities of pyruvate decarboxylase is depended on the help of coenzymes thiamine pyrophosphate and magnesium (Kutter *et al*., 2009; Pires and Branyik, 2015). Alcohol dehydrogenase later converts acetaldehyde to ethanol (Pires and Branyik, 2015). Pyruvate

decarboxylase is activated by phosphorylation when yeast are exposed to high glucose content. Pyruvate decarboxylase has it highest activity at logarithm phase when the organism is doubling itself but its activity decreases when glucose is exhausted (Pires and Branyik, 2015). Alcohol dehydrogenase is used by yeast to either form ethanol or degrade it. The expression of alcohol dehydrogenase is correlated to the amount of initial concentration of sugar that is present in the fermentation medium and temperature at which the sugar is fermented (Saerens *et al*., 2008; Pires and Branyik, 2015).

Pyruvate ----------> Acetadehyde ----------> Ethanol
(Alcoholic fermentation)

2.7.2 Organisms Used in Fermentation

There are criteria that microorganisms to be considered for industrial production of bioethanol from lignocellulose must meet. They have to show high fermentative activity of pentoses and hexoses with high ethanol yield. They should be resistant to environmental stress (such as high temperature and others) and inhibitors formed during industrial production (especially, 5-hydroxymethyl furfuraland furfural) (Robak and Balcerek, 2018). They should be able to grow fast on various substrates of lignocellulose and suitable for genetic manipulation. Fermentative microorganisms may be isolated from various sources like palm wine, distillery waste and others (Robak and Balcerek, 2018).

2.7.2.1 Bacteria

Examples of bacteria that have been used for production of ethanol are *Zymomonas mobilis*(Liu *et al*., 2017; Ferreira*et al*., 2018) and *Escherichia coli*(Sun *et al*. 2018). Some bacteria that have also been reported for production of ethanol are *Thermoanaerobacterium thermosaccharolyticum* (Pang *et al*., 2018), *Clostridium acetobutilicum*, *Clostridium thermocellum*(Hon *et al*., 2018), *Klebsiella oxytoca*(Sharma *et al*., 2018), *Klebsiella pneumonia* (Oh *et al*., 2011), *Lactobacillus pentoaceticus*(Gamage *et al*., 2010) among others.

Escherichia coli fermented glycerol to ethanol under aerobic condition (Pranata *et al*., 2018). Ethanol was produced by *Clostridium ragsdalei* through syngas fermentation (Patankar *et al*., 2018). Ethanol production from vegetable peels has been reported (Promon *et al*., 2018). Fruits wastes such as mango peels, papaya peels, pineapple peels and banana peels have been utilized for ethanol production (Pranata *et al*., 2018; Promon *et al*., 2018). Inoculum size, temperature, substrates, pH, nitrogen source and period of fermentation are parts of the conditions that affect production of ethanol by bacteria (Patankar *et al*., 2018; Sharma*et al*., 2018).

A Gram-negative bacterium, *Zymomonas mobilis*is efficient in converting glucose to ethanol and the process is rapid. *Zymomonas mobilis* is generally regarded as being safe when used for ethanol production (Gamage *et al*., 2010). Wild type *Zymomonas mobilis* is not appropriate for producing ethanol from lignocellulose because it ferments only sucrose, fructose and glucose (Robak and Balcerek, 2018). Many *Zymomonas mobilis* genetic strains which ferment five carbon sugar like arabinose and xylose to ethanol have been created. Conversion of arabinose and xylose to ethanol will improve the overall ethanol yield derived from lignocellulose (Gamage *et al*., 2010). Significant characteristics of *Zymomonas mobilis* is its resistance ability against up to one hundred and twenty gram per liter ethanol content, low nutritional requirement for growth and capacity of homofermentative production of ethanol (Robak and Balcerek, 2018).

Escherichia coli is commonly used in the industries and laboratories for genetic studies, protein expression and molecular biology (Gamage *et al*., 2010; Pranata *et al*., 2018). It does not require high growth factors. Wide range of sugars is fermented by *Escherichia coli*.Use of *Escherichia coli* for producing ethanol requires careful monitoring of culture conditions because it performs well within low range of pH and it has low tolerance to ethanol. Additionally, it produces mixtures of ethanol and other organic acid compounds. Wild type of *E. coli* does not ferment xylose but fermentation of xylose could be achieved through genetical manipulation of its genetic materials. There has been creation of a strain which prefers fermentation of pentose sugars to ethanol even when hexose sugars are present (Trinh *et al*., 2008; Gamage *et al*., 2010).

2.7.2.2 Yeasts

Yeast are basidiomycetous or ascomycetous fungi whose spores are not enclosed in a fruiting body and capable of reproducing by fission or budding. They are identified by their morphological, physiological and genetic characteristics (Azhar *et al*., 2017). Yeast are isolated from plants, animals, soil, water, atmosphere, drinks and foods. There is diversity of yeast cell like size, shape and colour. Genetic makeup and growth conditions influence the sizes of yeast cell. Azhar *et al*. (2017) observed that the size of brewing strains is larger than the laboratory size.

Bioethanol production by yeast is based on its ability to convert six carbon compoundlike glucose to two carbon molecules like ethanol with no further oxidation to final product (carbon dioxide). Yeast like*Saccharomyces cerevisiae* have been utilized for ethanol production in wine and brewery industries for long years. Yeasts are now utilized to convert renewable energy source such as lignocellulose to bioethanol (Azhar *et al*., 2017). *Kluyveromyces fagilis* (Kf1), *S. cerevisiae* (RL-11) and*Pichia stipitis* (NRRL-Y-7124) are some of the yeasts reported to have produced high yield of ethanol from reducing sugars (Mussato *et al*., 2012).*Pachysolen tannophilus* was used to produce ethanol using banana peels hydrolysate as substrate (Ferreira *et al*., 2018).Use of *Candida intermedia*, *Zygosaccharomyces bailii*, *Saccharomyces paradoxus* and*Kluyveromyces marxianus* for producing ethanol have also been reported (Perez-Cadena *et al*., 2018; Guilherme *et al*., 2019).

Saccharomyces cerevisiae is the traditionalutilized yeast in the production of ethanol in the industries because of it is able to withstand varying ranges of pH. Increase in temperature and ethanol content beyond 35 °C and 20 % respectively are some of the common difficulties experienced during ethanol production (Tofighi, 2014). Ability to ferment pentose sugar is another challenge of ethanol production. The traditionally used yeast (*Saccharomyces cerevisiae*) can only convert hexose (6-carbon sugar) to ethanol but not pentose (5-carbon sugar). Some yeasts that are able to ferment pentose to bioethanol are from genera *Pachysolen,Schizosaccharomyces*, *Pichia* and*Candida*(Mussato*et al*., 2012).

2.7.2.3 Filamentous Fungi

There are reports that filamentous fungi areengaged in the production of bioethanol. Millati *et al*. (2005) used*Rhizopus*, *Rhizomucor* and *Mucor* to produce ethanol. *Mucor indicus* has been used to produce ethanol (Asachi *et al*., 2011).

2.7.2.4 Mushroom

Production of ethanol by mushrooms has been recorded by some researchers (Mizuno *et al*., 2009; Mori *et al*., 2019). Mori *et al*. (2019) used a white rot fungus (*Phanarochaete sordida* YK-624) to ferment a six carbon sugar (glucose) to ethanol in the absence of oxygen. It has been reported that *Flammulina velutipes* has both lignocellulose degradation and ethanol fermentation abilities (Mizuno *et al*.,2009).

2.7.2.5 Recombinant Fermentative Microorganisms

Wild yeasts with other isolated microorganisms fail to meet the necessary requirements for bioethanol production industrially from lignocellulose despite diversity of fermentative microorganisms in the environment (Robak and Balcerek, 2018). The requirements include ability to grow in lignocellulosic hydrolysate, high tolerance to elevated temperatures and effective utilization of xylose to bioethanol (Li *et al*., 2015). Most wild microbes are unable to convert xylose and other pentose sugar to bioethanol and do not produce high yield of ethanol (Robak and Balcerek, 2018). Microorganisms are modified so as to boost ethanol production by improving theresistance of microorganisms to inhibitors that are produced during pretreatment,resistance to conditions of fermentation as well as their tolerance to high sugar and ethanol content (Lee and Kuan, 2015).

Modification of microorganisms increases the range of sugars that are utilized for ethanol production and makes production of ethanol cost effective (Achinas and Euverink, 2016). Progress has been made in the modification of microbes to utilize both hexoses and pentoses. A gene responsible for the utilization of a sugar to be added to the gene of a traditional ethanol-producing organisms like*Zymomonas mobilis*and *Saccharomyces cerevisiae*(Ragauskas *et al*., 2014). That is, genes created for metabolism of xylose are inserted into *Saccharomyces cerevisiae* or other organisms as the host. Genetic

manipulation has improved ethanol production by ethanol-producing bacteria like*Escherichia coli*and *Zymomonas mobilis*(Robak and Balcerek, 2018).

Use of genetic engineering to use mixed sugar simultaneous for production of ethanol have been attempted on *Saccharomyces cerevisiae*, *Clostridium cellulolyticum*, *Lactobacillus casei*, *Zymomonas mobilis*, *Klebsiella oxytoca* and*Escherichia coli* (Robak and Balcerek, 2018). Some of the methods for getting microorganisms with the ability of simultaneous utilizing xylose and glucose include mutagenesis (Robak and Balcerek, 2018), introduction of heterologous metabolic pathway for xylose utilization into ethanol production strains (Ko *et al*., 2016; Wilkinson *et al*., 2017) and introduction of cellulase-encoding genes into specific species (Kricka *et al*., 2014).

Rate of utilizing pentose to hexose for ethanol production by genetic engineered microbes is relatively low. Recombinant microbes prefer the use of hexose instead of pentose when present together for ethanol production (Robak and Balcerek, 2018). Pentoses and hexoses are not fermentedat the same time by recombinant yeasts but first utilize glucose only after which consummation of xylose occur which led to prolonged fermentation period and this means it is not economical (Oreb *et al*., 2012; Robak and Balcerek, 2018). Specific genetic engineering methods of fermenting microbes are required to co-ferment pentoses and hexoses in lignocelluloses to ethanol (Robak and Balcerek, 2018). Genetic engineering added to this sector but inadequacy in intermediate processes and efficient conversion of pentose to ethanolis still observed. There are still limitations to ethanol yields obtained from pentose when compared to hexoses (Robak and Balcerek, 2018).

2.7.3 Hydrolysis and Fermentation Strategies

2.7.3.1 Separate Hydrolysis and Fermentation (SHF)

This is a technique where enzymatic hydrolysis is carried out separately from fermentation. The enzymatic hydrolysis is first performed which is followed by fermentation. Ability to execute each step under optimum conditions is an advantages of SHF (Balat, 2011).Glucose released during hydryolysis inhibits some enzymes(β-glucosidase and cellulase) which is counted as the disadvantage of this method (Balat, 2011).

2.7.3.2 Simultaneous Saccharification and Fermentation (SSF)

Simultaneous saccharification and fermentation (SSF) combines hydrolysis of pretreated lignocellulose and fermentation of liberated sugar in a single step to ethanol. SSF needsconducivesaccharification and fermentation condition which means that anoptimum substrate concentration, temperature and pH is necessary for both saccharification and fermentation (Balat, 2011). Sun and Cheng (2002) described major advantages of SSF as reduction in enzyme requirement; shorter process period; increase in the rate of hydrolysis by converting the sugar hindering activity of cellulase immediately to ethanol; lower reactor volume; lower requirement for sterilization since released glucose is converted immediately to bioethanol without being removed from the reactor and higher yield of ethanol during production. Difference in the temperature of saccharification and fermentation is the major challenge of this method (Krishna *et al*., 2001).

2.7.3.3 Simultaneous Saccharification and Cofermentation (SSCF)

This process involves neutralization of pretreated lignocellulose and exposure of neutralized pretreated lignocellulose to enzymes and microbes that are able to hydrolyze holocellulose to fermentable sugar at the same time ferment pentoses and hexoses to ethanol in a single step. One of the main challenges in this method is that pentoses fermenting organisms prefer hexoses as substrate. There is always competition when pentose-fermenting organisms and hexose-fermenting organisms are together. Sequentially fermentation of hexose and pentose have been planned where hexose-fermenting microorganisms are first introduced and pentose-fermenting organisms are introduced after complete fermentation of hexoses in the same reactor. Production of ethanol in this sequential fermentation is low (El-Naggar *et al*., 2014).

2.7.3.4 Direct Microbial Conversion (DMC)

Direct microbial conversion is also called consolidated bioprocessing (CBP). This process combines production of cellulases, hydrolysis of cellulose by produced cellulases to glucose and fermentation glucose to bioethanol in one single step (Balat, 2011). This method is attractive because there is reduction in the number of reactors that are used and cost of chemicals are also reduced. There is no capital or operating cost allocated for

enzyme production with this method. Low bioethanol yields, low tolerance of microbes to ethanol and poor growth of microbes infermentation medium are the disadvantages of this single step of production of bioethanol from lignocellulose (Zaldivar *et al*., 2001).

2.8 Distillation

Distillation is used to recover ethanol from fermentation medium. Membrane separation, liquid extraction hybrid, ordinary distillation, extractive distillation, azeotrophic distillation and absorption are different separation techniques utilized in recoveringbioethanol from fermentation medium (Adekunle *et al*., 2016). Efficiency of hydrolysis and fermentation have great effect on the cost of distillation. The amount spent on distillation is in inverse proportion to ethanol concentration, the lower the concentration of ethanol the higher the cost of distillation (Farias *et al*., 2017).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Sample Collection

3.1.1 Collection of Lignocellulosic Wastes

All lignocellulosic biomass were collected from Oyo state (8.1196° N, 3.4196° E). Groundnut shell was collected from Saki,maize cob from Ajegunle, Oyo town while maize straw was collected from Okunlola's farm, Ilora. Sugar cane bagasse was obtained from Akunlemu, Oyo town andrice straw was collected from InternationalInstitute of Tropical Agriculture (IITA), Ibadan. All the samples were dried andmilled with a milling machine in Oyo town and taken to the Laboratory of Department of Biological Sciences, Ajayi Crowther University, Oyo town.

3.1.2 Collection of White Rot Fungi

Three white rot fungi (*Pleurotus ostreatus*, *Pleurotus tuber-regium* and *Lentinus squarrosulus*) were collected from Department of Botany, University of Ibadan, Ibadan. They were screened for their ability to produce cellulase, hemicellulase and lignase/laccase.

3.1.3 Yeast Collection

Yeasts were isolated from palm wine sample obtained from Akinmorin farm, Oyo State. It was fermented for 5 days and samples were taken every 24 hours. The samples were serially diluted and 1 mL of appropriate dilutions was introduced into a sterile Petri dish. Cool molten sterilized yeast extract agar supplemented with streptomycin and 2% glucose was added to the Petri dish and swirled clockwise and anticlockwise for even distribution. It was incubated at 28±2 °C for 48 hours. Distinct colonies were sub-cultured severally until pure cultures were obtained. Pure isolates were kept at 4 °C in a refrigerator for further use. About 0.5 g of commercial yeast was inoculated into 50 mL of sterilized yeast

extract peptone dextrose broth and incubated at 28±2 °C for 24 hours. Commercial yeast from yeast

extract peptonedextrose broth was streaked on yeast extract agar supplemented with 2 % glucose and incubated at 28±2 °C for 24 hour. A distinct colony of commercial yeast was sub-cultured in yeast extract agar (supplemented in 2 % glucose) slant and kept in refrigerator for further use.

3.2 Screening of White Rot Fungi for Enzymes Production

3.2.1 Screening of White Rot Fungi for Cellulase Production

Potato Dextrose Agar was prepared and supplemented with 1 % of Carboxyl Methyl Cellulose (CMC). It was sterilized at 121 °C and 1.05kg cm^{-2} for 15 minutes. It was dispensed into sterile Petri dish and allowed to solidify.Each plate was inoculated with *Pleurotus ostreatus*, *Pleurotus tuber-regium* and *Lentinus squarrosulus*separately and incubated at 28 ±2 °C for five days.There were five sets for each white rot fungus. Each set was taken every 24 hours,flooded with 2% (w/v) aqueous congo red and left for 15 minutes. Excess stain was poured off after 15 minutes, washed with distilled water and appearance of yellow-opaque area around colonies against a red colour for undegraded CMC indicates cellulase production (Pointing, 1999).Relative cellulase activities were determined by dividing the diameter of hydrolysed CMC by the diameter of organism.

3.2.2 Screening of White Rot Fungi for Xylanase Production

Xylan is the main component of hemicellulose. One percent (1% w/v) xylan was used to supplement potato dextrose agar and was sterilized at 121 °C and 1.05kg cm^{-2} for 15 minutes. It was dispensed into sterile Petri dish and allowed to gel. *Pleurotus ostreatus*, *Pleurotus tuber-regium* and *Lentinus squarrosulus* were inoculated into each plate separately and incubated at 28 ±2 °C for five days. There were five sets for each organism. Each set was taken every 24 hours and flooded with iodine stain (0.25% w/v aqueous I_2 and KI) and left for 5 minutes. Stain was poured off the plate after 5 minutes and was washed with distilled water. Appearance of yellow-opaque area against a blue/reddish purple colour shows xylanase activities (Pointing, 1999).Relative xylanase activitieswere determined by dividing the diameter of hydrolysed xylan by the diameter of organism.

3.2.3 Screening of White Rot Fungi for Lignase Production

Tannic acid agar was prepared by supplementing potato dextrose agar with one percent tannic acid. It was sterilized at 121 °C and 1.05kg cm^{-2} for 15 minutes. It was dispensed into sterile Petri dish and allowed to solidify. Each plate was inoculated separately with *Pleurotus ostreatus*, *Pleurotus tuber-regium* and *Lentinus squarrosulus* and incubated at 28 ±2°C. Growth and colour were observed every 24 hours for five days. A brown oxidation zone around colonies indicates lignin degradation (Pointing, 1999). Relative lignaseactivities were determined by dividing the diameter of degraded lignin by the diameter of organism.

3.2.4 Screening for Laccase Production

Laccase is one of the enzymes involved in lignin degradation.Potato dextrose agar was supplemented with 0.1% (w/v) of 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS). It was sterilized at 121 °C and 1.05kg cm^{-2} for 15 minutes, dispensed into sterilePetri dish, allowed to solidify and inoculated separately with *Pleurotus ostreatus*, *Pleurotus tuber-regium* and *Lentinus squarrosulus* and incubated at 28 ±2°C. The plates were observed every 24 hour for five days for development of green or purple coloration around the colonies indicating production of laccase (Pointing, 1999). Relative laccase activities was determined by dividing the diameter of oxidized ABTS by the diameter of organism.

3.3 Pretreatment of Lignocellulose with White Rot Fungi

The five lignocellulosic wastes (maize cob, maize straw, rice straw, groundnut shell and sugar cane bagasse) were pretreated with two selected white rot fungi (*Pleurotus ostreatus* and *Lentinus squarrosulus*). The two mushrooms were selected based on theirability to produce cellulase, xylanase, lignase and laccase. One hundred (100) gram of each lignocellulosic material were weighed into separate bottles and 300 mL of distilled water was added and mixed. They were sterilized at 121 °C and 1.05kg cm^{-2} for 15 minutes and allowed to cool. Each cooled biomass was inoculated separately with eight agar plugs (7mm in diameter) of*Pleurotus ostreatus* and *Lentinus squarrosulus* separately and consortium (4 agar plugs each of *Pleurotus ostreatus*and *Lentinus squarrosulus*)

(Adenipekun and Fasidi, 2005). They were incubated at 28 ±2°C for 70 days. Samples were taken from degrading substrate every 7 days and were analyzed for cellulose, hemicellulose, lignin, extractives and reducing sugar contents.

3.3.1 Determination of Extractives, Hemicellulose, Lignin and Cellulose

Solvent extraction (60 mL of acetone for 1 g of dried biomass sample) was used to determine the extractives in each biomass. The temperature was maintained at 56°C for 2 hours. The samples were later dried at 105 °C until a constant weight was obtained. The different in weight before and after extraction is the amount of extractives that is present in the biomass (Blasi *et al*., 1999; Lin *et al*., 2010).

Four (4) milliliters of 0.5mol/LNaOH was added to 0.4g of extractive-free dried biomass and maintained at 80 °C for three and half hours. The sample was washed with distilled water until the pH of the solution reach 7 and dried in hot air oven. The difference between the weight of the sample before and after treatment is the hemicellulose content (Blasi *et al*., 1999; Lin *et al*., 2010).

Twelve (12) milliliters of 98% suphuric acid was added to 0.4g of extractive-free dried biomass and left at room temperature for 24 hours. After 24 hours, the sample was diluted with 80 mL of distilled water and later boiled at 100 °C for 1 hour. The mixture was allow to cool, filtered and the residue was washed until there was no detectable sulphate ion in the filtrate. The residue was dried to a constant weight. The weight of the residue is the lignin content (Blasi *et al*., 1999; Lin *et al*., 2010).

The amount of cellulose was calculated by the difference assuming that the biomass are made up of only cellulose, lignin, hemicellulose and extractive (Blasi *et al*., 1999; Li *et al*., 2004;Lin*et al*., 2010).

Cellulose content (%) = Weight of total biomass – (lignin content + hemicellulose content + extractive content)

3.3.2 Determination of Reducing Sugar Content

Extraction of reducing sugar from degraded substrate was done by adding 1 g of degraded substrate to 20 mL of distilled water and was homogenized every 15 minutes for 2 hours. It was filtered and the filtrate was used to determine the reducing sugar content.

The reducing sugar content was determined using Dinitrosalicylic acid (DNS) method. The reagent was prepared by adding 1 g of 3, 5-Dinitrosalicylic acid to 20 mL of distilled water. It was heated in boiling water until it dissolved. Twenty (20) milliliter of 2M sodium hydroxide was added followed by sodium-potassium tartrate (30g) and stirred until complete dissolution. The volume was made up to 100 mL, boiled and allowed to cool down.

Filtrate was added to DNS reagent in ratio 1:1. It was boiled at 100 °C for 5 minutes and allowed to cool down. Absorbance was taken at 540nm. Different concentrations of glucose was also prepared and mixed with DNS reagent in ratio 1:1. It was boiled at 100 °C for 5 minutes and allowed to cool down. Absorbance of different concentrations of glucose taken at 540nm was used to plot standard graph. Reducing sugar concentration offiltrates from degraded substrates was extrapolated from the standard graph(Miller, 1959).

3.4 Determination of Reducing Sugar Contents of Lignocellulosic Biomass

Two hundred grammes of each of maize straw, rice straw and sugar cane bagasse was mixed with 600 mL of distilled water and packed in polythene bag. It was sterilized at 121 °C and 1.05kg cm^{-2} for 15 minutes and allowed to cool. Each bag was inoculated with full plate of white rot fungi (*Pleurotus ostreatus* and *Lentinus squarrosulus*) separately and in combination and allowedto undergo degradation for 35 days.Half plate of *Pleurotus ostreatus* and half plate of *Lentinus squarrosulus* was used in consortium of the two(Adenipekun and Fasidi, 2005). Samples were taken every 7 days and reducing sugar content determined. Reducing sugar was extracted by adding 5 % of degraded substrates into acetate buffer (0.1M, pH 5). It was boiled, filtered and filtrate was used to determine the reducing sugar content using DNS method (Miller, 1959).

3.5 Screening of Yeast for Ethanol Production

Yeast extract peptone dextrose broth (1% yeast extract, 2% peptone and 2% glucose) was prepared and dispensed into test tube with inverted Durham tube. It was sterilized at 121 °C and 1.05kg cm^{-2} for 15 minutes. Two percent (2%) of 1.0 MacFarland standard of the inoculum was inoculated into the test tube and incubated at 28±2°C for 5 days. Ability of yeast to produce ethanol was determined by measuring the height of gas production in Durham tube (Dung *et al*., 2012).

3.6 Identification of Selected Yeasts

Yeasts (isolate P412 and commercial yeast) cells (100mg) suspended in two hundred mirolitre isotonic bufferwas put in lysis tubeseparately. Seven hundred and fifty microliter of lysis solution was poured into each tube.Bead fitted with two milliliter tube holder was used to secure each sample. These were processed for 5 minutes at maximum speed. They were centrifuged in microcentrifuge for one minute at 10000 x g. Supernatant (400 µl) was poured into a Zymo-Spin IV Spin Filter in a collection tube. These were then centrifuged for a period of one minute at 7000 x g. One thousand two hundred (1200) microliters of fungal DNA binding buffer was poured to the filtrate. Eight hundred microliter of the mixtures was poured to a Zymo-Spin IIC Column in a collection tube. This was centrifuged for one minute at 10000 x g. Two hundred microliter of DNA pre-wash buffer (200 µl) was added into a new collection tube containingZymo-Spin IIC Column. This was centrifuged for one minute at 10000 x g. Five hundred microliters of fungal DNA wash buffer was added to Zymo-Spin IIC Column. This was centrifuged for one minute at 10000 x g. One hundred microliter of DNA elution buffer was poured to column matrix containing microcentrifuge of the Zymo-Spin IIC Column. It was centrifuged for 30 seconds at 10000 x g in order to elute the DNA(Garner *et al*., 2010; Angelov *et al*., 2015)

Extracted DNA was amplified in PCR. The cocktail mix is made up ofH_2O (3.1 µl),10ng/µl DNA (2.0 µl), Taq 5u/ul (0.1 µl),2.5Mm DNTPs (0.8 µl), DMSO (1.0 µl), 5pMol forward primer (0.5 µl) 5pMol reverse primer (0.5 µl),25mM $MgCl_2$ (1.0 µl) and10 x PCR buffer (1.0 µl). The total volume was 10 µl. The forward primer is ITS4 TCCTCCGCTTATTGACATGS while reverse primer is ITS5 GGAACTAAAAGTCGTAACAAGG. The initial denaturation was executed at ninety

four degree Celsius for five minutes and another denaturation was done for thirty seconds. Annealing was carried out at fifty four degree Celsius for thirty seconds while extension was carried out at seventy two degree Celsius for forty five seconds. There were thirty six cycles and final extension was carried out at seventy two degree Celsius for seven minutes and was held at ten degree Celsius.

Amplicon of PCR was loaded on 1.5% agarose gel. The ladder used was 1kb plus from Invitrogen (USA). The PCR product was purified by adding 20 µl of absolute ethanol to the PCR product. This was incubated at room temperature for 15 minutes and was spun down at 10,000 rpm for 15 minutes. Supernatant was decanted and spun down at 10,000 rpm for 15 minutes. Seventy percent ethanol (40 µl) was added and supernatant was decanted and air dried. Ten microliters of ultra-pure water was added and amplicon was checked on 1.5% agarose. The PCR product was use for sequencing reaction. The sequencing reaction was purified and purified product was loaded on the 3130xl analyzer from applied biosystems to get sequences.

The sequence obtained was subjected to sequence comparison through BLAST nucleotide search tooland identified at National Centre for Biotechnology Information (NCBI). The sequence was sent to gene bank of NCBI in order to get accession number. The molecular phylogenetic analysis was done by maximum likelihood method (Tamura and Nei, 1993; Kumar *et al*., 2018).

3.7 Fermentation of Degraded Substrates by *Saccharomyces cerevisiae* SA01 and *Saccharomyces cerevisiae* SA02

Acetate buffer (0.1M, pH 5.0) was used to extract fermentable reducing sugar from degraded substrates and was subjected to two different treatments. In the first treatment, 5% degraded substrates inacetate buffer was sterilized at 121 °C and 1.05kg cm^{-2} for 15 minutes. In the second treatment, 5 % degraded substrate was boiled in acetate buffer, filtered and the filtrates sterilized at 121 °C and 1.05kg cm^{-2} for 15 minutes. The two treatments were inoculated with 2 % of 1.0 MacFarland standard of *Saccharomyces cerevisiae* SA01 and *Saccharomyces cerevisiae* SA02 separately. They were incubated at 30 °C for 7 days. Samples were taken every day to determine the reducing sugar content.

3.8 Fermentation ofSelected Degraded Substrates by *Saccharomyces cerevisiae* SA01 and *Saccharomyces cerevisiae* SA02for Ethanol Production

Five percent of selected degraded substrates (maize straw, rice straw and sugarcane bagasse) by *Pleurotus ostreatus* and sugarcane bagasse degraded by consortium of *Pleurotus ostreatus* and *Lentinus squarrosulus* were mixed with acetate buffer (0.1M, pH 5.0), boiled and filtered. The filtrates were inoculated with 2 % inoculum of 1.0 MacFarland standard of *Saccharomyces cerevisiae* SA01 and *Saccharomyces cerevisiae* SA02 separately. They were incubated at 30 °C for 5 days. Samples were taken every 24 hours for the determination of ethanol content, reducing sugar and pH.

3.9 Determination of Ethanol Content

Ethanol content was determined by gravimetric method. Sixty milliliters of filtrates was distilled by heating mantle. The weight of dry, empty 25 mL density bottle was taken. Distillate was poured into the emptied density bottle and the weight was also taken. Equal volume of distilled water was added to density bottle and the weight was taken. The specific gravity was determined by dividing the weight of distillate with equal weight of distilled water. Specific gravity was used to determine the ethanol content with ethyl alcohol conversion table (AOAC, 1990; Wakil *et al*., 2013).

3.10 Pretreatment with Sodium Hydroxide and *Pleurotus ostreatus*

Ten percent maize straw (substrate with highest ethanol content throughout fermentation period) was added to 2.5% sodium hydroxide solution for 1 hour. This was later autoclaved at 121 °C and 1.05kg cm^{-2} for 30 minutes. The autoclaved sample was filtered and washed severally with distilled water until when the pH of the filtrate is about 7. The residue was then dried at 105 °C until constant weight was obtained (Irfan *et al*., 2011; Nadeem *et al*., 2015).

The dried sample was then degraded with *Pleurotus ostreatus* according to the method of Adenipekun and Fasidi(2005) as earlier explained(3.4)for 21 days. The samples were dried after the degradation and kept in an airtight nylon for further use. The reducing sugar content was determined using DNS Method (Miller, 1959).

3.11 Sugar Profile Analysis of Combined Pretreated Maize Straw

Sugar profile of combined pretreated maize straw was analysed with High Performance Liquid Chromatography (HPLC) (Agilent 1200 series). Refractive index detector (RID) was used as detector with injection volume of 5.0 µl and flow rate of 1.0 ml/min at 25 °C. Membrane filter (0.45 µm) was used to filter and degas freshly prepared mobile phase (75acetonitrile:25ultra-pure water) via vacuum filtration. Standardsolutions of needed standard concentrations were prepared from a prepared standard stock solution of relatively 8 g/L. Appropriate and accurate weights of standards were added to volumetric flask in order to prepare different calibrations standards. Deionized water was added to the volumetric flask till it reached the mark. Combined pretreated maize straw (2.5 g) was weighed into standard volumetric flask making up to 500 mL mark. Membrane filters (cellulose-acetate) were used to filter and degas all samples through vacuum filtration.Syringe filters were fitted on all syringes before injecting into the column(Valliyodan *et al*., 2015).

3.12 Fermentation of Single Pretreated and Combined Pretreated Maize Straw

Filtrate extracted from *Pleurotus ostreatus*-pretreated maize straw and combined (sodium hydroxide and *Pleurotus ostreatus*) pretreated maize straw were extracted with acetate buffer (0.1M, pH 5.0) and sterilized at 121 °C and 1.05kg cm^{-2} for 15 minutes. It was inoculated with 2 % 1.0 MacFarland standard of *Saccharomyces cerevisiae* SA01 and *Saccharomyces cerevisiae* SA02 separately and incubated at 30 °C for 5 days. Ethanol content, reducing sugar content and pH were determined every 24 hours.

3.13 Optimization Conditions for Ethanol Production

Different parameters affect production of ethanol. Among these parameters are pH of the medium, temperature of incubation, sugar concentration, nitrogen source and its concentration, inoculum size and load.

3.13.1 Effect of pH of Acetate Buffer on Ethanol Production

Effect of pH of acetated buffer was studied on combined (sodium hydroxide and *Pleurotus ostreatus*) pretreated maize straw which had higher ethanol than *Pleurotus*

ostreatus-pretreated maize straw. Different pH (4.0, 4.5, 5.0 and 5.5) of 0.1 M of acetate buffer was used to extract reducing sugar from combined-pretreated maize straw.Filtrates were sterilized and inoculated with 2 % of 1.0 MacFarland standard ofthe 2 strains of *Saccharomyces cerevisiae*(*Saccharomyces cerevisiae* SA01 and *Saccharomyces cerevisiae* SA02) for 3 days. The reducing sugar concentration, pH and ethanol content were determined after the fermentation period.

3.13.2 Effect of Temperature on Ethanol Production

Acetate buffer (0.1M, pH 5.5) was used to extract reducing sugar from maize straw that was pretreated with both sodium hydroxide and *Pleurotus ostreatus* together. Filtrate was dispensed into different fermentation bottles. They weresterilized at 121 °C and 1.05kg cm^{-2} for 15 minutes.Each filtrate was inoculated with 2% of 1.0 MacFarland standard of two strains of *Saccharomyces cerevisiae* (*Saccharomyces cerevisiae* SA01 and *Saccharomyces cerevisiae* SA02) and incubated at varying temperatures (30, 35, 40 and 45) °C for 3 days. Reducing sugar content, pH and ethanol concentration were determined at the end of the fermentation.

3.13.3 Effect of Sugar Concentration on Ethanol Production

Five percent (5%)of combined-pretreated maize strawwas boiled in 0.1 M acetate buffer of pH 5.5. It was filtered and the filtrate was supplemented with different concentrations of glucose (0, 1, 2, 3, 4 and 5%) and fructose (0, 1, 2, 3, 4 and 5 %). The supplemented substrates were sterilized and inoculated separately with the two strains (2% of 1.0 MacFarland standard) of *Saccharomyces cerevisiae*(*Saccharomyces cerevisiae* SA01 and *Saccharomyces cerevisiae* SA02). They were incubated at 30 °C for 3 days fermentation period. Ethanol content was determined after fermentation.

3.13.4 Effect of Nitrogen Sources on Ethanol Production

Combined pretreated maize straw (5%) was boiled in 0.1 M acetate buffer of pH 5.5. It was filtered and the filtrate was dispensed into Erlenmeyer flask. The filtrates were supplemented separately with 1 % of nitrogen sources (groundnut cake, soya meal, fish meal and blood meal). For corn steep liquor as nitrogen source, corn steep liquor was mixed with 0.1M acetate buffer (pH 5.5) in ratio 50:50 and 5 % of maize straw was boiled

in the mixture, filtered and the filtrate was used as maize straw supplemented with corn steep liquor. Two percent of glucose was added to all the filtrates. They were sterilized at 121 °C and 1.05kg cm^{-2} for 15 minutes. They were allowed to cool and inoculated with 2% of 1.0 MacFarland standard of *Saccharomyces cerevisiae* SA01 and *Saccharomyces cerevisiae* SA02. They were incubated at 30 °C for 3 days. Ethanol content, reducing sugar concentration and pH were determined at the end of the fermentation.

3.13.5 Effect of Different Concentration of Corn Steep Liquor on Ethanol Production

Different ratio (90/10, 80/20, 70/30, 60/40 and 50/50) of acetate buffer (0.1M, pH 5.5) to corn steep liquor (from yellow maize) were prepared. These were used to extract sugar from combined–pretreated maize straw. Five (5) percent of the pretreated maize straw was boiled with different ratio of acetate buffer to corn steep liquor. They were filtered and supplemented with 2% glucose. The supplemented filtrate was subjected to two different treatments.Firstly, pH was adjusted to 5.5 after supplementation with glucose while the other set'spH was not adjusted. They were sterilized at 121 °C and 1.05kg cm^{-2} for 15 minutes and allowed to cool. These were later inoculated with 2 % of 1.0 MacFarland standard of *Saccharomyces cerevisiae*SA01 and *Saccharomyces cerevisiae* SA02. They were incubated at 30±2 °C for 3 days. Ethanol content and pH were determined after fermentation.

3.13.6 Effect of Different Inoculum Sizes and Loads on Ethanol Production

Acetate buffer (0.1M, pH 5.5) were mixed with corn steep liquor (from yellow maize) in ratio 60/40. Combined-pretreated maize straw (5%) was boiled in the mixture. It was filtered and supplemented with 2% glucose. It was then dispensed into different fermentation bottles and sterilized at 121 °C and 1.05kg cm^{-2} for 15 minutes. Different inoculum sizes (1, 1.5, 2, 2.5 and 3 %) of 1.0 MacFarland standard of *Saccharomyces cerevisiae*SA01 and *Saccharomyces cerevisiae* SA02 were used to inoculate the filtrates. Two percent of different inoculum load (0.5, 1, 2 and 3 MacFarland standard) of *Saccharomyces cerevisiae* SA01 and *Saccharomyces cerevisiae* SA02 were also inoculated into another set. They were incubated at 30 °C for 3 days. The ethanol concentration, reducing sugar content and the pH were determined.

3.14 Statistical Analysis

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

CHAPTER FOUR

RESULTS

The three white rot fungi (*Lentinus squarrosulus*, *Pleurotus ostreatus* and *Pleurotus tuber-regium*)screened for cellulase, xylanase, lignase and laccase showed ability to produce one, two, three or all the four enzymes. The appearance of a yellow opaque areas around fungus growth against a red colour of undegraded carboxymethyl cellulose (CMC) as shown by *Lentinus squarrosulus* indicated cellulase production (Plate 4.1a). A xylanase-producing *Lentinus squarrosulus* on potato dextrose agar supplemented with xylan is as shown in Plate 4.1b.

Plate 4.1c shows *Pleurotus ostreatus* with the ability to degrade lignin on tannic agar. The brown coloration around the growing fungus confirms its ability to produce lignase. Lignase combines all the enzymes involves in lignin degradation.Laccase-producing *Pleurotus tuber-regium* on potato dextrose agar supplemented with 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) is shown in Plate 4.1d. Development of purple coloration indicated ability of the fungus to produce laccase.

The three white rot fungi (*Lentinus squarrosulus*, *Pleurotus ostreatus* and *Pleurotus tuber-regium*)were able to produce cellulase, xylanase, lignase and laccase with the exception of *Pleurotus tuber-regium* that could not produce cellulase and xylanase as shown in Table 4.1. Relative activities of lignase and laccase by *Pleurotus tuber-regium* (1.68 and 1.42) >*Pleurotus ostreatus* (1.48 and 1.24) >*Lentinus squarrosulus* (1.27 and 1.12) and were significantly different ($P \leq 0.05$) from one another. Highest relative cellulase activity (1.08) was obtained by *Lentinus squarrosulus* and same relative xylanase activity (1.14) was recorded by *Lentinus squarrosulus* and *Pleurotus ostreatus*

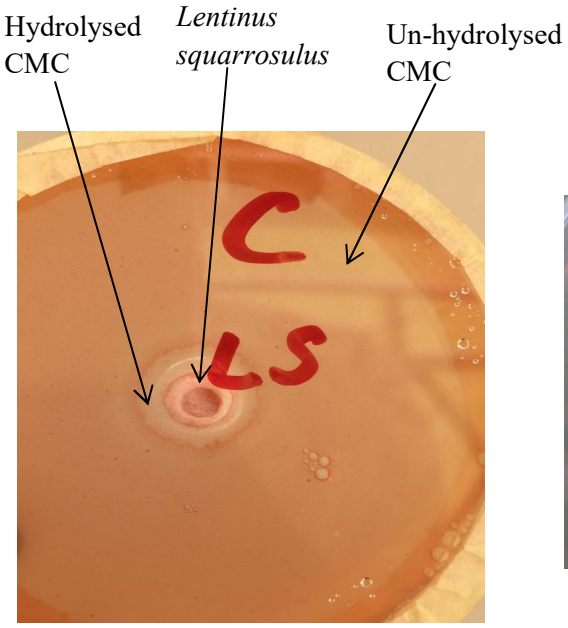

Plate 4.1a: Cellulase-Producing *Lentinus squarrosulus* on Carboxymethyl Cellulose Agar

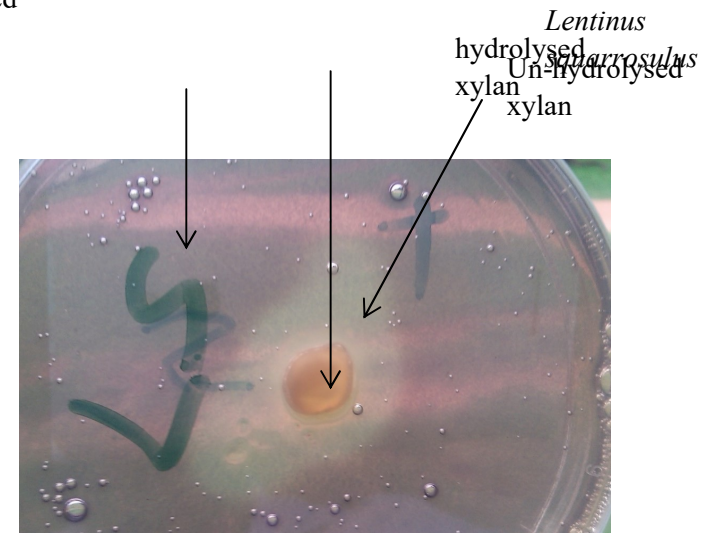

Plate 4.1b: Xylanase-Producing *Lentinus squarrolulus* on Potato Dextrose Agar Supplemented with Xylan

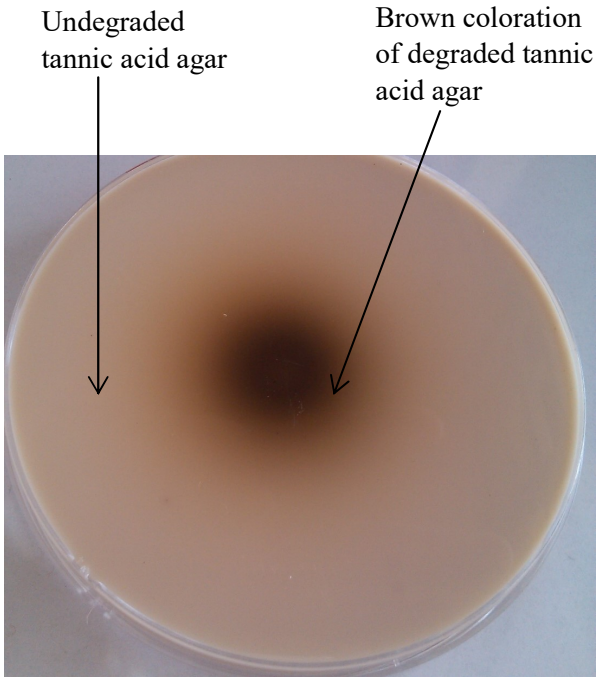

Plate 4.1c: Lignase-Producing *Pleurotus ostreatus* on Tannic Acid Agar

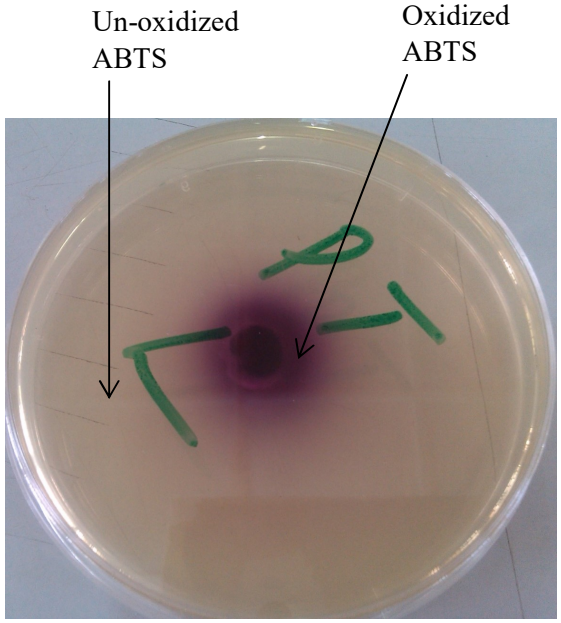

Plate 4.1d: Lacasse-Producing *Pleurotus tuber-regium* on Potato Dextrose Agar Supplemented with 2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS)

Table 4.1: Relative Activities of Lignocellulolytic Enzymes of White Rot Fungi

Enzymes Produce	Relative Enzymes Activities		
	Lentinus squarrosulus	***Pleurotus ostreatus***	***Pleurotus tuber-regium***
Cellulase	1.08^{b}	1.03^{a}	-
Xylanase	1.14^{a}	1.14^{a}	-
Lignase	1.27^{a}	1.48^{b}	1.68^{c}
Laccase	1.12^{a}	1.24^{b}	1.42^{c}

Mean values with different superscripts across the row are significantly different ($P \leq 0.05$)

Component (extractive, hemicellulose, lignin and cellulose) of groundnut shell pretreated with *Pleurotus ostreatus* and *Lentinus squarrosulus* is shown in Table 4.2. Extractives of groundnut shell degraded by *Pleurotus ostreatus* (PO), *Lentinus squarrosulus*(LS) and consortium of *Pleurotus ostreatus* and *Lentinus squarrosulus*(POLS) ranged from 2.09 – 9.99 %; 2.02 – 9.99 % and 2.31 – 9.99 % with the least recorded at 21, 28 and 28 days of degradation respectively and their highest extractives were recorded before degradation. Highest hemicellulose content of groundnut shell degraded by *Pleurotus ostreatus* (30.39 %), *Lentinus squarrosulus* (26.31 %) and consortium of *Pleurotus ostreatus* and *Lentinus squarrosulus* (26.07 %) were obtained on14 days of degradation and least (15.59 %) was obtained before degradation. The least lignin contents (33.43, 17.60 and 18.78 %) of all treatments (PO, LS and POLS) of groundnut shell were observed in 49-day degraded sample respectively. Cellulose content of groundnut shell degraded by PO, LS and POLS ranged from 18.55 – 37.68 %, 23.44 – 52.61 % and 21.00 – 52.69 % respectively. The least and highest cellulose content by all degrading fungi were obtained at 14 and 49 days of degradation respectively. Statistical analysis revealed that the values of extractives, hemicellulose, lignin and cellulose of degraded groundnut shell by *Pleurotus ostreatus*, *Lentinus squarrosulus* and consortium of the two were significantly different ($P \leq 0.5$) with days of degradation.

The highest component of degraded maize cob by all the treatments was hemicellulose followed by lignin and the least component being extractivesas shown in Table 4.3. Thehighest extractives of PO(8.78 %), LS(8.59 %) and POLS (10.33 %)were recorded in 7-day degraded maize cob. Highest hemicellulose content of 40.70 %, 43.92 % and 44.88 % were recorded when maize cob was degraded by PO, LS and POLS at 14, 63 and 14 days of degradation respectively. Lignin content of maize cob degraded by *Pleurotus ostreatus*, *Lentinus squarrosulus* and consortium of *Pleurotus ostreatus* and *Lentinus squarrosulus* ranged from 24.29 – 35.66 %, 26.45 – 35.37 % and 22.65 – 35.85 % respectively. The initial cellulose content (29.08 %) of undegraded maize cob was higher than those degraded with the exception of maize cob degraded by PO for 49 days (29.13 %) which was not significantly different ($P > 0.05$) from the undegraded. Least cellulose content was observed at 14 days of degradation by PO (21.34 %)and POLS (15.07%). Maize cob degraded byLS had least cellulose content (16.22 %) at 49 day of degradation

which was not significantly different (P>0.05) from cellulose content (16.68 %) obtained at 14 day of degradation.

Table 4.2: Component of Groundnut Shell Pretreated with *Pleurotus ostreatus* and *Lentinus squarrosulus*Singly and Combined

Days	Extractives (%)			Hemicellulose (%)			Lignin (%)			Cellulose (%)		
	PO	LS	POLS	PO	LS	POLS	PO	LS	POLS	PO	LS	POLS
0	9.99^{e}	9.99^{d}	9.99^{d}	15.59^{a}	15.59^{a}	15.59^{a}	40.79^{c}	40.79^{c}	40.79^{c}	33.64^{f}	33.64^{g}	33.64^{f}
7	8.19^{d}	9.55^{d}	7.09^{bc}	28.41^{e}	26.18^{g}	24.44^{g}	43.06^{de}	35.38^{b}	37.13^{b}	20.34^{b}	28.90^{de}	31.35^{de}
14	3.01^{a}	2.90^{a}	3.13^{a}	30.39^{e}	26.31^{g}	26.07^{h}	48.05^{f}	47.35^{f}	49.80^{f}	18.55^{a}	23.44^{a}	21.00^{a}
21	2.09^{a}	3.47^{a}	3.41^{a}	21.40^{c}	20.91^{ef}	20.75^{de}	50.24^{g}	48.79^{f}	49.41^{f}	26.27^{c}	26.83^{bc}	26.43^{b}
28	2.45^{a}	2.02^{a}	2.31^{a}	16.81^{a}	16.81^{ab}	17.25^{b}	49.21^{fg}	51.57^{g}	50.20^{f}	31.52^{e}	29.59^{de}	30.24^{d}
35	5.74^{b}	6.31^{b}	6.31^{b}	18.88^{b}	18.89^{cd}	15.09^{a}	41.64^{cd}	43.27^{de}	41.53^{cd}	33.74^{f}	31.53^{f}	37.07^{g}
42	6.28^{bc}	7.62^{bc}	6.38^{b}	18.58^{b}	18.01^{bc}	19.60^{cd}	48.87^{fg}	48.50^{f}	47.84^{e}	26.27^{c}	25.87^{b}	26.18^{b}
49	7.95^{d}	7.58^{bc}	7.71^{bc}	20.94^{c}	22.21^{f}	20.81^{de}	33.43^{a}	17.60^{a}	18.78^{a}	37.68^{g}	52.61^{h}	52.69^{h}
56	7.68^{cd}	8.54^{cd}	8.23^{c}	23.92^{d}	21.02^{ef}	22.70^{f}	42.24^{cde}	42.33^{d}	40.94^{c}	26.16^{c}	28.10^{cd}	28.13^{c}
63	6.28^{bc}	7.14^{bc}	7.16^{bc}	24.43^{d}	19.84^{de}	18.34^{bc}	43.60^{e}	43.88^{e}	42.58^{d}	25.69^{c}	29.15^{de}	31.93^{e}
70	7.19^{bcd}	7.89^{c}	7.47^{bc}	24.86^{d}	21.19^{ef}	21.25^{e}	38.74^{b}	40.71^{c}	41.05^{c}	29.21^{d}	30.21^{ef}	30.24^{d}

Mean values with different superscripts along the column are significantly different ($P \leq 0.05$)

Keys:

PO: *Pleurotus ostreatus*

LS: *Lentinus squarrosulus*

POLS: Consortium of *Pleurotus ostreatus* and *Lentinus squarrosulus*

Table 4.3: Component of Maize Cob Pretreated with *Pleurotus ostreatus* and *Lentinus squarrosulus*Singly and Combined

Days	Extractives (%)			Hemicellulose (%)			Lignin (%)			Cellulose (%)		
	PO	LS	POLS	PO	LS	POLS	PO	LS	POLS	PO	LS	POLS
0	8.26^{bc}	8.26^{c}	8.26^{c}	35.27^{ab}	35.27^{a}	35.27^{a}	27.38^{b}	27.38^{ab}	27.38^{b}	29.08^{e}	29.08^{f}	29.08^{e}
7	8.78^{c}	8.59^{c}	10.33^{d}	37.37^{cde}	42.80^{de}	39.73^{cd}	27.77^{b}	28.52^{b}	28.66^{bc}	26.08^{c}	20.09^{b}	21.28^{b}
14	3.59^{a}	5.10^{b}	4.20^{ab}	40.70^{g}	42.84^{de}	44.88^{f}	34.37^{de}	35.37^{e}	35.85^{h}	21.34^{a}	16.68^{a}	15.07^{a}
21	3.20^{a}	3.88^{b}	5.38^{b}	39.43^{fg}	40.02^{c}	39.54^{cd}	35.66^{e}	32.99^{d}	34.82^{gh}	21.72^{ab}	23.11^{d}	20.27^{b}
28	3.24^{a}	2.33^{a}	2.92^{a}	34.02^{a}	39.01^{c}	37.22^{b}	34.55^{de}	33.91^{de}	33.79^{fg}	28.20^{de}	24.74^{e}	26.07^{d}
35	8.62^{c}	7.39^{c}	7.40^{c}	36.39^{bc}	42.86^{de}	35.73^{a}	32.13^{c}	28.00^{b}	32.01^{e}	22.86^{b}	21.75^{cd}	24.86^{d}
42	6.93^{b}	8.12^{c}	7.34^{c}	36.71^{bc}	36.91^{b}	38.45^{bc}	33.37^{cd}	33.74^{d}	33.02^{ef}	22.99^{b}	21.22^{bc}	21.19^{b}
49	7.72^{bc}	7.64^{c}	7.79^{c}	38.86^{ef}	41.75^{d}	41.49^{e}	24.29^{a}	34.39^{de}	29.39^{cd}	29.13^{e}	16.22^{a}	21.33^{b}
56	7.90^{bc}	8.55^{c}	8.60^{c}	38.54^{def}	38.91^{c}	42.05^{e}	26.72^{b}	31.23^{c}	28.29^{bc}	26.83^{cd}	21.31^{bc}	21.07^{b}
63	6.95^{b}	7.61^{c}	7.61^{c}	38.22^{def}	43.92^{e}	38.98^{c}	26.68^{b}	26.45^{a}	30.57^{d}	28.15^{de}	22.02^{cd}	22.84^{c}
70	7.03^{b}	7.56^{c}	7.87^{c}	37.22^{cd}	36.91^{b}	40.91^{de}	27.29^{b}	27.60^{ab}	22.65^{a}	28.46^{e}	27.93^{f}	28.57^{e}

Mean values with different superscripts along the column are significantly different ($P \leq 0.05$)

Keys:

PO: *Pleurotus ostreatus*

LS: *Lentinus squarrosulus*

POLS: Consortium of *Pleurotus ostreatus* and *Lentinus squarrosulus*

The Extractives, hemicellulose, lignin and cellulose contents of degraded maize straw was as shown in Table 4.4. The highest extractive (10.72 %) was recorded in undegraded maize straw and lowest extractives of 1.12 %, 2.00 % and 4.98 % were observed in PO, LS and POLS-degraded maize strawat 28, 28 and 7 days of degradation respectively. Hemicellulose content ranged from 42.05 – 54.16 %, 41.14 – 46.47 % and 33.40 – 43.77 % in PO, LS and POLS degraded maize straw respectively. Hemicellulose content of PO-degraded maize straw was higher than LS and POLS-degraded maize straw throughout the period of degradation. The observed highest lignin content by maize straw degraded by PO (37.42 %), LS (38.99 %) and POLS (35.76%) were recorded at 28, 21 and 42 days of degradation respectively. It was observed that maize straw degraded for 7 days had highest cellulose content for PO (22.66 %), LS (23.56 %) and POLS (37.24 %). Days of degradation had significant effect($P \leq 0.05$) on extractives, hemicellulose, lignin and cellulose of degraded maize straw.

Component of pretreated sugarcane bagasse is as shown in Table 4.5. Undegraded sugarcane bagasse had highest extractives when compared with those degraded by selected white rot fungi. The least extractive observedin sugarcane bagasse degraded by PO, LS and POLS were 3.01 %, 2.35 % and 2.19 % at 28, 14, and 28 days of degradation respectively. Hemicellulose content of 35.21 – 43.66 %, 36.15 – 44.17 % and 36.15 – 45.03 % were observed in PO, LS and POLS-degraded sugarcane bagasse with their highest content at 70, 63 and 21 days of degradation respectively. While highest lignin content of PO-degraded sugarcane bagasse (34.23 %) was observed at 42 days of degradation, highest lignin contents of LS (34.70 %) and POLS (35.77%) -degraded sugarcane bagasse were recorded at 14 days of degradation. Least cellulose content obtained when sugar cane bagasse was degraded by PO, LS and POLSwere 16.83 %, 17.83 % and 14.95 % at 49, 49 and 21 days of degradation respectively. There were significant differences ($P \leq 0.05$) in the values of extractives, hemicellulose, lignin and cellulose with day of degradation.

Table 4.4: Component of Maize Straw Pretreated with *Pleurotus ostreatus* and *Lentinus squarrosulus*Singlyand Combined

Days	Extractives (%)			Hemicellulose (%)			Lignin (%)			Cellulose (%)		
	PO	LS	POLS	PO	LS	POLS	PO	LS	POLS	PO	LS	POLS
0	10.72^{g}	10.72^{f}	10.72^{e}	42.05^{a}	42.05^{ab}	42.05^{def}	29.52^{e}	29.52^{b}	29.52^{c}	17.71^{g}	17.71^{ef}	17.71^{a}
7	2.87^{b}	3.18^{a}	4.98^{a}	48.29^{cd}	42.73^{bc}	39.35^{c}	26.18^{b}	30.53^{b}	18.42^{a}	22.66^{h}	23.56^{g}	37.24^{e}
14	4.59^{c}	5.22^{b}	5.30^{ab}	47.55^{bc}	46.47^{f}	36.14^{b}	31.00^{f}	35.99^{d}	33.44^{d}	16.86^{fg}	12.33^{ab}	25.12^{d}
21	7.39^{de}	6.66^{bc}	6.40^{abc}	46.34^{b}	43.41^{bcd}	40.77^{d}	32.81^{g}	38.99^{f}	35.23^{e}	13.46^{d}	10.94^{a}	17.60^{a}
28	1.12^{a}	2.00^{a}	13.97^{f}	49.78^{e}	45.11^{ef}	33.40^{a}	37.42^{h}	37.59^{e}	35.13^{e}	11.68^{c}	15.30^{d}	17.50^{a}
35	9.05^{f}	8.67^{e}	8.05^{d}	46.57^{b}	41.14^{a}	43.77^{g}	29.12^{de}	27.14^{a}	25.70^{b}	15.27^{e}	23.05^{g}	22.48^{c}
42	7.06^{de}	6.93^{cd}	6.65^{bcd}	50.66^{ef}	43.55^{cd}	41.40^{de}	36.69^{h}	36.06^{d}	35.76^{e}	5.60^{a}	13.46^{bc}	16.19^{a}
49	7.65^{ef}	8.34^{de}	7.00^{cd}	51.62^{f}	43.70^{cde}	42.78^{efg}	30.98^{f}	33.42^{c}	33.68^{d}	9.75^{b}	14.55^{cd}	16.54^{a}
56	7.62^{ef}	7.32^{cde}	7.47^{cd}	49.5^{de}	44.34^{de}	42.97^{fg}	27.77^{cd}	29.51^{b}	29.73^{c}	15.12^{e}	18.84^{f}	19.83^{b}
63	6.03^{d}	6.30^{bc}	6.62^{bcd}	53.33^{g}	46.35^{f}	43.54^{fg}	27.05^{bc}	30.65^{b}	26.41^{b}	13.59^{d}	16.70^{e}	23.43^{c}
70	8.29^{ef}	7.01^{cd}	6.94^{cd}	54.16^{g}	43.59^{cd}	42.75^{efg}	21.84^{a}	30.97^{b}	30.16^{c}	15.71^{ef}	18.43^{f}	20.15^{b}

Mean values with different superscripts along the column are significantly different ($P \leq 0.05$)

Keys:

PO: *Pleurotus ostreatus*

LS: *Lentinus squarrosulus*

POLS: Consortium of *Pleurotus ostreatus* and *Lentinus squarrosulus*

Table 4.5: Component of Sugarcane Bagasse Pretreated with *Pleurotus ostreatus* and *Lentinus squarrosulus*Singlyand Combined

Days	Extractives (%)			Hemicellulose (%)			Lignin (%)			Cellulose (%)		
	PO	LS	POLS	PO	LS	POLS	PO	LS	POLS	PO	LS	POLS
0	11.12^{f}	11.12^{e}	11.12^{g}	36.15^{a}	36.15^{a}	36.15^{a}	29.71^{b}	29.71^{c}	29.71^{e}	23.01^{def}	23.01^{c}	23.01^{d}
7	4.45^{ab}	3.52^{ab}	4.48^{b}	35.44^{a}	38.33^{b}	39.34^{b}	27.27^{a}	27.49^{b}	26.10^{bc}	32.85^{h}	30.66^{f}	30.08^{g}
14	3.67^{ab}	2.35^{a}	2.43^{a}	35.21^{a}	39.46^{bc}	42.17^{cd}	33.35^{c}	34.70^{e}	35.77^{g}	27.78^{g}	23.48^{c}	19.62^{c}
21	4.83^{bc}	4.39^{b}	5.38^{bc}	40.51^{c}	40.36^{cd}	45.03^{f}	32.86^{c}	30.00^{c}	34.63^{g}	21.80^{cd}	25.25^{d}	14.95^{a}
28	3.01^{a}	3.27^{ab}	2.19^{a}	39.02^{b}	42.25^{e}	41.12^{c}	33.64^{c}	32.10^{d}	32.46^{f}	24.33^{f}	22.38^{c}	24.23^{de}
35	8.73^{e}	7.17^{cd}	4.80^{b}	41.84^{cd}	39.67^{bcd}	43.66^{ef}	25.88^{a}	29.88^{c}	27.91^{d}	23.55^{ef}	23.29^{c}	23.63^{de}
42	6.72^{d}	7.08^{cd}	6.31^{cd}	40.63^{c}	40.50^{cd}	41.55^{c}	34.23^{c}	32.21^{d}	33.07^{f}	18.41^{b}	20.21^{b}	19.07^{c}
49	9.26^{e}	7.96^{d}	7.81^{ef}	40.56^{c}	41.21^{de}	43.84^{ef}	33.35^{c}	33.01^{d}	31.62^{f}	16.83^{a}	17.83^{a}	16.73^{b}
56	9.98^{ef}	11.17^{e}	8.92^{f}	42.57^{de}	40.98^{cde}	41.43^{c}	26.88^{a}	26.89^{b}	24.95^{b}	20.57^{c}	20.97^{b}	24.70^{e}
63	6.00^{cd}	6.00^{c}	7.40^{de}	42.52^{de}	44.17^{f}	41.32^{c}	29.01^{b}	26.41^{b}	27.16^{cd}	22.47^{de}	23.42^{c}	24.12^{de}
70	6.77^{d}	6.87^{cd}	7.35^{de}	43.66^{e}	40.69^{cd}	43.06^{de}	26.98^{a}	23.91^{a}	23.21^{a}	22.58^{de}	28.53^{e}	26.38^{g}

Mean values with different superscripts along the column are significantly different ($P \leq 0.05$)

Keys:

PO: *Pleurotus ostreatus*

LS: *Lentinus squarrosulus*

POLS: Consortium of *Pleurotus ostreatus* and *Lentinus squarrosulus*

Table 4.6 shows the extractives, hemicellulose, lignin and cellulose contents of PO, LS and POLS-degraded rice straw. The extractives of rice straw degraded by PO, LS and POLS ranged from 3.20 – 8.86 %, 3.84 – 8.86 % and 3.07 – 8.86 % respectively. Hemicellulose content of 39.61 – 56.70 %, 39.61 – 50.38 % and 36.95 – 51.66 % were recorded in PO, LS and POLS-degraded rice straw respectively. During degradation, 20.91 – 31.54 %, 24.91 – 34.85 % and 24.37 – 35.19 % ranges of lignin content were obtained in PO, LS and POLS-degraded rice straw respectively. Cellulose content of rice straw degraded by PO ranged from 12.16 to 27.97 % with the least and highest at 70 and 7 days of degradation respectively. Cellulose content range of 8.06 – 25.25 % and 15.31 – 30.46 % were recorded in LS and POLS degraded rice straw respectively. Statistical analysis revealed values of extractives, hemicellulose, lignin and cellulose of degraded rice is significantly different ($P \leq 0.05$) with days of degradation.

The reducing sugar content of lignocellulosic samples (groundnut shell, maize cob, maize straw, sugar cane bagasse and rice straw) determined every 7 days during 70 days of degradationis as shown in Table 4.7. Reducing sugar of PO-pretreated groundnut shell ranged from 2.61 to 11.23 mg/g with the least and highest at 56 and 49 days of degradation respectively. The least reducing sugar (0.78 mg/g) of groundnut shell degraded by LS was obtained after 70 days of degradation and highest (11.83 mg/g) at 21 days of degradation. Reducing sugar content of POLS-degraded groundnut shell ranged from 2.90 to 8.01 mg/g with the least and highest at 7 and 49 days of degradation and were not significantly different ($P>0.05$). Reducing sugar of maize cob degraded by PO, LS and POLS ranged from 0.37 – 8.16 mg/g, 3.64 – 13.32 mg/g and 3.76 – 13.25 mg/g respectively. Highest reducing sugar of PO, LS and POLS-degraded maize straw were 27.03 mg/g, 20.41 mg/g and 19.70 mg/g at 14, 35 and 0 days respectively. The reducing sugar content of PO-degraded maize straw was generally higher than the reducing sugar of LS and POLS-degraded maize straw.Sugarcane bagasse degraded by PO, LS and POLS have reducing sugar in the range of 9.08 – 28.70 mg/g, 11.75 – 25.58 mg/g and 10.28 – 31.94 mg/g with their highest content at 7, 7 and 49 days respectively. Lowest reducing sugar (6.88 mg/g) was recorded in rice straw before degradation. There was increase in reducing sugar of rice straw with increase in the degradation time. The highest reducing sugar of rice straw degraded by PO(37.96 mg/g), LS (17.01 mg/g) and POLS (28.74

mg/g) were recorded at 49, 42 and 63 days respectively. Statistical analysis revealed that there was no significant difference ($P>0.05$) in the reducing sugar content of rice straw that was degraded by *Lentinus squarrosulus* with days of degradation.

Table 4.6: Component of Rice Straw Pretreated with *Pleurotus ostreatus* and *Lentinus squarrosulus*Singlyand Combined

Days	Extractives (%)			Hemicellulose (%)			Lignin (%)			Cellulose (%)		
	PO	LS	POLS	PO	LS	POLS	PO	LS	POLS	PO	LS	POLS
0	8.86^{d}	8.86^{d}	8.86^{c}	39.61^{a}	39.61^{a}	39.61^{b}	26.95^{f}	26.95^{c}	26.95^{b}	24.59^{f}	24.59^{fg}	24.59^{d}
7	4.26^{a}	3.84^{a}	3.58^{a}	46.87^{c}	39.97^{a}	36.95^{a}	20.91^{a}	30.99^{d}	29.01^{c}	27.97^{g}	25.20^{g}	30.46^{e}
14	3.31^{a}	6.71^{bc}	3.40^{a}	51.37^{d}	50.38^{f}	43.23^{cd}	31.54^{h}	34.85^{e}	35.19^{f}	13.79^{b}	8.06^{a}	18.18^{b}
21	6.53^{b}	4.86^{a}	4.46^{a}	45.29^{b}	48.96^{ef}	48.18^{f}	25.61^{ef}	31.54^{d}	31.19^{d}	22.57^{e}	14.64^{c}	16.17^{a}
28	3.20^{a}	4.53^{a}	3.07^{a}	52.40^{de}	49.44^{ef}	45.91^{e}	30.16^{gh}	34.79^{e}	32.93^{e}	14.24^{b}	11.24^{b}	18.09^{b}
35	8.19^{cd}	7.85^{bcd}	7.60^{bc}	51.37^{d}	48.52^{e}	48.29^{f}	23.68^{cd}	26.72^{bc}	28.79^{c}	16.76^{c}	16.91^{d}	15.31^{a}
42	8.21^{cd}	6.94^{bc}	7.97^{bc}	45.36^{b}	45.33^{d}	44.17^{d}	29.41^{g}	34.13^{e}	32.17^{de}	17.03^{c}	13.61^{c}	15.70^{a}
49	7.16^{bc}	6.50^{b}	7.22^{b}	47.86^{c}	41.41^{b}	42.08^{c}	24.88^{de}	30.27^{d}	30.75^{d}	20.11^{d}	21.82^{e}	19.95^{c}
56	8.17^{cd}	8.18^{cd}	7.77^{bc}	53.66^{e}	42.80^{bc}	46.18^{e}	24.96^{de}	27.17^{c}	26.90^{b}	13.21^{ab}	21.85^{e}	19.15^{bc}
63	7.98^{bcd}	6.73^{bc}	8.34^{bc}	53.65^{e}	42.62^{b}	48.77^{f}	22.08^{ab}	25.40^{ab}	26.23^{b}	16.29^{c}	25.25^{g}	16.66^{a}
70	7.97^{bcd}	7.21^{bc}	8.11^{bc}	56.70^{f}	44.14^{cd}	51.66^{g}	23.17^{bc}	24.91^{a}	24.37^{a}	12.16^{a}	23.73^{f}	15.86^{a}

Mean values with different superscripts along the column are significantly different ($P \leq 0.05$)

Keys:

PO: *Pleurotus ostreatus*

LS: *Lentinus squarrosulus*

POLS: Consortium of *Pleurotus ostreatus* and *Lentinus squarrosulus*

Table 4.7: Reducing Sugar Content (mg/g) of Lignocelluloses Degraded by *Lentinus squarrosulus* and *Pleurotus ostreatus*Singlyand Combined

Period	GS			MC			MS			SB			RS		
(days)	PO	LS	POLS	PO	LS	POLS	PO	LS	POLS	PO	LS	POLS	PO	LS	POLS
0	3.99^{ab}	3.99^{ab}	3.99^{a}	3.76^{abcd}	3.76^{a}	3.76^{a}	19.70^{a}	19.70^{b}	19.70^{b}	16.88^{abc}	16.88^{abc}	16.88^{ab}	6.88^{a}	6.88^{a}	6.88^{a}
7	6.37^{abc}	4.54^{ab}	2.90^{a}	7.06^{cd}	13.26^{b}	10.67^{a}	14.76^{a}	11.64^{ab}	12.39^{ab}	28.70^{d}	25.58^{c}	23.68^{abc}	17.80^{bc}	15.68^{a}	15.74^{ab}
14	4.20^{abc}	5.16^{ab}	3.12^{a}	3.32^{abcd}	8.45^{ab}	6.68^{a}	27.03^{a}	15.16^{ab}	9.14^{a}	22.66^{cd}	14.53^{ab}	10.28^{a}	19.04^{bcd}	11.19^{a}	15.80^{ab}
21	7.14^{bc}	11.83^{c}	6.36^{a}	4.58^{abcd}	6.49^{ab}	8.59^{a}	22.26^{a}	18.81^{ab}	13.29^{ab}	16.23^{abc}	22.66^{bc}	23.13^{abc}	31.84^{ef}	11.57^{a}	20.70^{ab}
28	3.42^{ab}	8.56^{bc}	7.88^{a}	4.45^{abcd}	3.85^{a}	5.82^{a}	18.24^{a}	15.91^{ab}	14.12^{ab}	11.52^{ab}	22.17^{bc}	12.58^{ab}	25.88^{cde}	10.27^{a}	15.17^{ab}
35	8.08^{cd}	3.44^{a}	7.03^{a}	4.74^{abcd}	9.34^{ab}	9.66^{a}	24.94^{a}	20.41^{b}	18.93^{b}	19.51^{bc}	18.90^{abc}	17.68^{abc}	30.44^{ef}	13.46^{a}	19.27^{ab}
42	5.67^{abc}	1.71^{a}	4.98^{a}	0.37^{a}	13.32^{b}	10.73^{a}	18.14^{a}	16.29^{ab}	14.27^{ab}	9.61^{a}	17.59^{abc}	13.03^{ab}	11.93^{ab}	17.01^{a}	17.92^{ab}
49	11.23^{d}	4.08^{ab}	8.01^{a}	5.76^{bcd}	7.37^{ab}	8.92^{a}	11.93^{a}	16.76^{ab}	11.80^{ab}	16.00^{abc}	19.88^{abc}	31.94^{c}	37.96^{f}	12.55^{a}	11.38^{ab}
56	2.61^{a}	5.64^{ab}	7.99^{a}	2.89^{abc}	4.92^{ab}	5.20^{a}	15.54^{a}	16.70^{ab}	11.86^{ab}	11.08^{ab}	20.26^{abc}	21.73^{abc}	19.85^{bcd}	14.00^{a}	17.45^{ab}
63	3.55^{ab}	1.51^{a}	7.26^{a}	8.16^{d}	7.62^{ab}	13.25^{a}	17.48^{a}	13.72^{ab}	11.25^{ab}	18.60^{abc}	11.75^{a}	26.37^{bc}	27.47^{de}	13.37^{a}	28.74^{b}
70	5.50^{abc}	0.78^{a}	6.25^{a}	0.76^{ab}	3.64^{a}	7.12^{a}	16.96^{a}	9.66^{a}	12.58^{ab}	9.08^{a}	15.01^{ab}	20.80^{abc}	18.79^{bcd}	8.34^{a}	18.14^{ab}

Mean values with different superscripts along the column are significantly different ($P \leq 0.05$)

Keys

GS: Groundnut Shell
MC: Maize Cob
MS: Maize Straw
SB: Sugarcane Bagasse
RS: Rice Straw
PO: *Pleurotus ostreatus*
LS: *Lentinus squarrosulus*
POLS: Consortium of *Pleurotus ostreatus* and *Lentinus squarrosulus*

The effect of degradation time on released reducing sugar in selected lignocellulose(rice straw, maize straw and sugarcane bagasse) degraded for 35 days is shown in Table 4.8. The reducing sugar of rice straw degraded by PO, LS and POLS ranged from 15.50 – 16.89 mg/g, 14.70 – 16.51 mg/g and 15.50 – 16.63 mg/g with their highest at 21, 28 and 35 days of degradation respectively. Maize straw degraded by PO, LS and POLS had their reducing sugar ranging from 14.40 – 16.79 mg/g, 14.18 – 16.55 mg/g and 15.52 – 16.36 mg/g with their highest observed at 21, 35, and 28 days of degradation respectively. The highest reducing sugar released when sugarcane bagasse was degraded by PO (16.94 mg/g), LS (17.04 mg/g) and POLS (16.98 mg/g) were recorded at 35, 35 and 14 days of degradation respectively. Highest reducing sugar released in rice straw (16.89 mg/g), maize straw (16.79 mg/g) and sugarcane bagasse (16.98 mg/g) were observed on 21 (degraded by PO), 21 (degraded by PO) and 14 (degraded by POLS) days of degradation respectively. There was significant difference ($P \leq 0.05$) in the released reducing sugar from the selected lignocellulose with degradation time. There was no significant difference ($P > 0.05$) in the reducing sugar released from sugarcane bagasse by POLS at 14 days (16.98 mg/g) and 21 days (16.91 mg/g). Statistical analysis also revealed that there was no significant difference($P > 0.05$) in the reducing sugar released from sugarcane bagasse when degraded by PO. Rice straw, maize straw and sugarcane bagasse degraded by PO for 21 days and sugarcane bagasse degraded by POLS for 21 days were selected for further studies based on their reducing sugar content.

Sixty four (64) yeasts were isolated from palm wine and screened for their ability to produce ethanol through height of carbon dioxide in Durham tube per time. Isolate P412 produced maximum height (3.6 cm) of carbon dioxide in less than 60 hours and was selected for further studies.

Table 4.8: Effect of Degradation Time on the Amount ofReleased Reducing Sugar inSelectedLignocellulose

Period of Degradation (Days)	Rice straw			Maize straw			Sugarcane Bagasse		
	PO	LS	POLS	PO	LS	POLS	PO	LS	POLS
0	15.50^{a}	15.50^{b}	15.50^{a}	15.90^{bc}	15.90^{c}	15.90^{b}	16.66^{a}	16.66^{a}	16.66^{a}
7	16.62^{b}	16.15^{c}	16.11^{c}	14.40^{a}	15.81^{c}	15.52^{a}	16.78^{a}	16.87^{ab}	16.93^{ab}
14	16.67^{b}	14.70^{a}	15.81^{b}	15.81^{b}	15.57^{b}	15.68^{a}	16.75^{a}	16.79^{ab}	16.98^{b}
21	16.89^{c}	16.45^{d}	16.13^{c}	16.79^{d}	15.79^{c}	15.58^{a}	16.82^{a}	16.96^{ab}	16.91^{ab}
28	16.77^{bc}	16.51^{d}	16.32^{d}	16.02^{c}	14.18^{a}	16.36^{d}	16.89^{a}	16.89^{ab}	16.87^{ab}
35	16.63^{b}	16.15^{c}	16.63^{e}	15.81^{b}	16.55^{d}	16.17^{c}	16.94^{a}	17.04^{b}	16.97^{ab}

Mean values with different superscripts along the column are significantly different ($P \leq 0.05$)

Keys:

PO: *Pleurotus ostreatus*

LS: *Lentinus squarrosulus*

POLS: Consortium of *Pleurotus ostreatus* and *Lentinus squarrosulus*

Selected yeast isolate was identified as *Saccharomyces cerevisiae*when subjected to sequence comparison through BLAST nucleotide search tool at National Centre for Biotechnology Information (NCBI). Accession number of the selected yeast was MK038975 with strain name SA01. The commercial yeast used was identified as *Saccharomyces cerevisiae* SA02 with accession number of MN491900.

Phylogenic Analysis of *Saccharomycescerevisiae* SA01 and *Saccharomyces cerevisiae* SA02 is shown in Figure 4.1.*Saccharomyces cerevisiae* SA01 and *Saccharomyces cerevisiae* SA02 are closely related but have distant relationship with *Pichia membranifaciens* CBS107 and *Brettanomyces bruxellensis* CBS72.

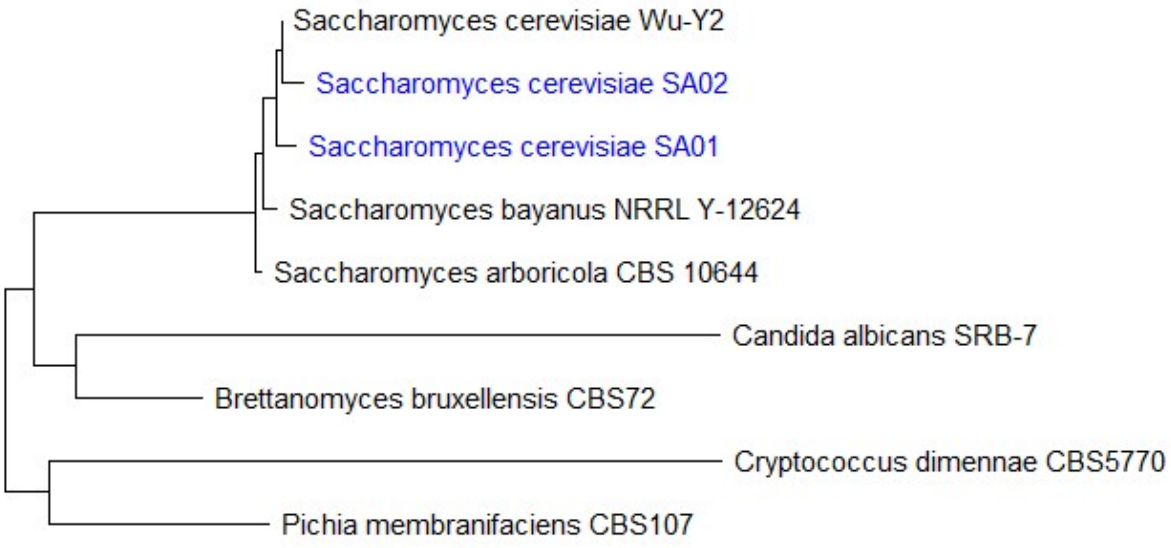

Figure 4.1: Phylogenic Analysis of *Saccharomycescerevisiae* SA01 and *Saccharomyces cerevisiae* SA02

The results of the reducing sugar of 7-days degraded substrates submerged in acetate buffer (0.1M, pH 5.0) fermented by two strains of *Saccharomyces cerevisiae* is shown in Table 4.9. Generally, there was decrease in reducing sugar content with increase in fermentation day. Reducing sugar of rice straw (RS), maize straw (MS) and sugarcane bagasse (SB) degraded by *Pleurotus ostreatus* and fermented by *Saccharomyces cerevisiae* SA01 ranged from 12.83 – 15.05 mg/g, 13.56 – 14.70 mg/g and 14.13 – 15.83 mg/g respectively. Reducing sugar content of 7-day *Lentinus squarrosulus* degraded substrates and fermented by *Saccharomyces cerevisiae* SA01 ranged from 13.19 – 16.09 mg/g with the least and highest in rice straw (after 168 hours fermentation) and sugar cane bagasse (before fermentation) respectively. Highest (16.79 mg/g) and lowest (13.10 mg/g) reducing sugar in substrates degraded for 7 days by consortium of *Pleurotus ostreatus* and *Lentinus squarrosulus*(POLS) and fermented by *Saccharomyces cerevisiae* SA01 was recorded in sugar cane bagasse (before fermentation) and rice straw (after 168 hours of fermentation) respectively. Reducing sugar of substrates degraded for 7 days by *Pleurotus ostreatus*, *Lentinus squarrosulus* and consortium of the two and fermented by *Saccharomyces cerevisiae*SA02 ranged from 12.57 – 16.00 mg/g, 12.99 – 16.09 mg/g and 12.87 – 16.14 mg/g respectively. Generally, there was significant difference ($P \leq 0.05$) on the reducing sugar content with increase in the period of fermentation by both *Saccharomyces cerevisiae* SA01 and *Saccharomyces cerevisiae* SA02.

Table 4.9: Reducing Sugar Content (mg/g) of Submerged 7-Day Degraded Substrates Fermented by *Saccharomyces cerevisiae*

Period of fermentation (hours)	Substrates	*Saccharomyces cerevisiae* SA01			*Saccharomyces cerevisiae* SA02		
		PO	LS	POLS	PO	LS	POLS
0	RS	14.70^{f}	15.07^{i}	15.77^{mn}	15.53^{i}	14.70^{fg}	15.04^{jk}
	MS	14.70^{f}	14.93^{hi}	15.00^{hi}	14.18^{ef}	14.29^{de}	14.46^{fg}
	SB	15.83^{k}	16.09^{k}	16.79^{o}	16.00^{j}	16.09^{k}	16.14^{o}
24	RS	14.65^{f}	14.60^{efg}	14.67^{efg}	15.56^{i}	14.73^{fg}	15.09^{jkl}
	MS	14.09^{de}	14.60^{efg}	14.58^{defg}	14.01^{de}	14.37^{de}	14.06^{cd}
	SB	14.32^{e}	15.92^{k}	15.92^{n}	15.90^{j}	16.00^{k}	15.81^{n}
48	RS	14.97^{gh}	14.75^{fgh}	15.08^{ij}	15.30^{hi}	14.52^{ef}	14.88^{ij}
	MS	14.32^{e}	14.35^{cde}	14.48^{cdef}	13.61^{c}	14.25^{de}	14.56^{gh}
	SB	15.39^{j}	15.58^{j}	15.60^{lm}	15.57^{i}	15.58^{ij}	15.34^{lm}
72	RS	14.69^{f}	14.62^{egf}	15.04^{ij}	14.97^{g}	14.35^{de}	14.73^{hi}
	MS	13.74^{bc}	14.48^{def}	14.47^{cdef}	13.84^{cd}	14.29^{de}	14.17^{de}
	SB	15.30^{ij}	15.41^{j}	15.47^{kl}	15.37^{hi}	15.73^{j}	15.49^{m}
96	RS	14.85^{fg}	14.67^{fg}	15.03^{hij}	15.23^{h}	14.85^{g}	14.74^{hi}
	MS	13.59^{b}	14.54^{efg}	14.36^{cd}	13.71^{c}	13.86^{c}	14.06^{cd}
	SB	15.15^{hij}	15.35^{j}	15.28^{jk}	15.33^{hi}	15.45^{hi}	15.42^{m}
120	RS	15.05^{ghi}	14.77^{gh}	15.15^{ij}	15.43^{hi}	14.36^{de}	14.73^{hi}
	MS	13.91^{cd}	14.48^{def}	14.73^{fg}	13.67^{c}	13.91^{c}	13.87^{c}
	SB	15.15^{hij}	15.37^{j}	15.26^{ijk}	15.34^{hi}	15.30^{h}	15.26^{klm}
144	RS	14.63^{f}	14.22^{cd}	14.78^{gh}	14.75^{g}	14.21^{d}	14.31^{defg}
	MS	13.56^{b}	13.78^{b}	13.61^{b}	12.83^{b}	13.55^{b}	12.87^{a}
	SB	14.29^{e}	14.37^{cde}	14.43^{cde}	14.43^{f}	14.48^{def}	14.39^{efg}
168	RS	12.83^{a}	13.19^{a}	13.10^{a}	12.57^{a}	12.99^{a}	12.92^{a}
	MS	13.60^{b}	13.25^{a}	13.63^{b}	13.76^{cd}	13.53^{b}	13.40^{b}
	SB	14.13^{de}	14.12^{c}	14.28^{c}	14.24^{ef}	14.37^{de}	14.24^{def}

Mean values with different superscripts along the column are significantly different ($P \leq 0.05$)

Key:

RS: Rice straw; MS: Maize straw; SB: Sugarcane bagasse

PO: *Pleurotus ostreatus*; LS: *Lentinus squarrosulus*:

POLS: Consortium of *Pleurotus ostreatus* and *Lentinus squarrosulus*

Table 4.10 shows the results of the amount of reducing sugar present in the filtrates extracted from 7-day pretreated substrates and fermented by the two (2) strains of *Saccharomyces cerevisiae*. From the table, the highest reducing sugar contents of PO-degraded substrates were observed after 24 hours fermentation with *Saccharomyces cerevisiae* SA01. The value ranges from 15.90 mg/g in sugarcane bagasse to 14.01 mg/g in maize straw and the values were significantly different ($P \leq 0.05$) from all other fermentation time except for unfermented (0 hour) and 96 hours fermented filtrate for rice straw (RS) and unfermented filtrate of SB. For LS-degraded substrates, the highest quantity of reducing sugar observed at 0 hour which ranges from 15.98 mg/g in SB to 14.97 mg/g in MS filtrates was significantly different ($P \leq 0.05$) from the amount of the reducing sugar at all other fermentation period. While for POLS-degraded substrates, the highest amount of reducing sugar was recorded in unfermented (0 hour) filtrates of MS (14.89 mg/g) and SB (15.87 mg/g) and 96-hour fermented filtrate of RS (15.50 mg/g). For filtrates fermented with *Saccharomyces cerevisiae* SA02, the highest reducing sugar of PO-degraded substrates was observed in unfermented filtrates. The value ranges from 13.90 mg/g in maize straw to 16.90 mg/g in sugarcane bagasse and the values were significantly different ($P \leq 0.05$) from all other fermentation except for 24, 48 and 72-hour fermented maize straw. For LS-degraded filtrates, the highest reducing sugar content was recorded in unfermented filtrates and the values were significantly different ($P \leq 0.05$) from all other fermentation period. The highest reducing sugar of POLS-degraded substrates were observed in unfermented (0 hour) filtrate of RS (14.96 mg/g), MS (14.43 mg/g) and SB (16.22 mg/g). Generally, highest reducing sugar contents was recorded at all fermentation time in SB-filtrate by the two *Saccharomyces cerevisiae* strains.

Reducing sugar content of submerged-14-day degraded substrates fermented by two strains of *Saccharomyces cerevisiae* is as shown in Table 4.11. For substrates fermented by *Saccharomyces cerevisiae* SA01, the reducing sugar content of PO, LS and POLS-degraded substrates ranged from 12.85 – 16.21 mg/g, 10.91 – 16.21 mg/g, and 12.54 – 16.81 mg/g respectively while those fermented by *Saccharomyces cerevisiae* SA02 ranged from 11.28 – 16.07 mg/g, 12.04 – 16.09 mg/g and 12.73 – 16.15 mg/g respectively. Their highest reducing sugar content were all obtained in degraded sugarcane bagasse before fermentation and were significantly different ($P \leq 0.05$) from all

other fermentation period. The least reducing sugar content of substrates degraded by PO and LS and fermented by *Saccharomyces cerevisiae* SA01 and *Saccharomyces cerevisiae* SA02 were all recorded in maize straw after 168 hours of fermentation. Substrates degraded by POLS and fermented by *Saccharomyces cerevisiae* SA01 and *Saccharomyces cerevisiae* SA02 had least reducing sugar in rice straw after 168 hours of fermentation.

Table 4.10: Reducing Sugar Content (mg/g) of Filtrate Extracted from 7-Day Degraded Substrates Fermented by *Saccharomyces cerevisiae*

Period of fermentation (hours)	Substrates	*Saccharomyces cerevisiae* SA01			*Saccharomyces cerevisiae*SA02		
		PO	LS	POLS	PO	LS	POLS
0	RS	15.08^{ij}	15.53^{j}	15.34^{ijk}	15.49^{m}	15.37^{hi}	14.96^{i}
	MS	13.68^{cde}	14.97^{ef}	14.89^{gh}	13.90^{fg}	14.41^{f}	14.43^{gh}
	SB	15.84^{l}	15.98^{k}	15.87^{m}	16.09^{n}	16.26^{k}	16.22^{m}
24	RS	15.09^{ij}	15.15^{fgi}	15.09^{hi}	15.07^{jk}	14.93^{g}	14.52^{h}
	MS	14.01^{f}	14.50^{cd}	14.47^{ef}	13.90^{fg}	14.37^{ef}	14.09^{ef}
	SB	15.90^{l}	15.56^{j}	15.76^{lm}	15.91^{n}	15.83^{j}	15.90^{l}
48	RS	14.88^{hi}	14.86^{e}	14.84^{gh}	14.86^{ij}	14.73^{g}	14.03^{e}
	MS	13.79^{def}	14.37^{cd}	14.27^{de}	13.78^{f}	14.13^{de}	13.71^{d}
	SB	15.54^{k}	15.54^{j}	15.50^{jk}	15.37^{lm}	15.50^{i}	15.50^{k}
72	RS	14.88^{hi}	14.99^{ef}	14.17^{d}	14.74^{i}	14.86^{g}	14.32^{fgh}
	MS	13.60^{bcd}	14.51^{cd}	14.70^{fg}	13.65^{ef}	14.39^{ef}	14.14^{ef}
	SB	15.23^{j}	15.39^{ij}	15.60^{kl}	15.53^{m}	15.43^{hi}	15.30^{jk}
96	RS	15.22^{j}	15.09^{efg}	15.50^{jk}	15.12^{kl}	13.49^{c}	14.43^{gh}
	MS	13.40^{b}	14.37^{cd}	13.76^{c}	13.14^{c}	14.39^{ef}	14.14^{ef}
	SB	15.22^{j}	15.26^{gi}	15.41^{jk}	15.19^{kl}	15.34^{hi}	15.46^{k}
120	RS	14.82^{h}	15.05^{efg}	14.78^{g}	14.71^{i}	14.74^{g}	14.22^{efg}
	MS	13.90^{ef}	14.46^{cd}	14.12^{d}	13.33^{cd}	14.17^{def}	13.56^{d}
	SB	15.11^{ij}	15.13^{fg}	15.33^{ij}	15.23^{kl}	15.23^{h}	15.11^{ij}
144	RS	14.41^{g}	14.55^{d}	14.35^{de}	14.13^{gh}	13.97^{d}	13.60^{d}
	MS	12.85^{a}	13.80^{b}	13.45^{b}	12.89^{b}	13.45^{c}	13.12^{c}
	SB	14.36^{g}	14.40^{cd}	14.32^{de}	14.33^{h}	14.33^{ef}	14.32^{fgh}
168	RS	12.92^{a}	13.31^{a}	12.99^{a}	12.36^{a}	12.51^{a}	11.96^{a}
	MS	13.53^{bc}	13.27^{a}	13.48^{b}	13.49^{de}	13.06^{b}	12.47^{b}
	SB	14.27^{g}	14.24^{c}	14.16^{d}	14.20^{h}	14.28^{ef}	14.10^{ef}

Mean values with different superscripts along the column are significantly different ($P \leq 0.05$)

Key:

RS: Rice straw; MS: Maize straw; SB: Sugarcane bagasse

PO: *Pleurotus ostreatus*; LS: *Lentinus squarrosulus*:

POLS: Consortium of *Pleurotus ostreatus* and *Lentinus squarrosulus*

Table 4.11: Reducing Sugar Content (mg/g) of Submerged 14-Day Degraded Substrates Fermented by *Saccharomyces cerevisiae*

Period of fermentation (hours)	Substrates	*Saccharomyces cerevisiae* SA01			*Saccharomyces cerevisiae* SA02		
		PO	LS	POLS	PO	LS	POLS
0	RS	15.94^{n}	15.15^{m}	14.90^{l}	15.88^{o}	15.52^{l}	14.84^{m}
	MS	14.96^{k}	13.79^{hi}	15.01^{l}	13.04^{f}	14.70^{ij}	15.13^{no}
	SB	16.21^{o}	16.21^{n}	16.81^{n}	16.07^{o}	16.09^{m}	16.15^{p}
24	RS	15.22^{lm}	14.39^{kl}	14.16^{ij}	15.13^{mn}	14.90^{jk}	14.22^{ijk}
	MS	14.20^{ghi}	13.11^{de}	14.46^{k}	12.43^{e}	13.93^{fg}	14.37^{kl}
	SB	15.37^{m}	15.34^{m}	15.43^{m}	15.33^{n}	15.53^{l}	15.35^{o}
48	RS	15.13^{klm}	13.95^{ij}	13.94^{ghi}	15.08^{mn}	13.95^{fg}	13.99^{ghi}
	MS	14.40^{ij}	13.31^{ef}	14.43^{k}	11.87^{c}	13.61^{e}	14.44^{kl}
	SB	15.11^{kl}	15.09^{m}	15.04^{l}	14.99^{m}	15.13^{k}	14.97^{mn}
72	RS	14.51^{j}	13.75^{hi}	13.84^{fgh}	14.62^{l}	14.22^{h}	13.69^{ef}
	MS	13.61^{cd}	12.61^{b}	13.88^{gh}	11.60^{b}	13.25^{d}	14.25^{jk}
	SB	14.52^{j}	14.50^{l}	14.33^{jk}	14.51^{kl}	14.62^{i}	14.51^{l}
96	RS	14.31^{hij}	13.61^{gh}	13.49^{cd}	14.20^{ij}	13.88^{f}	13.41^{cd}
	MS	13.60^{cd}	12.78^{bc}	13.75^{efg}	11.89^{c}	12.80^{b}	13.40^{cd}
	SB	14.12^{gh}	14.32^{kl}	14.33^{jk}	14.35^{jk}	14.35^{h}	14.56^{l}
120	RS	14.13^{gh}	13.26^{ef}	13.27^{bc}	13.99^{hi}	13.61^{e}	13.27^{c}
	MS	13.42^{bc}	12.69^{bc}	13.61^{def}	13.00^{f}	12.15^{a}	13.65^{de}
	SB	14.08^{fgh}	14.14^{jk}	14.06^{hi}	14.18^{ij}	14.17^{gh}	14.09^{hij}
144	RS	13.99^{efg}	13.38^{fg}	13.27^{bc}	13.80^{gh}	13.72^{ef}	13.36^{c}
	MS	13.33^{b}	12.81^{bc}	13.52^{cde}	12.19^{d}	13.18^{cd}	13.03^{b}
	SB	13.75^{de}	13.78^{hi}	13.83^{fgh}	13.91^{gh}	13.86^{ef}	13.78^{efg}
168	RS	13.75^{de}	12.92^{cd}	12.54^{a}	13.72^{g}	12.97^{bc}	12.73^{a}
	MS	12.85^{a}	10.91^{a}	13.07^{b}	11.28^{a}	12.04^{a}	12.87^{ab}
	SB	13.86^{def}	13.93^{ij}	13.83^{fgh}	14.02^{hi}	13.87^{ef}	13.94^{fgh}

Mean values with different superscripts along the column are significantly different ($P \leq 0.05$)

Key:

RS: Rice straw; MS: Maize straw; SB: Sugarcane bagasse

PO: *Pleurotus ostreatus*; LS: *Lentinus squarrosulus*:

POLS: Consortium of *Pleurotus ostreatus* and *Lentinus squarrosulus*

Table 4.12 shows the quantity of reducing sugar present in the filtrate extracted from 14-day degraded substrate. For filtrate fermented by *Saccharomyces cerevisiae* SA01, the highest amount of reducing sugar of PO-degraded rice straw (15.53 mg/g), maize straw (15.05 mg/g) and sugarcane bagasse (16.00 mg/g) were all recorded in unfermented filtrates and the values were significantly different ($P \leq 0.05$) from all other fermentation time. The highest reducing sugar content of LS-degraded substrates was observed in unfermented rice straw (15.00 mg/g) and sugarcane bagasse (16.05 mg/g) and 120-hour fermented maize straw (13.79 mg/g). The highest quantity of reducing sugar in POLS-degraded filtrates was recorded in unfermented filtrate. The value ranges from 14.66 mg/g in rice straw to 16.03 mg/g in sugarcane bagasse and the values were significantly different ($P \leq 0.05$) from all other fermentation time. For filtrates fermented with *Saccharomyces cerevisiae* SA02, the highest amount of reducing sugar of filtrates of PO-degraded substrates were recorded in unfermented filtrates. The values ranged from 14.85 mg/g in maize straw to 15.90 mg/g in sugarcane bagasse.For LS-degraded substrates, highest amount of reducing sugar was recorded in filtrate of unfermented rice straw (14.41 mg/g) and sugarcane bagasse (16.01 mg/g) and 96-hour fermented filtrate of maize straw. Highest quantity of reducing sugar in filtrate of POLS-degraded substrates were recorded in unfermented filtrate and the value ranged from 14.31 mg/g in rice straw to 16.03 mg/g in sugarcane bagasse which are significantly different ($P \leq 0.05$) to all other fermentation period.

The reducing sugar content of submerged 21-day degraded substrates fermented by two strains of *Saccharomyces cerevisiae* is shown in Table 4.13. Reducing sugar content of PO, LS and POLS-degraded substrates fermented by *Saccharomyces cerevisiae* SA01 ranged from 14.47 – 16.71 mg/g, 11.11 – 16.81 mg/g, and 10.86 – 16.90 mg/g with their highest values in non-fermented sugarcane bagasse. The highest reducing sugar content (16.90 mg/g) of PO-degraded substrates fermented by *Saccharomyces cerevisiae* SA02 was obtained in sugar cane bagasse followed by 16.85 mg/g (rice straw) and least (11.82 mg/g) was recorded in rice straw after 96 hours of fermentation. LS-degraded substrates fermented by *Saccharomyces cerevisiae* SA02 had reducing sugar ranging from 12.74 mg/g (120-hour fermented maize straw) to 16.15 mg/g (24-hour fermented sugarcane bagasse) while the reducing sugar of POLS-degraded substrate fermented by

Saccharomyces cerevisiae SA02 ranged from 12.19 in 168-hour fermented rice straw to 16.90 mg/g in unfermented sugarcane bagasse. There was generally decrease in the reducing sugar content with increase in fermentation period and fermentation period had significant effect ($P \leq 0.05$) on the amount of reducing sugar present.

Table 4.12: Reducing Sugar Content (mg/g) of Filtrate Extracted from 14-Day Degraded Substrates Fermented by *Saccharomyces cerevisiae*

Period of fermentation (hours)	Substrates	*Saccharomyces cerevisiae* SA01			*Saccharomyces cerevisiae* SA02		
		PO	LS	POLS	PO	LS	POLS
0	RS	15.53^{k}	15.00^{j}	14.66^{jk}	15.35^{n}	14.41^{l}	14.31^{h}
	MS	15.05^{ij}	13.64^{f}	15.22^{l}	14.85^{kl}	12.42^{e}	15.05^{ij}
	SB	16.00^{l}	16.05^{l}	16.03^{m}	15.90^{o}	16.10^{n}	16.03^{j}
24	RS	14.77^{h}	14.52^{i}	14.17^{gh}	14.63^{jk}	13.90^{j}	13.38^{d}
	MS	14.36^{g}	13.25^{e}	14.66^{jk}	14.31^{hi}	12.30^{e}	14.40^{h}
	SB	15.19^{j}	15.33^{k}	15.27^{l}	15.22^{mn}	15.27^{m}	15.20^{j}
48	RS	14.99^{hij}	13.82^{f}	13.93^{fg}	14.56^{ij}	13.59^{hi}	13.27^{d}
	MS	13.98^{def}	12.24^{c}	14.52^{ij}	13.93^{ef}	12.06^{d}	14.21^{gh}
	SB	14.82^{hi}	15.01^{j}	14.86^{k}	15.01^{lm}	15.05^{m}	14.90^{i}
72	RS	14.43^{g}	15.22^{jk}	13.49^{de}	14.03^{fg}	13.46^{h}	12.97^{c}
	MS	13.72^{cd}	12.04^{bc}	14.16^{gh}	13.56^{cd}	11.24^{b}	13.88^{ef}
	SB	14.47^{g}	14.31^{hi}	14.51^{ij}	14.46^{hij}	14.36^{l}	14.41^{h}
96	RS	13.99^{ef}	13.27^{e}	13.22^{bc}	14.03^{fg}	13.15^{g}	12.91^{c}
	MS	13.42^{b}	11.83^{b}	13.82^{f}	13.29^{b}	13.08^{g}	12.64^{b}
	SB	14.39^{g}	14.27^{hi}	14.39^{hi}	14.33^{hi}	14.20^{kl}	14.22^{gh}
120	RS	13.75^{cde}	13.26^{e}	12.99^{b}	13.38^{bc}	11.56^{c}	13.72^{e}
	MS	13.46^{b}	13.79^{f}	13.75^{f}	13.48^{bc}	11.18^{b}	13.67^{e}
	SB	14.06^{f}	14.08^{gh}	14.12^{g}	13.97^{ef}	13.99^{jk}	14.02^{fg}
144	RS	13.61^{bc}	13.34^{e}	13.36^{cd}	13.74^{de}	12.80^{f}	12.40^{a}
	MS	13.36^{b}	12.11^{c}	13.41^{cd}	13.76^{de}	10.31^{a}	13.22^{d}
	SB	13.82^{cdef}	13.83^{fg}	13.71^{ef}	14.24^{gh}	14.03^{jk}	13.82^{ef}
168	RS	13.61^{bc}	12.72^{d}	12.66^{a}	13.23^{b}	11.63^{c}	12.20^{a}
	MS	12.83^{a}	11.09^{a}	13.40^{cd}	12.89^{a}	11.05^{b}	13.42^{d}
	SB	13.84^{cdef}	13.88^{fg}	13.84^{f}	13.83^{ef}	13.82^{ij}	13.86^{ef}

Mean values with different superscripts along the column are significantly different ($P \leq 0.05$)

Key:

RS: Rice straw; MS: Maize straw; SB: Sugarcane bagasse

PO: *Pleurotus ostreatus*; LS: *Lentinus squarrosulus*:

POLS: Consortium of *Pleurotus ostreatus* and *Lentinus squarrosulus*

Table 4.13: Reducing Sugar Content (mg/g) of Submerged 21-Day Degraded Substrates Fermented by *Saccharomyces cerevisiae*

Period of fermentation (hours)	Substrates	*Saccharomyces cerevisiae* SA01			*Saccharomyces cerevisiae* SA02		
		PO	LS	POLS	PO	LS	POLS
0	RS	16.60^{l}	15.20^{hi}	15.90^{ij}	16.85^{l}	15.60^{kl}	16.71^{l}
	MS	16.53^{l}	14.58^{e}	14.65^{g}	16.71^{l}	15.13^{i}	14.08^{f}
	SB	16.71^{l}	16.81^{m}	16.90^{k}	16.90^{l}	15.45^{jk}	16.90^{l}
24	RS	16.11^{k}	15.54^{j}	15.46^{h}	15.84^{ij}	14.81^{g}	14.60^{g}
	MS	15.80^{j}	14.66^{ef}	14.50^{fg}	15.96^{jk}	14.86^{gh}	13.72^{d}
	SB	16.06^{k}	16.22^{l}	16.15^{j}	16.14^{k}	16.15^{m}	16.17^{k}
48	RS	15.43^{hi}	14.90^{fg}	15.13^{h}	15.54^{gh}	14.77^{fg}	14.67^{g}
	MS	15.79^{j}	14.59^{e}	14.63^{g}	15.65^{hi}	14.66^{fg}	14.05^{f}
	SB	15.64^{ij}	15.81^{k}	15.90^{ij}	15.84^{ij}	15.84^{l}	15.98^{k}
72	RS	15.30^{fgh}	15.00^{gh}	14.54^{fg}	15.33^{fg}	14.52^{f}	14.13^{f}
	MS	15.45^{hi}	11.11^{a}	14.20^{d}	15.45^{fgh}	14.08^{e}	13.53^{d}
	SB	15.73^{j}	15.80^{k}	15.73^{i}	15.68^{hi}	15.73^{l}	15.71^{j}
96	RS	15.13^{ef}	14.74^{ef}	10.86^{a}	11.82^{a}	13.48^{c}	13.99^{ef}
	MS	15.16^{efg}	12.99^{d}	13.29^{b}	13.48^{b}	13.88^{e}	13.53^{d}
	SB	15.39^{ghi}	15.28^{i}	15.34^{gh}	15.33^{fg}	15.34^{ij}	15.46^{i}
120	RS	14.90^{cde}	14.55^{e}	13.78^{c}	14.96^{e}	13.83^{de}	13.76^{de}
	MS	14.96^{cde}	12.19^{c}	13.90^{c}	14.63^{d}	12.74^{a}	12.92^{c}
	SB	14.89^{cde}	15.19^{hi}	15.24^{hi}	15.24^{f}	15.22^{ij}	15.22^{h}
144	RS	14.60^{ab}	13.03^{d}	14.37^{ef}	14.37^{c}	13.61^{cd}	13.59^{d}
	MS	14.82^{bcd}	11.93^{b}	13.30^{b}	14.77^{de}	13.98^{e}	13.03^{c}
	SB	14.97^{de}	15.16^{hi}	15.18^{h}	15.28^{fg}	15.18^{i}	15.19^{h}
168	RS	14.47^{a}	12.80^{d}	13.12^{b}	14.63^{d}	13.19^{b}	12.19^{a}
	MS	14.70^{abc}	11.98^{bc}	13.29^{b}	14.74^{de}	12.83^{a}	12.43^{b}
	SB	15.12^{ef}	15.08^{ghi}	15.16^{h}	15.24^{f}	15.08^{hi}	15.07^{h}

Mean values with different superscripts along the column are significantly different ($P \leq 0.05$)

Key:

RS: Rice straw; MS: Maize straw; SB: Sugarcane bagasse

PO: *Pleurotus ostreatus*; LS: *Lentinus squarrosulus*:

POLS: Consortium of *Pleurotus ostreatus* and *Lentinus squarrosulus*

Table 4.14 shows the reducing sugar content of filtrate extracted from 21-day degraded substrates and fermented by two strains of *Saccharomyces cerevisiae*. The highest amount of sugar recorded in PO-degraded substrates fermented by *Saccharomyces cerevisiae* SA01 was observed in unfermented maize straw (16.48 mg/g) and sugarcane bagasse (16.71 mg/g) and 24-hour fermented rice straw (16.28 mg/g). For LS-degraded substrate fermented by *Saccharomyces cerevisiae* SA01, the highest quantity of reducing sugar was recorded in unfermented substrate which ranged from 15.62 mg/g in maize straw to 16.62 mg/g in sugarcane bagasse. The values were significantly different ($P \leq 0.05$) from all other fermentation period except with 24-hour fermented filtrate of rice straw. The highest amount of reducing sugar of POLS-degraded substrates fermented by *Saccharomyces cerevisiae* SA01 were observed in unfermented filtrate and ranged from 15.34 mg/g in maize straw to 16.82 mg/g in rice straw. The reducing sugar values were significantly different ($P \leq 0.05$) from all other fermentation period except with 24-hour fermented filtrate of sugarcane bagasse. For filtrates fermented with *Saccharomyces cerevisiae* SA02, PO-degraded substrates recorded their highest amount of reducing sugar in unfermented filtrates ranging from 16.44 mg/g in maize straw to 16.63 mg/g in rice straw and sugarcane bagasse. LS-degraded substrates fermented by *Saccharomyces cerevisiae* SA02 recorded their highest amount of reducing sugar in unfermented filtrate. The value ranging from 15.34 mg/g in maize straw to 16.66 mg/g in sugarcane bagasse which are significantly different ($P \leq 0.05$) from all other fermentation period of the same substrate. POLS-degraded substrates fermented by *Saccharomyces cerevisiae* SA02 had their highest quantity of reducing sugar in unfermented substrates with highest (16.62 mg/g) in sugarcane bagasse followed by 15.12 mg/g in maize straw.

The reducing sugar content of submerged 28-day degraded substrates fermented by two strains of *Saccharomyces cerevisiae* is shown in Table 4.15.There was decrease in reducing sugar of filtrates with increase in the period of fermentation. Reducing sugar content of PO-degraded rice straw, maize straw and sugarcane bagasse fermented by *Saccharomyces cerevisiae* SA01 ranged from 13.18 – 16.36 mg/g, 12.13 – 15.70 mg/g and 15.01 – 16.86 mg/g respectively with the highest value of sugarcane bagasse in unfermented sample. Reducing sugar content of LS-degraded rice straw, maize straw and sugarcane bagasse fermented by *Saccharomyces cerevisiae* SA01 ranged from 12.88 –

15.83 mg/g, 11.64 – 15.16 mg/g and 15.16 – 16.87 mg/g respectively with their highest values in unfermented samples. The reducing sugar of POLS-degraded substrates fermented by *Saccharomyces cerevisiae* SA01 ranged from 14.39 – 16.93 mg/g with the least in maize straw after 144 hours of fermentation and highest in sugarcane bagasse before fermentation. The reducing sugar content of PO, LS and POLS-degraded substrates fermented by *Saccharomyces cerevisiae* SA02 ranged from 10.57 – 17.02 mg/g, 12.92 – 16.89 mg/g and 10.19 – 16.93 mg/g with their highest in unfermented sugarcane bagasse which were significantly different ($P \leq 0.05$) from all fermentation sample time.

Table 4.14: Reducing Sugar Content (mg/g) of Filtrate Extracted from 21-Day Degraded Substrates Fermented by *Saccharomyces cerevisiae*

Period of fermentation (hours)	Substrates	*Saccharomyces cerevisiae* SA01			*Saccharomyces cerevisiae* SA02		
		PO	LS	POLS	PO	LS	POLS
0	RS	15.31^{def}	15.64^{f}	15.94^{lm}	16.63^{m}	15.90^{m}	15.01^{hi}
	MS	16.48^{kl}	15.62^{f}	15.34^{ij}	16.44^{lm}	15.34^{jk}	15.12^{ij}
	SB	16.71^{l}	16.62^{i}	16.82^{m}	16.63^{m}	16.66^{n}	16.62^{m}
24	RS	16.28^{jk}	15.57^{f}	15.39^{j}	16.21^{l}	14.86^{gh}	14.97^{hi}
	MS	15.96^{hi}	15.13^{de}	14.86^{g}	15.87^{kl}	14.70^{fg}	14.50^{ef}
	SB	16.06^{ij}	16.14^{h}	16.14^{m}	15.96^{k}	15.98^{m}	16.06^{l}
48	RS	15.53^{fg}	15.08^{de}	15.03^{gh}	15.50^{ij}	14.94^{ghi}	14.67^{fg}
	MS	15.53^{fg}	14.92^{d}	14.62^{f}	15.68^{jk}	14.88^{gh}	14.80^{gh}
	SB	15.96^{hi}	15.92^{gh}	15.79^{kl}	15.81^{kl}	15.73^{lm}	15.87^{kl}
72	RS	15.72^{gh}	15.08^{de}	14.44^{ef}	15.72^{jkl}	14.78^{fg}	14.85^{gh}
	MS	15.64^{g}	14.65^{c}	14.31^{e}	15.18^{fg}	14.54^{ef}	14.13^{d}
	SB	15.68^{g}	15.72^{fg}	15.65^{k}	15.47^{hij}	15.57^{kl}	15.68^{k}
96	RS	14.93^{abc}	14.56^{c}	11.09^{a}	15.20^{fg}	13.61^{bc}	14.39^{e}
	MS	14.81^{ab}	13.31^{a}	13.86^{c}	13.33^{a}	13.56^{b}	13.88^{d}
	SB	15.34^{ef}	15.23^{e}	15.35^{ij}	15.35^{ghi}	15.34^{ij}	15.34^{j}
120	RS	14.92^{abc}	14.47^{c}	14.36^{e}	14.01^{b}	14.37^{de}	14.08^{d}
	MS	14.93^{abc}	13.78^{b}	14.02^{cd}	14.80^{cd}	13.26^{a}	13.30^{b}
	SB	15.16^{cde}	15.19^{e}	15.24^{hij}	15.23^{fgh}	15.47^{k}	15.19^{ij}
144	RS	14.96^{abc}	14.48^{c}	14.21^{de}	15.05^{def}	14.32^{de}	13.90^{d}
	MS	15.05^{bcd}	13.45^{a}	13.23^{b}	14.84^{d}	14.16^{d}	13.63^{c}
	SB	15.19^{cde}	15.19^{e}	15.13^{hij}	15.16^{efg}	15.08^{hi}	15.12^{ij}
168	RS	14.70^{a}	13.98^{b}	13.19^{b}	14.85^{d}	13.82^{c}	13.10^{ab}
	MS	14.85^{ab}	13.45^{a}	13.14^{b}	14.58^{c}	13.64^{bc}	12.95^{a}
	SB	15.15^{cde}	15.72^{fg}	15.12^{hi}	14.92^{de}	15.15^{ij}	15.12^{ij}

Mean values with different superscripts along the column are significantly different ($P \leq 0.05$)

Key:

RS: Rice straw; MS: Maize straw; SB: Sugarcane bagasse

PO: *Pleurotus ostreatus*; LS: *Lentinus squarrosulus*:

POLS: Consortium of *Pleurotus ostreatus* and *Lentinus squarrosulus*

Table 4.15: Reducing Sugar Content (mg/g) of Submerged 28-day Degraded Substrates Fermented by *Saccharomyces cerevisiae*

Period of fermentation (hours)	Substrates	*Saccharomyces cerevisiae* SA01			*Saccharomyces cerevisiae* SA02		
		PO	LS	POLS	PO	LS	POLS
0	RS	15.90^{kl}	15.83^{i}	16.05^{ef}	11.00^{b}	14.90^{fg}	10.19^{a}
	MS	15.16^{gh}	15.16^{g}	15.03^{b}	12.68^{d}	14.29^{de}	14.16^{d}
	SB	16.86^{p}	16.87^{kl}	16.93^{i}	17.02^{o}	16.89^{l}	16.93^{n}
24	RS	16.36^{mn}	15.77^{i}	16.22^{fgh}	14.73^{ij}	16.19^{jk}	15.61^{ijk}
	MS	15.70^{jk}	14.08^{e}	15.83^{de}	14.66^{i}	13.31^{b}	15.80^{k}
	SB	16.62^{o}	16.66^{kl}	16.77^{i}	16.71^{n}	16.64^{l}	16.68^{m}
48	RS	13.18^{d}	12.88^{d}	16.15^{fg}	15.85^{m}	14.50^{e}	15.18^{fgh}
	MS	14.58^{f}	11.94^{b}	15.88^{de}	14.24^{g}	12.92^{a}	15.72^{jk}
	SB	16.13^{lm}	16.38^{j}	16.45^{h}	16.66^{n}	16.67^{l}	16.64^{lm}
72	RS	16.28^{mn}	15.83^{i}	16.26^{fgh}	13.95^{f}	16.05^{ij}	15.41^{hi}
	MS	12.35^{ab}	12.09^{b}	15.16^{b}	10.57^{a}	15.23^{h}	15.03^{f}
	SB	16.24^{mn}	16.44^{jk}	16.45^{h}	16.70^{n}	16.38^{k}	16.40^{l}
96	RS	16.24^{mn}	15.39^{gh}	16.05^{ef}	15.77^{m}	15.90^{i}	12.69^{b}
	MS	13.74^{e}	14.06^{e}	15.65^{cd}	11.63^{c}	14.80^{f}	15.47^{ij}
	SB	16.44^{no}	16.36^{j}	16.40^{gh}	16.40^{n}	16.37^{k}	16.51^{lm}
120	RS	16.19^{mn}	14.69^{f}	15.43^{c}	14.92^{jk}	14.84^{f}	13.65^{c}
	MS	12.13^{a}	11.64^{a}	14.46^{a}	12.61^{d}	13.94^{c}	14.28^{de}
	SB	15.61^{ij}	15.68^{i}	15.80^{de}	15.88^{m}	15.85^{i}	15.79^{k}
144	RS	15.41^{hi}	14.69^{f}	15.11^{b}	14.56^{hi}	14.81^{f}	13.84^{c}
	MS	12.43^{bc}	12.42^{c}	14.39^{a}	12.83^{d}	14.08^{cd}	14.48^{e}
	SB	15.30^{h}	15.43^{h}	15.15^{b}	15.35^{l}	15.31^{h}	15.38^{ghi}
168	RS	15.00^{g}	14.44^{f}	14.96^{b}	14.33^{gh}	14.43^{e}	13.79^{c}
	MS	12.64^{c}	12.74^{d}	14.55^{a}	13.63^{e}	14.17^{cd}	14.22^{d}
	SB	15.01^{g}	15.16^{g}	15.09^{b}	15.13^{kl}	15.12^{gh}	15.13^{fg}

Mean values with different superscripts along the column are significantly different ($P \leq 0.05$)

Key:

RS: Rice straw; MS: Maize straw; SB: Sugarcane bagasse

PO: *Pleurotus ostreatus*; LS: *Lentinus squarrosulus*:

POLS: Consortium of *Pleurotus ostreatus* and *Lentinus squarrosulus*

The reducing sugar content of filtrates extracted from 28-day degraded substrates and fermented by two strains of *Saccharomyces cerevisiae* is as shown in Table 4.16. The highest amount of the reducing sugar obtained in filtrate of PO-degraded substrate fermented by *Saccharomyces cerevisiae* SA01 was recorded in 24-hour fermented filtrate. The values ranged from 15.80 mg/g (maize) to 16.74 mg/g (sugarcane bagasse) which are significantly different ($P \leq 0.05$) from all other fermentation time of the same substrate with the exception of unfermented filtrate of sugarcane bagasse, and 48-hour and 96-hour fermented sugarcane bagasse. The highest quantity of LS-degraded substrate fermented by *Saccharomyces cerevisiae* SA01 were recorded in unfermented filtrates for rice straw (16.70 mg/g) and sugarcane bagasse(16.67 mg/g) and 48-hour fermented filtrate for maize straw (16.09 mg/g). Filtrate of POLS-degraded substrate fermented by *Saccharomyces cerevisiae* SA01 had their highest amount of reducing sugar in unfermented filtrate which ranged from 15.62 mg/g in rice straw to 16.90 mg/g in sugarcane bagasse which are all significantly different ($P \leq 0.05$) from all other fermentation period of the same substrate except filtrate of 96-hour fermented rice straw. The highest quantity of reducing sugar in filtrate of PO-pretreated fermented by *Saccharomyces cerevisiae* SA02 was obtained in unfermented filtrates of rice straw (16.30 mg/g) and sugarcane bagasse (16.79 mg/g) and 24-hour fermented maize straw (15.15 mg/g). For LS-degraded substrate fermented by *Saccharomyces cerevisiae* SA02, the highest reducing sugar was observed in unfermented filtrate of sugarcane bagasse (16.85 mg/g), 24-hour fermented rice straw (16.32 mg/g) and 96-hour fermented maize straw (14.47 mg/g). For POLS-degraded substrates fermented by *Saccharomyces cerevisiae* SA02, highest amount of reducing sugar was obtained in unfermented filtrate ranging from 15.46 mg/g in rice straw to 16.89 mg/g in sugarcane bagasse and these values are significantly different ($P \leq 0.05$) from all other fermentation period of the same substrate except 24-hour and 96-hour fermented maize straw.

Table 4.16: Reducing Sugar Content (mg/g) of Filtrate Extracted from 28-Day Degraded Substrates Fermented by *Saccharomyces cerevisiae*

Period of fermentation (hours)	Substrates	*Saccharomyces cerevisiae* SA01			*Saccharomyces cerevisiae* SA02		
		PO	LS	POLS	PO	LS	POLS
0	RS	15.90^{k}	16.70^{k}	15.62^{jk}	16.30^{ij}	15.00^{e}	15.46^{f}
	MS	15.16^{fg}	15.03^{ef}	16.26^{m}	14.65^{de}	13.71^{ab}	15.75^{hi}
	SB	16.71^{m}	16.67^{k}	16.90^{o}	16.79^{l}	16.85^{l}	16.89^{l}
24	RS	16.36^{l}	16.06^{i}	12.59^{a}	16.11^{i}	16.32^{ij}	15.13^{de}
	MS	15.80^{jk}	14.73^{cd}	15.90^{l}	15.15^{fg}	13.88^{bc}	15.72^{ghi}
	SB	16.74^{m}	16.64^{k}	16.60^{n}	16.59^{kl}	16.67^{kl}	16.63^{k}
48	RS	15.60^{ij}	16.30^{ij}	13.04^{b}	16.32^{ij}	15.71^{fg}	14.93^{d}
	MS	15.42^{hi}	16.09^{i}	15.35^{hi}	14.90^{ef}	13.88	15.49^{fg}
	SB	16.56^{lm}	16.48^{jk}	16.45^{mn}	16.44^{jk}	16.57^{jk}	16.60^{jk}
72	RS	15.76^{jk}	16.29^{ij}	14.36^{ef}	14.71^{de}	15.94^{gh}	15.03^{d}
	MS	14.22^{c}	12.97^{a}	15.23^{h}	13.74^{c}	14.10^{c}	15.41^{f}
	SB	16.38^{l}	16.26^{ij}	16.37^{mn}	16.40^{jk}	16.34^{ij}	16.45^{jk}
96	RS	15.90^{k}	16.17^{i}	15.38^{hij}	15.65^{h}	16.13^{hi}	14.97^{d}
	MS	14.09^{c}	14.54^{c}	15.56^{ijk}	15.04^{f}	14.47^{d}	15.52^{fgh}
	SB	16.49^{lm}	16.33^{ij}	16.40^{mn}	16.32^{ij}	16.47^{jk}	16.37^{j}
120	RS	14.97^{ef}	15.60^{h}	13.84^{c}	14.70^{de}	15.66^{f}	14.05^{b}
	MS	13.75^{b}	13.41^{b}	14.59^{fg}	13.31^{b}	13.52^{a}	14.90^{d}
	SB	15.72^{jk}	15.72^{h}	15.80^{kl}	15.66^{h}	15.76^{fg}	15.81^{i}
144	RS	14.88^{e}	15.24^{fg}	14.08^{cd}	14.66^{de}	15.26^{e}	14.02^{b}
	MS	13.48^{a}	13.22^{ab}	14.71^{g}	12.99^{a}	13.53^{a}	14.43^{c}
	SB	15.33^{gh}	15.31^{g}	15.35^{hi}	15.30^{g}	15.26^{e}	15.30^{ef}
168	RS	14.54^{d}	14.96^{de}	13.94^{c}	14.52^{d}	15.00^{e}	13.57^{a}
	MS	13.40^{a}	13.21^{ab}	14.22^{de}	13.34^{b}	13.49^{a}	14.12^{b}
	SB	15.15^{fg}	15.16^{efg}	15.19^{h}	15.09^{fg}	15.11^{e}	15.04^{d}

Mean values with different superscripts along the column are significantly different ($P \leq 0.05$)

Key:

RS: Rice straw; MS: Maize straw; SB: Sugarcane bagasse

PO: *Pleurotus ostreatus*; LS: *Lentinus squarrosulus*:

POLS: Consortium of *Pleurotus ostreatus* and *Lentinus squarrosulus*

Table 4.17 shows the reducing sugar content of submerged 35-day degraded substrates fermented by two strains of *Saccharomyces cerevisiae*. Highest reducing sugar content in degraded rice straw (16.51 mg/g), maize straw (16.28 mg/g) and sugar cane bagasse (16.91 mg/g) fermented by *Saccharomyces cerevisiae* SA01 were recorded in samples degraded by POLS, LS, and PO respectively just before fermentation. Reducing sugar content of 35-day degraded substrates fermented by *Saccharomyces cerevisiae* SA01 decreased with increase in fermentation day. PO, LS and POLS-degraded substrates fermented by *Saccharomyces cerevisiae* SA02 for 168 hours had reducing sugar content that ranged from 13.14 mg/g (168-hour fermented maize straw) to 16.87 mg/g (unfermented sugarcane bagasse); 13.21 mg/g (168-hour fermented rice straw) to 16.98 mg/g (unfermented sugarcane bagasse) and 13.27 mg/g (168-hour fermented maize straw) to 16.91 mg/g (unfermented sugarcane bagasse) respectively. Generally, there was decrease in reducing sugar content with increase in fermentation period and statistical analysis revealed that period of fermentation had significant effect ($P \leq 0.05$) on the reducing sugar content.

The reducing sugar content of filtrates extracted from 35-day degraded substrates and fermented by two strains of *Saccharomyces cerevisiae* is shown in Table 4.18. The highest amount of reducing sugar obtained in PO-degraded substrates fermented by *Saccharomyces cerevisiae* SA01 was observed in unfermented filtrate in sugarcane bagasse (16.78 mg/g) and 24-hour fermented filtrate in rice straw (16.22 mg/g) and maize straw (13.74 mg/g). The highest quantity of reducing sugar in LS-degraded substrates fermented by *Saccharomyces cerevisiae* SA01 was recorded in unfermented filtrates which ranged from 16.07 mg/g in rice straw to 16.86 mg/g in sugarcane bagasse. The values were significantly different ($P \leq 0.05$) from all other fermentation time of the same substrate. For POLS-degraded substrates fermented by *Saccharomyces cerevisiae* SA01, highest amount of reducing sugar was observed in unfermented filtrate which ranged from 15.71 mg/g in maize straw to 16.71 mg/g in rice straw. These values were significantly different ($P \leq 0.05$) from all other fermentation period of the same substrate. For PO-degraded substrates fermented by *Saccharomyces cerevisiae* SA02, the highest quantity of reducing sugar was recorded in unfermented filtrate which ranged from 14.21 mg/g in maize straw to 16.74 mg/g in sugarcane bagasse. For LS-degraded substrates fermented

by *Saccharomyces cerevisiae* SA02, highest amount of reducing sugar was recorded in unfermented filtrate which ranged from 15.98 mg/g in rice straw to 16.60 mg/g in sugarcane bagasse. These values were significantly different ($P \leq 0.05$) from all other fermentation period of the same substrate. For POLS-degraded substrates fermented by *Saccharomyces cerevisiae* SA02, highest amount of reducing sugar was recorded in unfermented filtrate in rice straw (16.53 mg/g) and 16.81 mg/g in sugarcane bagasse and 48-hour fermented filtrate in maize straw (14.93 mg/g).

Table 4.17: Reducing Sugar Content (mg/g) of Submerged 35-Day Degraded Substrates Fermented by *Saccharomyces cerevisiae*

Period of fermentation (hours)	Substrates	*Saccharomyces cerevisiae* SA01			*Saccharomyces cerevisiae* SA02		
		PO	LS	POLS	PO	LS	POLS
0	RS	16.19^{mn}	16.41^{l}	16.51^{k}	16.47^{l}	15.24^{efg}	16.47^{n}
	MS	13.78^{bc}	16.28^{kl}	14.51^{cd}	13.80^{c}	16.53^{l}	13.75^{b}
	SB	16.91^{o}	16.85^{m}	16.85^{l}	16.87^{n}	16.98^{m}	16.91^{o}
24	RS	16.15^{m}	16.02^{j}	16.17^{j}	16.02^{ij}	15.04^{ef}	16.02^{lm}
	MS	14.06^{de}	15.85^{ij}	15.22^{g}	14.21^{d}	15.98^{ij}	14.51^{de}
	SB	16.41^{n}	16.41^{l}	16.28^{jk}	16.33^{kl}	16.29^{k}	16.22^{m}
48	RS	15.53^{ij}	15.56^{gh}	15.72^{i}	15.33^{gh}	14.36^{c}	15.62^{jk}
	MS	14.02^{cde}	15.43^{fg}	14.86^{ef}	14.21^{d}	15.47^{gh}	14.59^{def}
	SB	16.00^{lm}	16.07^{jk}	16.07^{j}	16.13^{jk}	16.18^{jk}	16.13^{m}
72	RS	15.26^{h}	15.39^{efg}	15.56^{hi}	15.52^{h}	14.37^{c}	15.43^{ij}
	MS	13.74^{b}	15.47^{fgh}	14.65^{cde}	13.76^{c}	15.60^{h}	14.36^{cd}
	SB	15.88^{kl}	15.84^{ij}	15.81^{i}	15.84^{i}	15.94^{ij}	15.83^{kl}
96	RS	15.30^{hi}	15.33^{defg}	15.46^{gh}	15.31^{gh}	14.52^{cd}	15.16^{h}
	MS	13.83^{bcd}	15.30^{defg}	14.52^{cd}	13.90^{c}	15.22^{efg}	14.18^{c}
	SB	15.73^{jk}	15.69^{hi}	15.73^{i}	15.85^{i}	15.85^{i}	15.79^{kl}
120	RS	15.19^{h}	15.13^{cde}	15.30^{g}	15.23^{g}	13.84^{b}	15.03^{gh}
	MS	13.69^{b}	15.08^{cd}	14.56^{cd}	13.19^{a}	15.00^{e}	14.16^{c}
	SB	15.26^{h}	15.28^{def}	15.27^{g}	15.31^{gh}	15.27^{fg}	15.26^{hi}
144	RS	14.63^{fg}	14.62^{b}	14.73^{def}	14.71^{ef}	13.90^{b}	14.50^{de}
	MS	13.44^{a}	14.51^{b}	13.98^{b}	13.48^{b}	14.35^{c}	13.64^{b}
	SB	14.75^{g}	14.92^{c}	14.93^{f}	14.92^{f}	14.69^{d}	14.65^{ef}
168	RS	14.12^{e}	14.18^{a}	14.40^{c}	14.50^{e}	13.21^{a}	14.82^{fg}
	MS	13.34^{a}	14.21^{a}	13.65^{a}	13.14^{a}	14.35^{c}	13.27^{a}
	SB	14.43^{f}	14.48^{b}	14.44^{c}	14.50^{e}	14.52^{cd}	14.52^{de}

Mean values with different superscripts along the column are significantly different ($P \leq 0.05$)

Key:

RS: Rice straw; MS: Maize straw; SB: Sugarcane bagasse

PO: *Pleurotus ostreatus*; LS: *Lentinus squarrosulus*:

POLS: Consortium of *Pleurotus ostreatus* and *Lentinus squarrosulus*

Table 4.18: Reducing Sugar Content (mg/g) of Filtrate Extracted from 35-Day Degraded Substrates Fermented by *Saccharomyces cerevisiae*

Period of fermentation (hours)	Substrates	*Saccharomyces cerevisiae* SA01			*Saccharomyces cerevisiae* SA02		
		PO	LS	POLS	PO	LS	POLS
0	RS	16.00^{jkl}	16.07^{k}	16.71^{k}	16.49^{k}	15.98^{no}	16.53^{l}
	MS	13.59^{c}	16.36^{l}	15.71^{g}	14.21^{e}	16.28^{p}	13.72^{ab}
	SB	16.78^{m}	16.86^{m}	16.70^{k}	16.74^{l}	16.60^{q}	16.81^{m}
24	RS	16.22^{l}	15.56^{hi}	16.37^{j}	15.98^{ij}	15.56^{kl}	16.10^{k}
	MS	13.74^{c}	15.76^{ij}	15.18^{ef}	13.79^{d}	15.72^{klm}	14.41^{de}
	SB	16.25^{l}	16.26^{kl}	16.10^{i}	16.17^{j}	16.26^{p}	16.25^{k}
48	RS	15.61^{ghi}	15.11^{ef}	15.76^{gh}	15.56^{h}	15.20^{hi}	15.60^{j}
	MS	13.18^{b}	15.43^{gh}	15.16^{ef}	13.15^{c}	15.50^{ijk}	14.93^{gh}
	SB	16.06^{kl}	16.17^{kl}	16.00^{hi}	16.15^{j}	16.15^{op}	16.03^{k}
72	RS	15.52^{gh}	15.09^{ef}	15.68^{g}	15.31^{g}	14.96^{gh}	15.58^{j}
	MS	13.22^{b}	15.24^{fg}	14.94^{de}	13.03^{c}	15.27^{ij}	14.74^{fg}
	SB	15.83^{ijk}	15.83^{j}	15.72^{g}	15.84^{i}	15.92^{mno}	15.72^{j}
96	RS	15.12^{f}	15.07^{ef}	15.34^{f}	15.20^{g}	14.80^{fg}	15.15^{hi}
	MS	13.08^{b}	15.19^{fg}	14.74^{cd}	13.18^{c}	15.27^{ij}	14.74^{fg}
	SB	15.77^{hij}	15.81^{ij}	15.77^{gh}	15.81^{i}	15.80^{lmn}	15.76^{j}
120	RS	15.01^{f}	14.67^{cd}	15.34^{f}	15.12^{g}	14.32^{cd}	15.13^{hi}
	MS	12.72^{a}	14.88^{de}	14.63^{bc}	12.77^{b}	14.55^{def}	13.95^{bc}
	SB	15.41^{g}	15.09^{ef}	15.18^{ef}	15.26^{g}	15.20^{hi}	15.27^{i}
144	RS	14.62^{e}	14.10^{a}	14.52^{bc}	14.47^{f}	14.13^{bc}	14.29^{d}
	MS	12.69^{a}	14.40^{b}	13.84^{a}	12.36^{a}	14.09^{bc}	13.79^{abc}
	SB	14.66^{e}	14.65^{bcd}	14.55^{bc}	14.67^{f}	14.66^{ef}	14.60^{ef}
168	RS	14.03^{d}	13.97^{a}	14.46^{b}	13.93^{d}	13.67^{a}	13.98^{c}
	MS	12.78^{a}	14.12^{a}	13.93^{a}	12.43^{a}	13.99^{b}	13.60^{a}
	SB	14.63^{e}	14.46^{bc}	14.51^{bc}	14.55^{f}	14.50^{de}	14.41^{de}

Mean values with different superscripts along the column are significantly different ($P \leq 0.05$)

Key:

RS: Rice straw; MS: Maize straw; SB: Sugarcane bagasse

PO: *Pleurotus ostreatus*; LS: *Lentinus squarrosulus*:

POLS: Consortium of *Pleurotus ostreatus* and *Lentinus squarrosulus*

Ethanol content of 21-day degraded substrates fermented by two strains of *Saccharomyces cerevisiae* is as shown in Table 4.19a. Highest ethanol content recorded by all substrates fermented by *Saccharomyces cerevisiae* SA01 was on day 3 except PO-degraded rice straw (RSPO) which had highest ethanol content (3.95 g/L) on day 5. Maize straw filtrates recorded highest ethanol contents at all-time except on day 4. Equal volume of ethanol (2.76 g/L) recorded by all substrates by *Saccharomyces cerevisiae* SA02 at different fermentation time with the shortest observed for RSPO and the longest for SBPO. Fermentation time had significant effect ($P \leq 0.05$) on the ethanol yield of *Saccharomyces cerevisiae* SA01 and *Saccharomyces cerevisiae* SA02.Maize straw was selected for further work based on its highest ethanol yield at most time of the fermentation period.

Table 4.19b shows the reducing sugar of 21-day degraded substrates fermented by two strains of *Saccharomyces cerevisiae*. The amount of reducing sugar released by all degraded substrates decreases as the fermentation time increases. Highest quantity of reducing sugar was recorded in SBPOLS by the 2 strains at all fermentation period. The highest (16.89 mg/g) and least (15.61 mg/g) amount of reducing sugar were recorded in filtrate of POLS-degraded sugarcane bagasse (before fermentation) and PO-degraded rice straw (after 5-day fermentation) fermented by *Saccharomyces cerevisiae* SA01 respectively. Period of fermentation had significant effect ($P \leq 0.05$) on the amount of reducing sugar.

High pH values was recorded in the filtrate of PO-degraded maize straw (MSPO)as shown in Table 4.19c. Low pH values was recorded for sugarcane bagasse degraded filtrates (SBPO and SBPOLS). The pH of the filtrates increased with fermentation time except for sugarcane bagasse-degraded filtrates (SBPO and SBPOLS) which appeared stable. There was no significant different ($P > 0.05$) in the pH of filtrate of SBPOLS with increase in fermentation period.

Table 4.19a: Ethanol Content (g/L) of 21-Day Degraded Substrates Fermented by *Saccharomyces cerevisiae*

Period of fermentation (day)	Fermenting Yeasts							
	***Saccharomyces cerevisiae* SA01**				***Saccharomyces cerevisiae* SA02**			
	RSPO	**MSPO**	**SBPO**	**SBPOLS**	**RSPO**	**MSPO**	**SBPO**	**SBPOLS**
0	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
1	2.37^{c}	2.76^{b}	0.79^{b}	1.97^{b}	2.76^{b}	1.58^{b}	1.18^{b}	1.18^{b}
2	2.76^{c}	3.16^{b}	2.37^{c}	2.37^{b}	1.97^{b}	2.76^{c}	1.97^{b}	2.76^{c}
3	1.97^{c}	3.95^{c}	3.95^{d}	2.76^{b}	1.97^{b}	1.18^{b}	2.76^{c}	2.76^{c}
4	0.79^{b}	0.39^{a}	2.37^{c}	2.37^{b}	1.97^{b}	1.58^{b}	1.58^{b}	1.97^{c}
5	3.95^{d}	2.37^{b}	2.37^{c}	2.37^{b}	2.37^{b}	1.58^{b}	0.00^{a}	0.39^{a}

Mean values with different superscripts along the column are significantly different ($P \leq 0.05$)

Keys: **RSPO:** Filtrate of rice straw degraded by *Pleurotus ostreatus*
MSPO: Filtrate of maize straw degraded by *Pleurotus ostreatus*
SBPO: Filtrate of sugarcane bagasse degraded by *Pleurotus ostreatus*
SBPOLS: Filtrate of sugarcane bagasse degraded by consortium of *Pleurotus ostreatus* and *Lentinus squarrosulus*

Table 4.19b: Reducing Sugar (mg/g) of 21-Day Degraded Substrates Fermented by *Saccharomyces cerevisiae*

Period of fermentation (day)	Fermenting Yeasts							
	***Saccharomyces cerevisiae*SA01**				***Saccharomyces cerevisiae* SA02**			
	RSPO	**MSPO**	**SBPO**	**SBPOLS**	**RSPO**	**MSPO**	**SBPO**	**SBPOLS**
0	16.48^{d}	16.52^{c}	16.78^{d}	16.89^{d}	16.60^{d}	16.48^{c}	16.74^{e}	16.82^{e}
1	16.55^{d}	16.45^{c}	16.79^{d}	16.78^{d}	16.41^{c}	16.40^{c}	16.56^{de}	16.71^{de}
2	16.26^{c}	16.37^{c}	16.44^{c}	16.52^{c}	16.30^{c}	16.30^{c}	16.48^{cd}	16.49^{cd}
3	16.15^{c}	16.15^{b}	16.28^{bc}	16.32^{bc}	16.09^{b}	16.07^{b}	16.24^{bc}	16.28^{bc}
4	15.90^{b}	15.99^{b}	16.15^{ab}	16.22^{b}	15.98^{b}	15.96^{ab}	16.13^{ab}	16.21^{b}
5	15.61^{a}	15.72^{a}	15.96^{a}	15.95^{a}	15.73^{a}	15.79^{a}	15.95^{a}	15.84^{a}

Mean values with different superscripts along the column are significantly different ($P \leq 0.05$)

Keys: **RSPO:** Filtrate of rice straw degraded by *Pleurotus ostreatus*
MSPO: Filtrate of maize straw degraded by *Pleurotus ostreatus*
SBPO: Filtrate of sugarcane bagasse degraded by *Pleurotus ostreatus*
SBPOLS: Filtrate of sugarcane bagasse degraded by consortium of *Pleurotus ostreatus* and *Lentinus squarrosulus*

Table 4.19c: pH of 21-Day Degraded Substrates Fermented by *Saccharomyces cerevisiae*

Period of fermentation (day)	Fermenting Yeasts							
	***Saccharomyces cerevisiae*SA01**				***Saccharomyces cerevisiae* SA02**			
	RSPO	**MSPO**	**SBPO**	**SBPOLS**	**RSPO**	**MSPO**	**SBPO**	**SBPOLS**
0	5.11^{ab}	5.20^{a}	4.93^{ab}	4.89^{a}	5.09^{ab}	5.21^{a}	4.93^{a}	4.89^{a}
1	5.09^{a}	5.24^{ab}	4.96^{b}	4.89^{a}	5.08^{a}	5.24^{ab}	4.92^{a}	4.88^{a}
2	5.10^{ab}	5.26^{b}	4.91^{a}	4.91^{a}	5.10^{ab}	5.26^{bc}	4.90^{a}	4.89^{a}
3	5.11^{ab}	5.27^{bc}	4.96^{b}	4.90^{a}	5.12^{ab}	5.27^{bc}	4.91^{a}	4.89^{a}
4	5.12^{ab}	5.31^{cd}	4.93^{ab}	4.89^{a}	5.13^{bc}	5.29^{cd}	4.98^{b}	4.89^{a}
5	5.14^{b}	5.34^{d}	4.93^{ab}	4.90^{a}	5.15^{c}	5.31^{d}	4.92^{a}	4.90^{a}

Mean values with different superscripts along the column are significantly different ($P \leq 0.05$)

Keys: **RSPO:** Filtrate of rice straw degraded by *Pleurotus ostreatus*
MSPO: Filtrate of maize straw degraded by *Pleurotus ostreatus*
SBPO: Filtrate of sugarcane bagasse degraded by *Pleurotus ostreatus*
SBPOLS: Filtrate of sugarcane bagasse degraded by consortium of *Pleurotus ostreatus* and *Lentinus squarrosulus*

Maize straw was selected for further studies based on its high ethanol content throughout the fermentation days. Figure 4.2 shows the reducing sugar content of maize pretreated with sodium hydroxide, *Pleurotus ostreatus* separately and combined pretreatment with sodium hydroxide followed by *Pleurotus ostreatus*. The reducing sugar content of non-pretreated (NP) maize straw was 15.98 mg/g while the reducing content of maize straw pretreated with sodium hydroxide (NaOH) was 2.88 mg/g. The reducing sugar content of maize straw pretreated with only *Pleurotus ostreatus*(PO) was 16.79 mg/g while 17.38 mg/g was recorded with combined pretreatment with sodium hydroxide and *Pleurotus ostreatus* (NaOHPO). The quantity of reducing sugar with different pretreated methods are in the descending order of NaOHPO>PO>NP>NaOH.

Figure 4.3 shows the chromatogram of some sugars and inhibitors in combined-pretreated maize straw. The sugars are ribose, xylose, arabinose, rhamnose, fructose, glucose, maltose, lactose and sucrose while the inhibitors there are Furfural and Hydroxymethylfurfural (HMF). Sugar profile of combined-pretreated maize straw is as shown in Table 4.20. Glucose was the highest sugar (850.599 mg/100g) followed by a five-carbon xylose (837.043 mg/100g) and the least was rhamnose (3.552×10^{-5} mg/100g). Furfural (63.122 mg/100g) and Hydroxymethylfurfural (40.648 mg/100g) were detected in the sample.

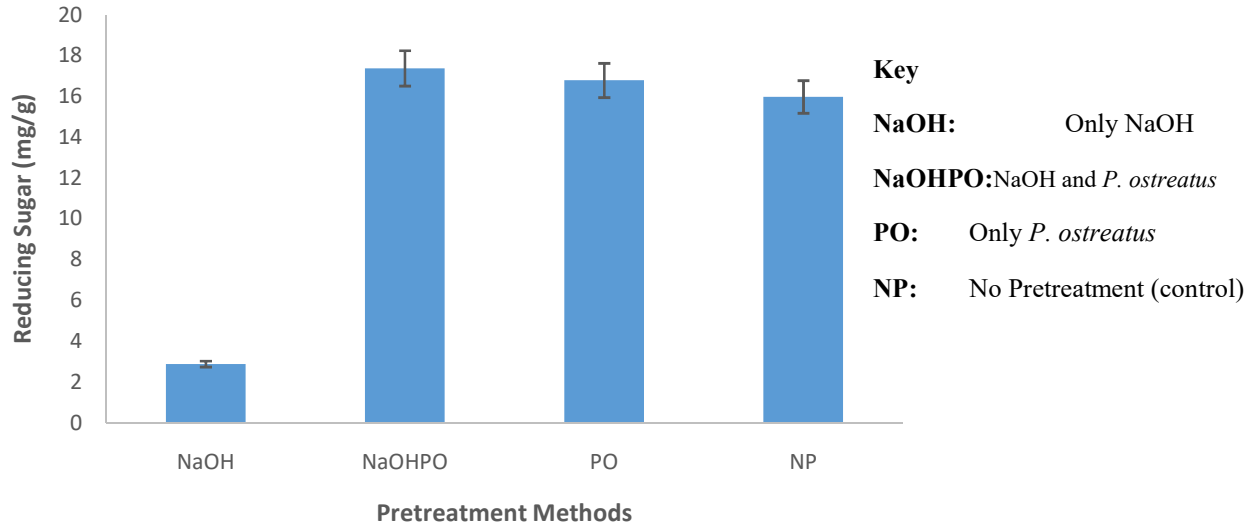

Figure 4.2: Reducing Sugar of Single and Combined Pretreated Maize Straw

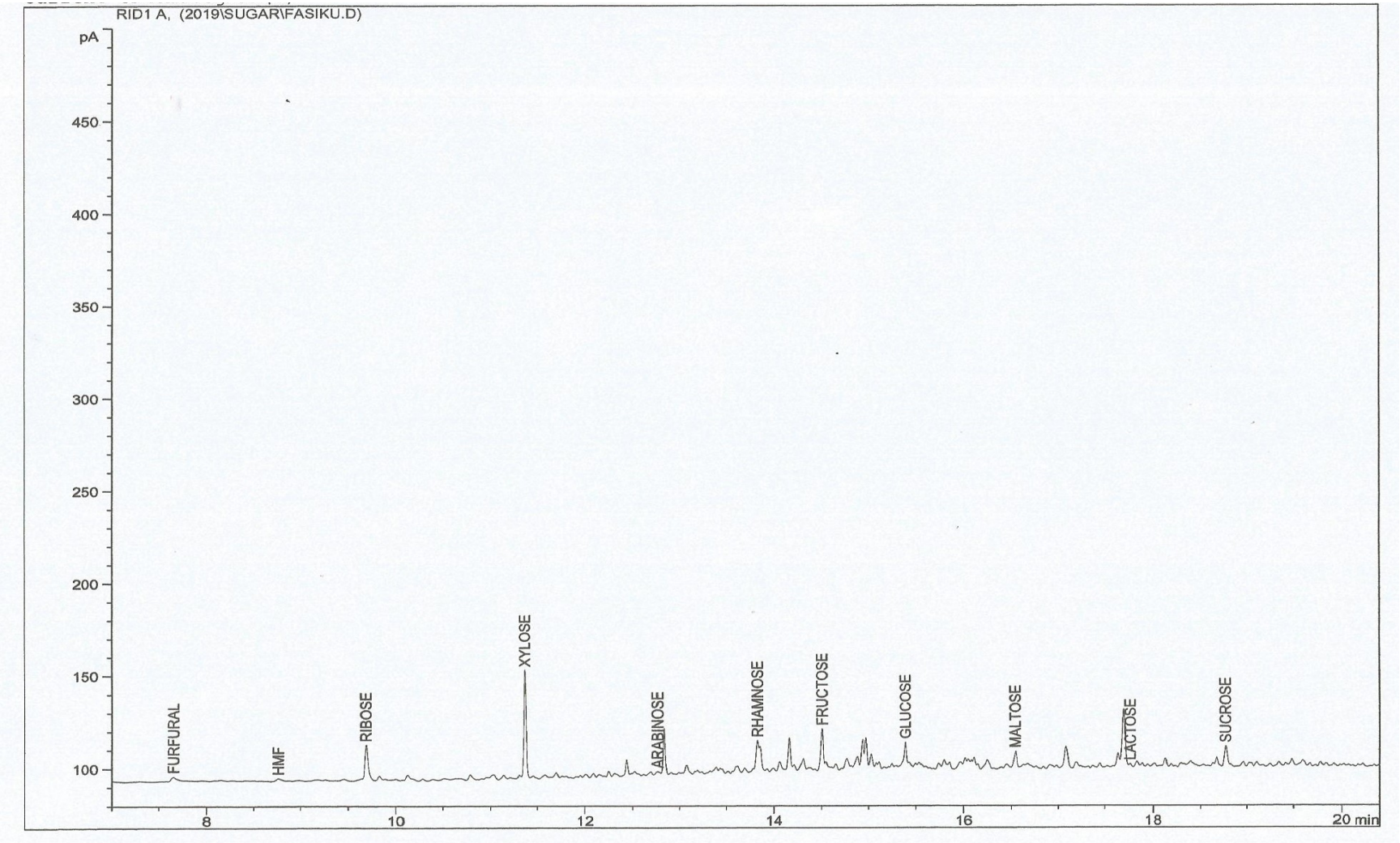

Figure 4.3: Chromatogram of Some Sugars in Combined-Pretreated Maize Straw

Table 4.20: Sugar Profile of Combined Pretreated Maize Straw

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

Mean values with different superscripts along the column are significantly different ($P \leq 0.05$)

Fermentation of combined (NaOHPO) and *Pleurotus ostreatus* (PO)-pretreated maize straw by two strains of *Saccharomyces cerevisiae* is shown in Figure 4.4a. Highest ethanol content (1.58 g/L) was observedin both NaOHPO and PO-pretreated maize fermented by *Saccharomyces cerevisiae* SA01 and *Saccharomyces cerevisiae* SA02 with the shortest observed in NaOHPO.There was increase in ethanol content of NaOHPO-pretreated maize straw fermented by *Saccharomyces cerevisiae* SA02 with increase in fermentation day with highest ethanol content (1.18 g/L) recorded after 2 days of fermentation, maintained till fourth day of fermentation and thereafter decreased. Volume of ethanol produced from PO-pretreated maize straw fermented by *Saccharomyces cerevisiae* SA02 ranged from 0.00 g/L (before fermentation) to 1.18 g/L (day 1 and 3).

Higher amount of reducing sugar was observed in NaOHPO-pretreated maize straw than PO-pretreated maize straw throughout the fermentation period as shown in Figure 4.4b. There was decrease in reducing sugar of both NaOHPO and PO-pretreated maize straw fermented by the two strains of *Saccharomyces cerevisiae*with increase in fermentation day. Highest reducing sugar (17.99 mg/g) was observed in unfermented NaOHPO-pretreated maize straw filtrate and least (15.81 mg/g) in 5-day *Saccharomyces cerevisiae* SA02 fermented PO-pretreated maize straw filtrate.

Figure 4.4c shows the pH of 21-day degraded maize straw fermented by two strains of *Saccharomyces cerevisiae*. Higher pH was recorded in PO-pretreated maize straw than NaOHPO-pretreated maize straw throughout the fermentation period by the two strains of *Saccharomyces cerevisiae*. Increase in pH was observed in PO-pretreated maize straw with increase in fermentation day whereas decrease in pH of NaOHPO-pretreated maize straw was recorded at day 4 of fermentation after initial increase.

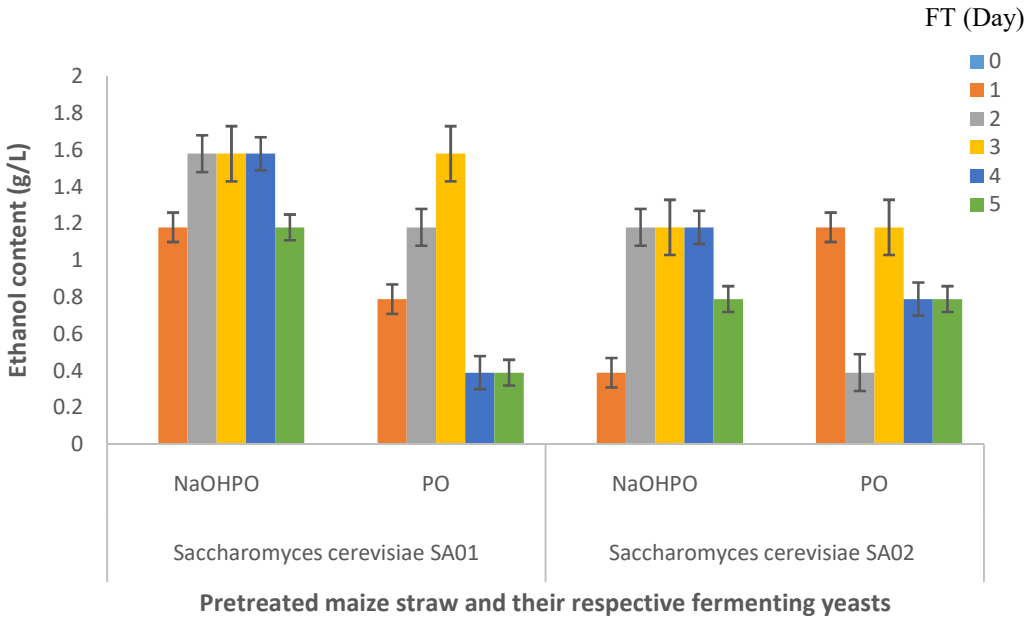

Figure 4.4a: Ethanol Content of 21-Day Degraded Substrates Fermented by *Saccharomyces cerevisiae*

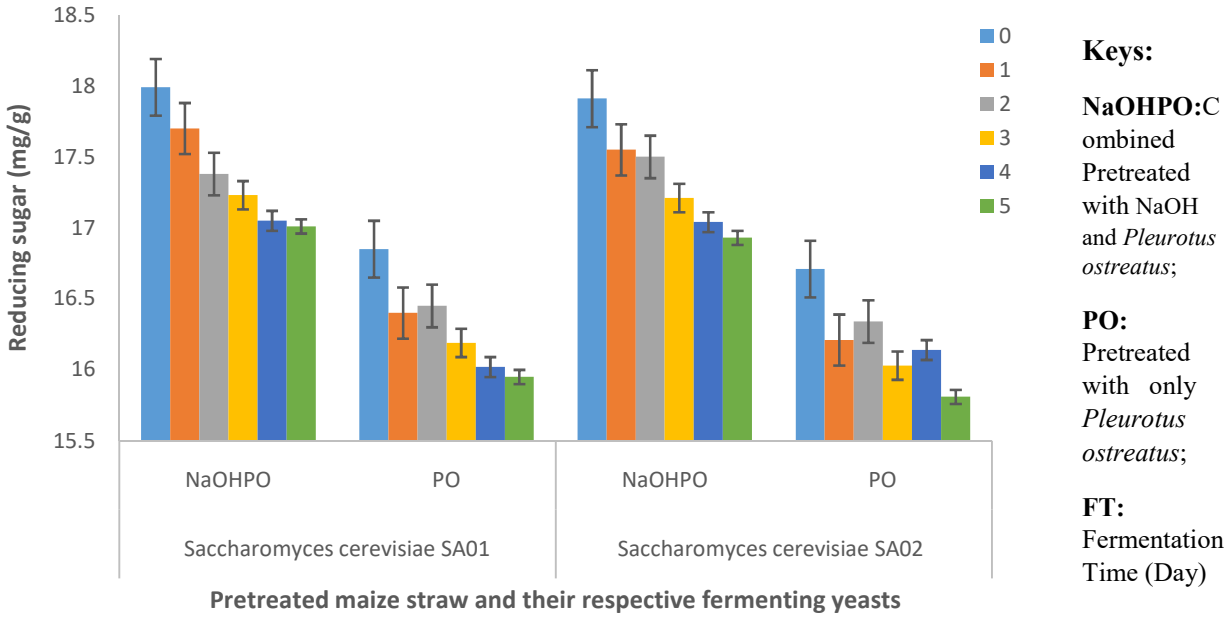

Figure 4.4b: Reducing Sugar of 21-Day Degraded Substrates Fermented by *Saccharomyces cerevisiae*

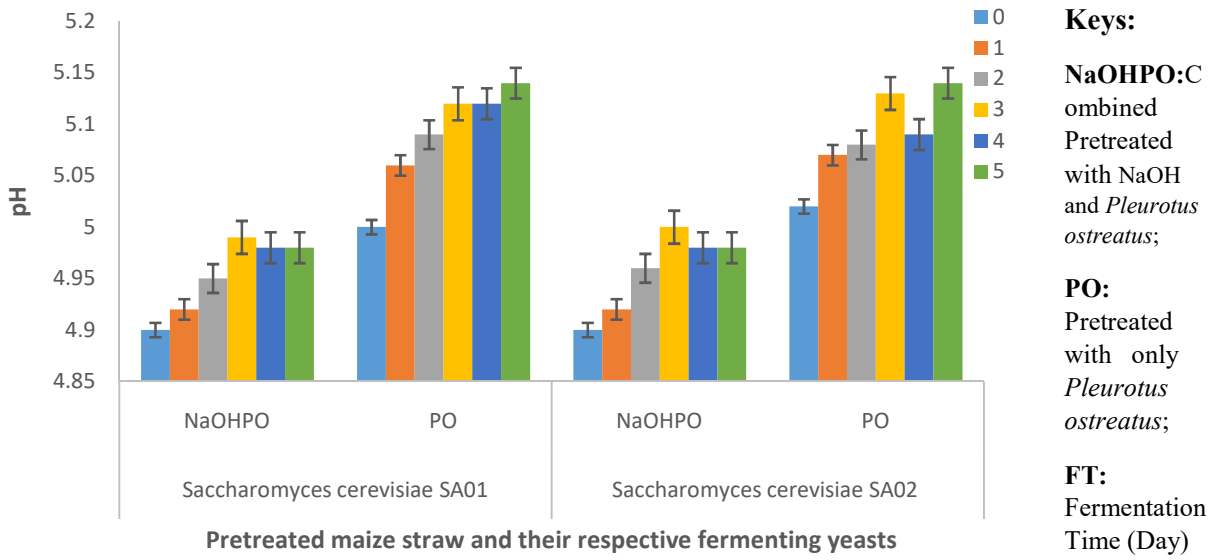

Figure 4.4c: pH of 21-Day Degraded Substrates Fermented by *Saccharomyces cerevisiae*

Effect of pH of buffer used to extract fermentable sugar from combined pretreated maize straw on ethanol content is as shown in Table 4.21a. No ethanol was produced at pH 4.0 and 4.5 of acetate buffer.There was increase in the ethanol content produced by the two strains of *Saccharomyces cerevisiae* with increase in the pH of the acetate buffer. Equal and highest volume of ethanol (1.97 g/L) was attained by the two strains of *Saccharomyces cerevisiae* at pH 5.5 and is not significantly different ($P>0.05$) from volume of ethanol (1.18 g/L) produced at pH 5.0. Acetate buffer (0.1M, pH 5.5) was used for further studies.

There was decrease in reducing sugar content of filtrates fermented by *Saccharomyces cerevisiae* for 3 days from 17.65 – 17.63 mg/g, 17.84 – 17.49 mg/g, 17.93 – 17.55 mg/g and 17.73 – 17.39 mg/g when acetate buffer was at 4.0, 4.5, 5.0 and 5.5 respectively (Table 4.21b). Decrease reducing sugar content was also observed when fermented by *Saccharomyces cerevisiae* SA02 with acetate buffer at pH 4.0, 4.5, 5.0 and 5.5 from 17.65 – 17.46 mg/g, 17.84 – 17.42 mg/g, 17.93 – 17.44 mg/g and 17.73 – 17.20 mg/g respectively.

From Table 4.21c, increase in the pH of the filtrates increased with increase of pH of acetate buffer that was used for extraction. Higher pH of filtrates was observed after fermentation with the exception of filtrates extracted with acetate buffer of 4.0 where slight decrease was observed from 3.98 to 3.97 and 3.95 by *Saccharomyces cerevisiae* SA01 and *Saccharomyces cerevisiae* SA02 respectively. Increase in pH of acetate buffer had significant effect ($P\leq0.05$) on the pH of filtrates.

Table 4.21a: Effect of pH on Ethanol Content (g/L) of Maize Straw's Filtrate Fermented byTwo Strains of *Saccharomyces cerevisiae* for 72 Hours

pH of acetate buffer	Fermenting Yeasts / Ethanol Content (g/L)			
	Saccharomyces cerevisiae SA01		*Saccharomyces cerevisiae* SA02	
	Before Fermentation	After fermentation	Before Fermentation	After Fermentation
4.0	0.00	0.00^{a}	0.00	0.00^{a}
4.5	0.00	0.00^{a}	0.00	0.00^{a}
5.0	0.00	1.18^{b}	0.00	1.18^{b}
5.5	0.00	1.97^{b}	0.00	1.97^{b}

Mean values with different superscripts along the column are significantly different ($P \leq 0.05$)

Table 4.21b: Effect of pH on Reducing Sugar Content (mg/g) of Maize Straw's Filtrate Fermented by Two Strains of *Saccharomyces cerevisiae* for 72 Hours

pH of acetate buffer	Fermenting Yeasts / Reducing Sugar (mg/g)			
	Saccharomyces cerevisiae SA01		*Saccharomyces cerevisiae* SA02	
	Before Fermentation	After fermentation	Before Fermentation	After Fermentation
4.0	17.65^{a}	17.63^{b}	17.65^{a}	17.46^{b}
4.5	17.84^{ab}	17.49^{a}	17.84^{ab}	17.42^{b}
5.0	17.93^{b}	17.55^{ab}	17.93^{b}	17.44^{b}
5.5	17.73^{a}	17.39^{a}	17.73^{a}	17.20^{a}

Mean values with different superscripts along the column are significantly different ($P \leq 0.05$)

Table 4.21c: Effect of pH of Acetate Buffer on pH of Maize Straw's Filtrates before and after 72 Hours of Fermentation by *Saccharomyces cerevisiae*

pH of acetate buffer	Fermenting Yeasts / pH			
	Saccharomyces cerevisiae SA01		*Saccharomyces cerevisiae* SA02	
	Before Fermentation	After fermentation	Before Fermentation	After Fermentation
4.0	3.98^{a}	3.97^{a}	3.98^{a}	3.95^{a}
4.5	4.49^{b}	4.50^{b}	4.49^{b}	4.50^{b}
5.0	5.10^{c}	5.11^{c}	5.10^{c}	5.10^{c}
5.5	5.68^{d}	5.73^{d}	5.68^{d}	5.73^{d}

Mean values with different superscripts along the column are significantly different ($P \leq 0.05$)

Table 4.22a shows effect of temperature on production of ethanol. Decrease in ethanol content was recorded with increase in incubation temperature. There was no significant difference (P>0.05) in the ethanol yield of *Saccharomyces cerevisiae* SA02 at different incubation temperature. Highest ethanol content was obtained by *Saccharomyces cerevisiae* SA01 (2.76 g/L) and *Saccharomyces cerevisiae* SA02 (2.37 g/L) at 30 °C and this temperature was used for further studies.

Effect of incubation temperature on reducing sugar of fermented filtrate is shown in Table 4.22b. Reduction in the reducing sugar content of filtrates was recorded after fermentation. After fermentation, highest reducing sugar by *Saccharomyces cerevisiae* SA01 (17.57 mg/g) and *Saccharomyces cerevisiae* SA02 (17.43 mg/g) was observed at 35 °C incubation temperature and were significantly different (P≤0.05) from the reducing sugar obtained at 45 °C by the two organisms

The pH of filtrate fermented by *Saccharomyces cerevisiae* SA01 increased from 5.63 to 5.69, 5.68, 5.69 and 5.67 at incubation temperature of 30, 35, 40 and 45 °C respectively while the pH of filtrate fermented with *Saccharomyces cerevisiae* SA02 also increased from 5.63 to 5.72, 5.74, 5.73 and 5.72 at incubation temperature of 30, 35, 40 and 45 °C respectively (Table 4.22c).Different incubation temperatures did not have significant effect (P>0.05) on the pH of filtrates fermented by the two strains of *Saccharomyces cerevisiae*.

Table 4.22a: Effect of Incubation Temperature on Ethanol Content (g/L) of Maize Straw's Filtrate Fermented for 72 Hours by *Saccharomyces cerevisiae*

Temperature °C	Fermenting Yeasts			
	***Saccharomyces cerevisiae* SA01**		***Saccharomyces cerevisiae* SA02**	
	Before Fermentation	**After fermentation**	**Before Fermentation**	**After Fermentation**
30	0.00	2.76^{b}	0.00	2.37^{a}
35	0.00	2.37^{ab}	0.00	1.58^{a}
40	0.00	1.58^{a}	0.00	1.97^{a}
45	0.00	1.97^{ab}	0.00	1.58^{a}

Mean values with different superscripts along the column are significantly different ($P \leq 0.05$)

Table 4.22b: Effect of Temperature Incubation on Reducing Sugar (mg/g) of Maize Straw's Filtrates Fermented for 72 Hours by *Saccharomyces cerevisiae*

Temperature °C	Fermenting Yeasts			
	***Saccharomyces cerevisiae* SA01**		***Saccharomyces cerevisiae* SA02**	
	Before Fermentation	**After fermentation**	**Before Fermentation**	**After Fermentation**
30	17.80^{a}	17.50^{bc}	17.80^{a}	17.32^{b}
35	17.80^{a}	17.57^{c}	17.80^{a}	17.43^{b}
40	17.80^{a}	17.30^{a}	17.80^{a}	17.24^{ab}
45	17.80^{a}	17.35^{ab}	17.80^{a}	17.12^{a}

Mean values with different superscripts along the column are significantly different ($P \leq 0.05$)

Table 4.22c: Effect of Incubation Temperature on pH of Maize Straw's Filtrates Fermented for 72 Hours by *Saccharomyces cerevisiae*

Temperature °C	Fermenting Yeasts			
	***Saccharomyces cerevisiae* SA01**		***Saccharomyces cerevisiae* SA02**	
	Before Fermentation	**After fermentation**	**Before Fermentation**	**After Fermentation**
30	5.63[a]	5.69[a]	5.63[a]	5.72[a]
35	5.63[a]	5.68[a]	5.63[a]	5.74[a]
40	5.63[a]	5.69[a]	5.63[a]	5.73[a]
45	5.63[a]	5.67[a]	5.63[a]	5.72[a]

Mean values with different superscripts along the column are significantly different ($P \leq 0.05$)

Effect of different concentrations of sugars is as shown in Table 4.23. Ethanol yield of *Saccharomyces cerevisiae* SA01 in filtrate supplemented with glucose ranged from 2.37 g/L to 2.76 g/L with highest content at 3 and 5 % of glucose while that of *Saccharomyces cerevisiae* SA02 ranged from 2.37 to 3.95 g/L with the highest at 2 % glucose. Ethanol yield of *Saccharomyces cerevisiae* SA01 and *Saccharomyces cerevisiae* SA02 with different concentration of fructose ranged from 1.97 to 2.76 g/L. Volume of ethanol (3.95 g/L) produced by *Saccharomyces cerevisiae* SA02 with 2 % glucose was significantly different ($P \leq 0.05$) from ethanol yield of other concentration of glucose with the exception of 5 % glucose. Glucose concentration of 2% was used for further studies.

Table 4:23: Effect of Different Concentration of Glucose and Fructose on Ethanol Production(g/L) from Filtrate of Maize Straw.

Sugar Concentration (%)	*Saccharomyces cerevisiae*SA01	*Saccharomyces cerevisiae* SA02	*Saccharomyces cerevisiae*SA01	*Saccharomyces cerevisiae* SA02
	Glucose		Fructose	
0	2.37^{a}	2.37^{a}	2.37^{ab}	2.37^{ab}
1	2.37^{a}	2.76^{a}	2.37^{ab}	2.76^{b}
2	2.37^{a}	3.95^{b}	1.97^{a}	1.97^{a}
3	2.76^{a}	2.76^{a}	2.37^{ab}	2.37^{ab}
4	2.37^{a}	2.76^{a}	2.76^{b}	2.76^{b}
5	2.76^{a}	3.16^{ab}	2.37^{ab}	2.76^{b}

Mean values with different superscripts along the column are significantly different ($P \leq 0.05$)

Figure 4.5a shows the effect of nitrogen sources on ethanol yield of the combined pretreated maize straw's filtrate fermented with *Saccharomyces cerevisiae* SA01 and *Saccharomyces cerevisiae* SA02. Supplementation with different wastes as nitrogen source (corn steep liquor, groundnut cake, soya meal, fish meal and blood meal) resulted in high ethanol yield. In filtrate fermented by *Saccharomyces cerevisiae* SA01, highest ethanol content (12.23 g/L) recorded for groundnut cake was not significantly different ($P>0.05$) from the ethanol yield observed for corn steep liquor (11.84 g/L) and Soya meal (11.84 g/L) while the least ethanol content (7.10 g/L) was recorded for blood meal. In filtrate fermented by *Saccharomyces cerevisiae* SA02, least ethanol content (9.47 g/L) was recorded in blood meal while highest ethanol yield (13.02 g/L) was observed in corn steep liquor and was not significantly different ($P>0.05$) from ethanol yield of groundnut cake (12.62 g/L).*Saccharomyces cerevisiae* SA02 had higher ethanol yield than *Saccharomyces cerevisiae* SA01 with all the nitrogen sources used. Corn steep liquor was used for further work.

Decrease in reducing sugar content of filtrates supplemented with various nitrogen source was observed after 72 hours of fermentation as shown in Figure 4.5b. Highest reducing sugar obtained after 72 hours of fermentation by *Saccharomyces cerevisiae* SA01 (91.91 mg/g) and *Saccharomyces cerevisiae* SA02 (73.62 mg/g) were recorded with blood meal as nitrogen source and were significantly different ($P\leq0.05$) from other reducing sugar after 72 hours of fermentation. Higher reducing sugar were recorded by *Saccharomyces cerevisiae* SA01 in corn steep liquor and blood meal after 72 hours of fermentation when compared with *Saccharomyces cerevisiae* SA02.

There was decrease in the pH (5.83-4.69) of combined pretreated maize straw filtrate supplemented by different nitrogen sources and fermented by *Saccharomyces cerevisiae* SA01 and *Saccharomyces cerevisiae* SA02 for 72 hours (Figure 4.5c). Lower pH was observed in *Saccharomyces cerevisiae* SA01 for all used nitrogen source after 72 hours of fermentation when compared with *Saccharomyces cerevisiae* SA02. Lowest pH before fermentation (4.82) was recorded in corn steep liquor while highest pH (5.83) was observed in fish meal. The lowest pH recorded after fermentation by *Saccharomyces*

cerevisiae SA01 (4.82) and *Saccharomyces cerevisiae* SA02 (4.75) was observed in corn steep liquor.

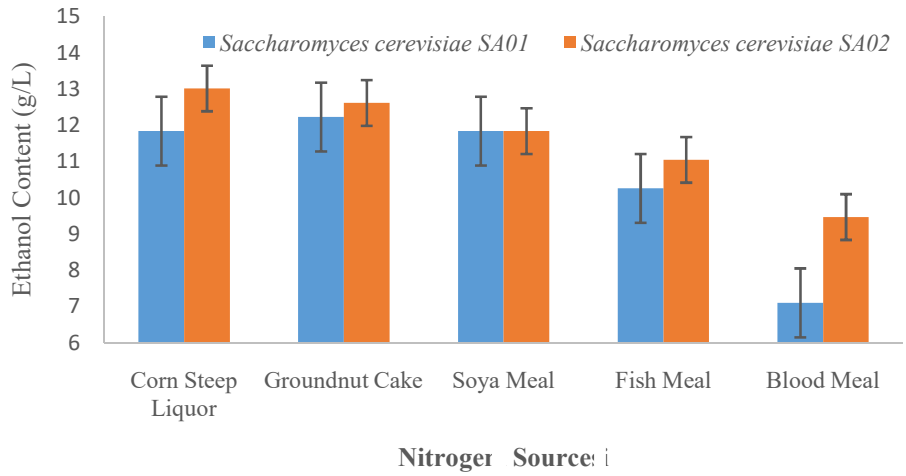

Figure 4.5a: Effect of Different Nitrogen Sources on Ethanol Content (g/L) of Combined-Pretreated Maize Straw's Filtrate

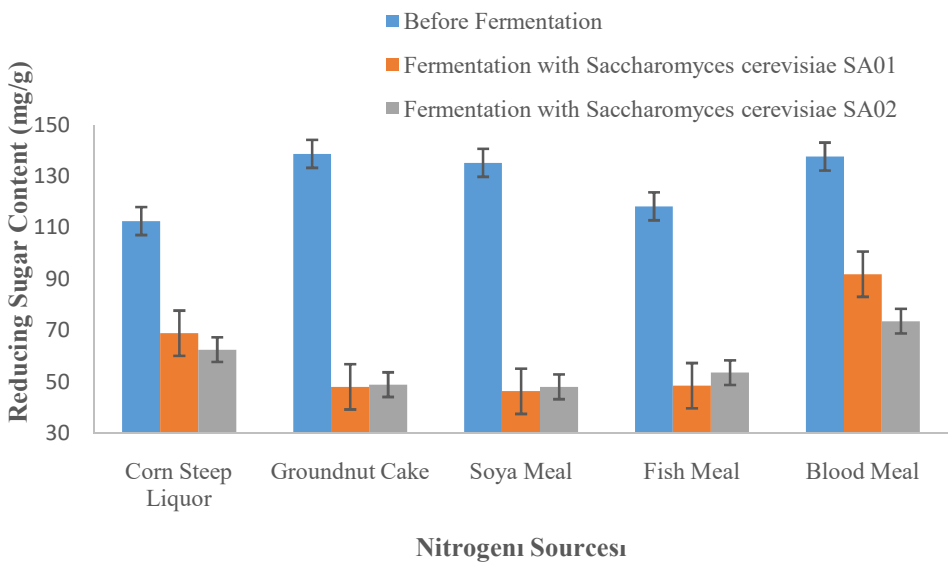

Figure 4.5b: Effect of Different Nitrogen Sources on Reducing Sugar Content (mg/g)of Combined-Pretreated Maize Straw's Filtrate

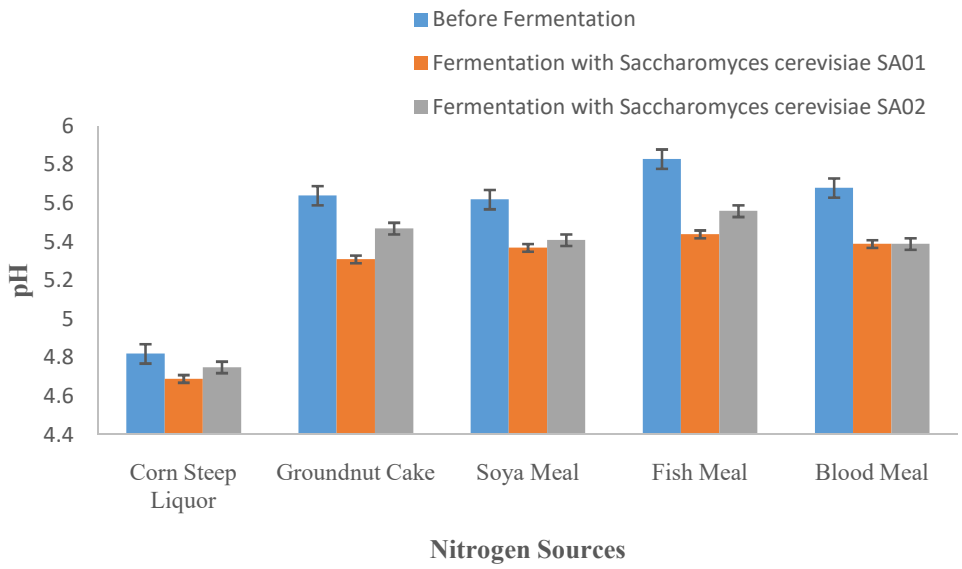

Figure 4.5c: Effect of Different Nitrogen Sources on pH of Combined-Pretreated Maize Straw's Filtrate

Effect of different ratio of buffer to corn steep liquor on ethanol content of maize straw filtrate fermented for 72 hours is as shown in Figure 4.6a. There was increase in ethanol content with increase in concentration of corn steep liquor by the two strains of *Saccharomyces cerevisiae*. Highest ethanol content (14.20 g/L) by *Saccharomyces cerevisiae* SA01 and *Saccharomyces cerevisiae* SA02 were recorded in filtrate with ratio 60 acetate buffer to 40 corn steep liquor and 50 acetate buffer to 50 corn steep liquor respectively. There was no significant difference ($P \leq 0.05$) in ethanol content obtained with *Saccharomyces cerevisiae* SA01 and *Saccharomyces cerevisiae* SA02 at 60/40 and 50/50 acetate buffer to corn steep liquor respectively. Ratio 60/40 acetate buffer to corn steep liquor was used for further work.

Before fermentation, decrease in pH of filtrates was observed with increase in concentration of corn steep liquor (Figure 4.6b). Decrease in pH was recorded in all filtrates after 72 hours of fermentation. Filtrates fermented by *Saccharomyces cerevisiae* SA02 had higher pH thanthose fermented by*Saccharomyces cerevisiae* SA01 in all concentration of corn steep liquor. The least pH by *Saccharomyces cerevisiae* SA01 (5.11) and *Saccharomyces cerevisiae* SA02 (5.15) was obtained in filtrate with ratio 50 acetate buffer to 50 corn steep liquor.

The pH of filtrates extracted with different concentration of corn steep liquor was adjusted to 5.5 and the effect on ethanol content was observed (Figure 4.7a). Increase in ethanol content was observed with increase in concentration of corn steep liquor. Higher ethanol content was recorded by *Saccharomyces cerevisiae* SA02 than *Saccharomyces cerevisiae* SA01 in all concentration of corn steep liquor. Highest amount of ethanol recorded by *Saccharomyces cerevisiae* SA01 (13.41 g/L) and *Saccharomyces cerevisiae* SA02 (13.81 g/L) were observed at ratio 70/30 and 50/50 concentration of buffer to corn steep liquor respectively.

The pH of extracted filtrates of combined pretreated maize straw with different concentration of corn steep liquor was adjusted to 5.5 and the effect on final pH after 72 hours of fermentation was observed as shown in Figure 4.7b.Decrease in pH was observed at all concentrations of corn steep liquor by the two strains of *Saccharomyces cerevisiae* after fermentation. Higher pH was recorded by *Saccharomyces cerevisiae* SA02 than *Saccharomyces cerevisiae* SA01 after fermentation in all concentration of corn steep liquor except ratio 90/10 buffer to corn steep liquor where pH of*Saccharomyces cerevisiae* SA01 (5.34) was higher than *Saccharomyces cerevisiae* SA02 (5.32).

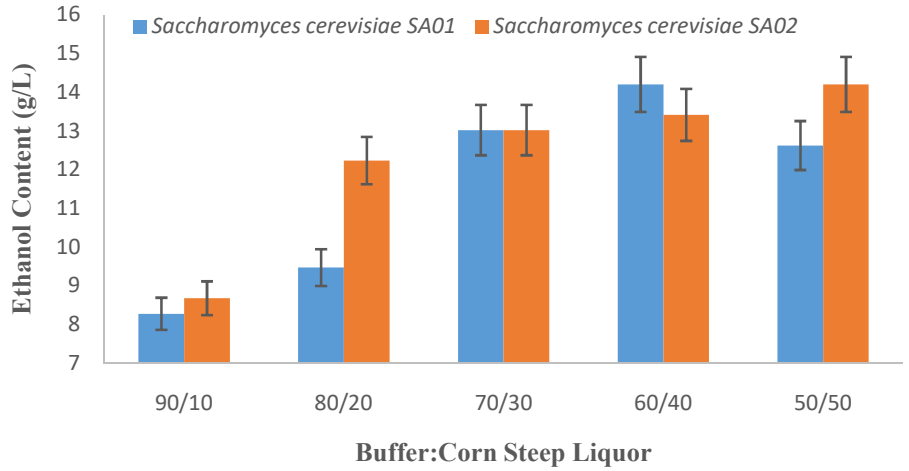

Figure 4.6a: Effect of Different Ratio of Buffer to Corn Steep Liquor on Ethanol Content of Combined-Pretreated Maize Straw's Filtrate

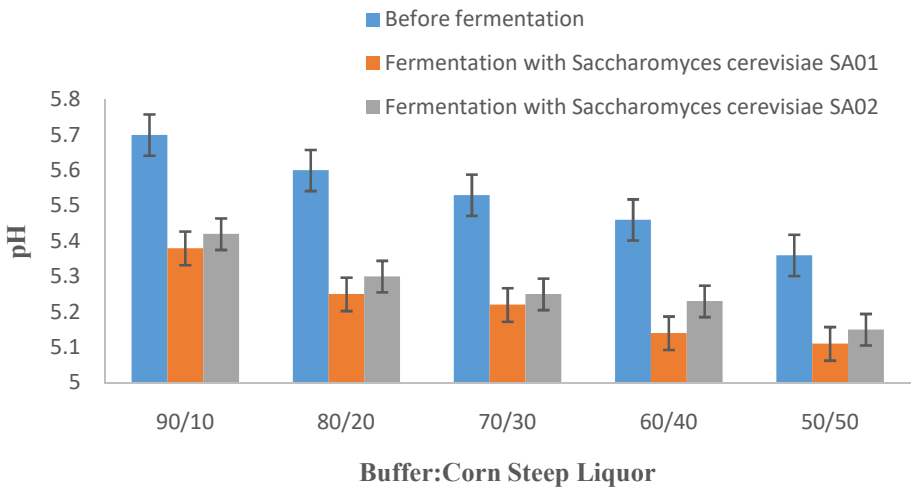

Figure 4.6b: Effect of Different Ratio of Buffer to Corn Steep Liquor on pH of Combined-Pretreatment Maize Straw's Filtrate

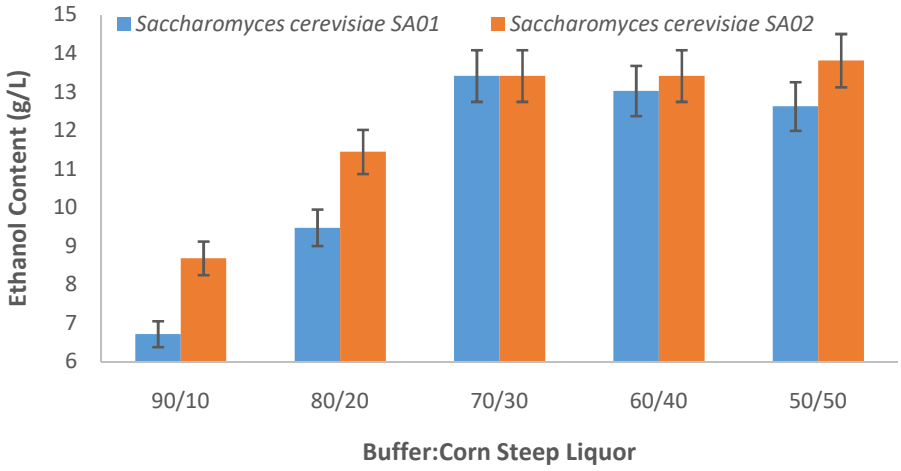

Figure 4.7a: Effect of pH 5.5 on Ethanol Yield of Combined-Pretreated Maize Straw's Filtrate

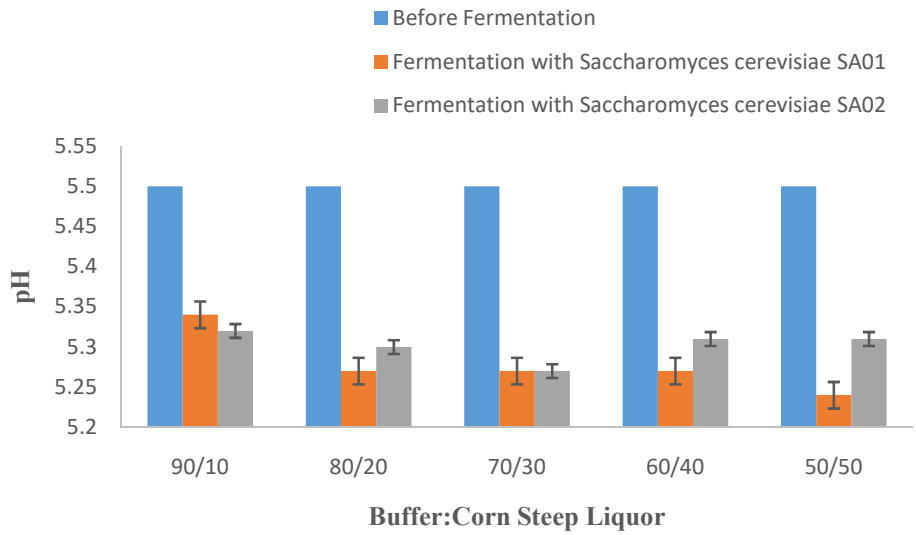

Figure 4.7b: Effect of pH 5.5 on pH of Fermented Combined-Pretreated Maize Straw's Filtrate

Table 4.24a shows the effect of inoculum load on ethanol content of combined pretreated maize straw's filtrates fermented by two strains of *Saccharomyces cerevisiae* for 72 hours. Increase in the amount of ethanol with increase in inoculum load was recorded by the two strains of *Saccharomyces cerevisiae*.Highest volume of ethanol (14.20 g/L) was attained by the two yeasts, while *Saccharomyces cerevisiae* SA01 attained it at inoculum load of 3.0 MacFarland standard, *Saccharomyces cerevisiae* SA02 attained it at lower inoculum load of 2.0 MacFarland standard. The least ethanol content recorded by *Saccharomyces cerevisiae* SA01 (13.02 g/L) and *Saccharomyces cerevisiae* SA02 (13.41 g/L) at inoculum load of 0.5 MacFarland standard were significantly different ($P \leq 0.05$) from their highest ethanol content (14.20 g/L).

Effect of inoculum load on reducing sugar of combined-pretreated maize straw's filtrate fermented by two strains of *Saccharomyces cerevisiae* for 72 hours is as shown in Table 4.24b. Highest quantity of reducing sugar (121.48 mg/g) was recorded before fermentation and decrease in reducing sugar was observed after the fermentation with both strains of *Saccharomyces cerevisiae*. The values of reducing sugar obtained with different inoculum loads by the two strains of *Saccharomyces cerevisiae* after

fermentation were not significantly different (P>0.05) at the different inoculum loads used.

Table 4.24c shows the effect of inoculum load on the pH of combined pretreated maize straw's filtrate fermented for 72 hours. Highest pH (5.40) was recorded before fermentation and values of pH decreased after 72 hours of fermentation with different inoculum load by the two strains of *Saccharomyces cerevisiae*. Higher pH was observed in filtrate fermented by *Saccharomyces cerevisiae* SA02 than *Saccharomyces cerevisiae* SA01. There was no significant difference (P>0.05) in the pH obtained with different inoculum load after fermentation with *Saccharomyces* SA01 however, pH 5.19 recorded after fermentation with inoculum load of 2.0 MacFarland standard of *Saccharomyces cerevisiae* SA02 is significantly different (P≤0.05) from pH 5.26 obtained with inoculum load of 3.0 MacFarland standard.

Table 4.24a: Effect of Inoculum Load on Ethanol Content (g/L) of Combined Pretreated Maize Straw's Filtrate Fermented by *Saccharomyces cerevisiae*

MacFarland Standard	*Saccharomyces cerevisiae* SA01		*Saccharomyces cerevisiae* SA02	
	Before	After	Before	After
0.5	0.00	13.02[a]	0.00	13.41[a]
1.0	0.00	13.41[ab]	0.00	13.81[ab]
2.0	0.00	13.81[ab]	0.00	14.20[b]
3.0	0.00	14.20[b]	0.00	14.20[b]

Mean values with different superscripts along the column are significantly different (P≤0.05)

Table 4.24b: Effect of Inoculum Load on Reducing Sugar (mg/g) of Combined Pretreated Maize Straw's Filtrate Fermented by *Saccharomyces cerevisiae*

MacFarland Standard	*Saccharomyces cerevisiae* SA01		*Saccharomyces cerevisiae* SA02	
	Before	**After**	**Before**	**After**
0.5	121.48[a]	24.83[a]	121.48[a]	17.79[a]
1.0	121.48[a]	21.48[a]	121.48[a]	22.15[a]
2.0	121.48[a]	21.48[a]	121.48[a]	22.15[a]
3.0	121.48[a]	21.48[a]	121.48[a]	21.48[a]

Mean values with different superscripts along the column are significantly different ($P \leq 0.05$)

Table 4.24c: Effect of Inoculum Load on pH of Combined-Pretreated Maize Straw's Filtrates Fermented by *Saccharomyces cerevisiae*SA01 and *Saccharomyces cerevisiae* SA02

MacFarland Standard	*Saccharomyces cerevisiae* SA01		*Saccharomyces cerevisiae* SA02	
	Before	After	Before	After
0.5	5.40^{a}	5.17^{a}	5.40^{a}	5.22^{ab}
1.0	5.40^{a}	5.20^{a}	5.40^{a}	5.22^{ab}
2.0	5.40^{a}	5.19^{a}	5.40^{a}	5.19^{a}
3.0	5.40^{a}	5.20^{a}	5.40^{a}	5.26^{b}

Mean values with different superscripts along the column are significantly different ($P \leq 0.05$)

Effect of inoculum size on ethanol yield of combined-pretreated maize straw filtrate is as shown in Table 4.25a. Equal and highest ethanol yield (14.99 g/L) was observed by *Saccharomyces cerevisiae* SA01 and *Saccharomyces cerevisiae* SA02 at 1 % inoculum size. Decrease in ethanol content was recorded by the two strains of *Saccharomyces cerevisiae* with increase in inoculum size. Ethanol yield of 14.99 g/L with inoculum size of 1.0 % of *Saccharomyces cerevisiae* SA01 is significantly different ($P \leq 0.05$) from ethanol yield from other inoculum size. Equal volume of ethanol (14.99 g/L) observed with inoculum size 1.0 % and 1.5 % of *Saccharomyces cerevisiae* SA02 was significantly different ($P \leq 0.05$) from other ethanol content recorded with other inoculum size of *Saccharomyces cerevisiae* SA02 except for ethanol obtained (14.20 g/L) from inoculum size of 3.0 % *Saccharomyces cerevisiae* SA02.

Effect of inoculum size on reducing sugar of combined-pretreated maize straw filtrate fermented by *Saccharomyces cerevisiae* SA01 and *Saccharomyces cerevisiae* SA02 is shown in Table 4.25b. Highest amount of reducing sugar (121.48 mg/g) was recorded before fermentation. Least reducing sugar (19.13 mg/g) was observed in filtrate fermented with inoculum size of 2.5 % of *Saccharomyces cerevisiae* SA01 and the value is significantly different ($P \leq 0.05$) from amount of reducing sugar (24.83 mg/g) obtained with inoculum size of 1.5 % and 3.0 % *Saccharomyces cerevisiae* SA01. The values of

reducing sugars obtained after 72 hours of fermentation by *Saccharomyces cerevisiae* SA02 were not significantly different (P>0.05) from each other.

Values of pH recorded after 72 hours of fermentation with different inoculum size were higher in filtrate fermented by *Saccharomyces cerevisiae* SA02 than *Saccharomyces cerevisiae* SA01 except pH of filtrate inoculated with inoculum size of 1.0% (Table 4.25c). Highest pH (5.23) obtained when filtrate was fermented by 2.0 % inoculum size of *Saccharomyces cerevisiae* SA01 for 72 hours was not significantly different (P>0.05) from other pH recorded with other inoculum size except 3.0% inoculum size. Highest pH (5.29) recorded after 72 hour of fermentation by 2.0 % inoculum size of *Saccharomyces cerevisiae* SA02 is significantly different (P≤0.05) with the pH obtained with 1.0 % and 1.5 % inoculum size.

Table 4.25a: Effect of Inoculum Size on Ethanol Content (g/L) of Combined Pretreated Maize Straw's Filtrate Fermented by *Saccharomyces cerevisiae*

Inoculum size (%)	***Saccharomyces cerevisiae* SA01**		***Saccharomyces cerevisiae* SA02**	
	Before	**After**	**Before**	**After**
1.0	0.00	14.99^{b}	0.00	14.99^{b}
1.5	0.00	13.41^{a}	0.00	14.99^{b}
2.0	0.00	13.02^{a}	0.00	13.81^{a}
2.5	0.00	13.02^{a}	0.00	13.81^{a}
3.0	0.00	12.62^{a}	0.00	14.20^{ab}

Mean values with different superscripts along the column are significantly different (P≤0.05)

Table 4.25b: Effect of Inoculum Size on Reducing Sugar (mg/g) of Combined Pretreated Maize Straw's Filtrate Fermented by *Saccharomyces cerevisiae*

Inoculum size (%)	*Saccharomyces cerevisiae* SA01		*Saccharomyces cerevisiae* SA02	
	Before	**After**	**Before**	**After**
1.0	121.48^{a}	21.14^{ab}	121.48^{a}	22.48^{a}
1.5	121.48^{a}	24.83^{b}	121.48^{a}	20.13^{a}
2.0	121.48^{a}	20.81^{ab}	121.48^{a}	22.82^{a}
2.5	121.48^{a}	19.13^{a}	121.48^{a}	22.15^{a}
3.0	121.48^{a}	24.83^{b}	121.48^{a}	22.48^{a}

Mean values with different superscripts along the column are significantly different ($P \leq 0.05$)

Table 4.25c: Effect of Inoculum Size on pH of Combined-Pretreated Maize Straw's Filtrate Fermented by *Saccharomyces cerevisiae*

Inoculum size (%)	*Saccharomyces cerevisiae* SA01		*Saccharomyces cerevisiae* SA02	
	Before	After	Before	After
1.0	5.40^{a}	5.22^{ab}	5.40^{a}	5.21^{a}
1.5	5.40^{a}	5.20^{ab}	5.40^{a}	5.21^{a}
2.0	5.40^{a}	5.23^{b}	5.40^{a}	5.29^{b}
2.5	5.40^{a}	5.20^{ab}	5.40^{a}	5.28^{b}
3.0	5.40^{a}	5.17^{a}	5.40^{a}	5.31^{b}

Mean values with different superscripts along the column are significantly different ($P \leq 0.05$)

CHAPTER FIVE

DISCUSSION

The white rot fungi used in this work had the ability to produce hydrolytic enzymes. Production of hydrolytic enzymes by*Pleurotus ostreatus* and *Lentinus squarrosulus*was confirmed by their cellulos-degradation potential in the formation of colourless halos aroung the fungi plugs against a pink Congo red-cellulose complex. An indication of their abilitiesto break down cellulose to simple sugars as reported by Huang *et al*.(2019). In the hydrolysis of xylan, the clear zone against theblue black colour observed is an indication of the fungal abilities to produce hemicellulase (Yu and Atalla, 2005; Mohammad and Ariffin, 2020). The fungi effectively converted xylan to hexoses and pentoseswhich resulted in clear zones around xylanase producing mushrooms on xylan agar. Production of lignase on tannic acid agar by *Pleurotus ostreatus*, *Pleurotus tuber-regium* and *Lentinus squarrosulus* in this work might be due to their abilities to utilise tannic acid as a source of energy which resulted in the brown coloration observed on the plates as earlier reported by Pointing (1999). Gramss *et al*. (2017) explained that laccase-producing organisms oxidized 2, 2'-Azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) that was colourless to $ABTS^{2+}$ (purple) and purple coloration that appeared on medium supplemented with ABTS in this work indicated abilities of mushroom to produce laccase.

Production of cellulase by *Pleurotus ostreatus*and *Lentinus squarrosulus*as observed in this studyhas been reported by some researchers to be due the ability of the organisms to excrete hydrolysing and oxidising enzymes which effectively broke down the cellulolytic component of lignocellulose (Atri and Sharma, 2011; Khalil *et al*., 2011; Karthikeyan, 2015; Debnath *et al*., 2018; Premkumar *et al*., 2018;Huang *et al*., 2019). However, the inability of *Pleurotus tuber-regium* to produce cellulase in the course of this work could

been an influence of environmental factors or genetic make-up of the organism which might make the degradation of cellulose difficult.

Hydrolytic capability of *Pleurotus ostreatus* and *Lentinus squarrosulus*in the production of xylanase and laccase recorded in this research has been confirmed by many researchers which could have been influenced by their genetic make-up (Pukahuta *et al*., 2004; Isikhuemhen *et al*., 2012; Radhika *et al*., 2013; Mukhopadhyay and Banerjee, 2015; Alvarez-Cervantes *et al*., 2016; Khvedelidze *et al*., 2018; Premkumar *et al*., 2018; Huang *et al*., 2019).

Pleurotus ostreatus and *Lentinus squarrosulus*selected were able to degrade agro lignocellulosic wastes because of their abilities to produce required enzymes (cellulase, xylanase, lignase) necessary for degrading lignocellulose. These findings were similar to the reports ofPukahuta *et al*.(2004), Isikhuemhen *et al*.(2012), Alvarez-Cervantes *et al*.(2016) and Huang *et al*. (2019) who also reported the abiilites of such mushrooms to adaptable growth and fruiting on wide range of agro lignocellulosic wastes.

Changes in chemical composition observed when differentlignocellulolytic substrates were degraded with *Pleurotus ostreatus* and *Lentinus squarrosulus*through solid state fermentationcould be due to the metabolites (cellulase, xylanase, lignase/laccase, etc.) produced by these organisms,which have ability to degrade different parts of lignocellulose. This observation corroborates the work of Issaka *et al*. (2013) and Wuanor *et al*. (2018) who degraded groundnut shell with *Pleurotus* species and reported changes in chemical composition of groundnut shell. Lower extractive recorded in most of the degraded substrates than non degraded ones might probably be due to utilization of the extractives as nutrient during degradation by these mushrooms as earlier reported by Vilanova *et al*.(2014).

Higher hemicellulose content observed in degraded lignocellulolytic substrates compared with non-degraded might be as a result of low required nutrients needed for the production of hemicellulases (xylanase and others) on the substratesthat could have converted hemicellose to glucose and xylose. This is in contrary to the findings of Issaka *et al*. (2013) and Wuanor *et al*. (2018) who recorded decrease in hemicellulose content after degrading groundnut shell with *Pleurotus* species. Percentage composition of

lignocellulolytic substrates differ from one another based on the class the substrate that is soft or hard wood. Generally, lower hemicellulose content was observed when degraded by co-culture of *Pleurotus ostreatus* and *Lentinus squarrosulus* than when degraded singly. This might be due to synergistic relationship between *Pleurotus ostreatus* and *Lentinus squarrosulus* in the utilization of hemicellulose. There have been reports that organisms performed differently when in consortium from when used singly (Wang *et al*., 2014).

The decrease in lignin content of groundnut shell observed after 49 days of degradation by *Pleurotus ostreatus*, *Lentinus squarrosulus* and consortium of *Pleurotus ostreatus* and *Lentinus squarrosulus* shows that these mushrooms have ability to remove lignin bonds that prevent holocellulose from being broken down to simple and fermentable sugar. This observation has been reported to be due to production of lignin-degrading enzymes by these organisms (Isikhuemhen *et al*., 2012; Issaka *et al*., 2013; Radhika *et al*., 2013; Wuanor *et al*., 2018). Similar observation of decreased lignin content of degraded groundnut shell by *Pleurotus ostreatus* for 5 weeks (Issaka *et al*., 2013) and 30 days(Wuanor *et al*.,2018) has also been reported. The observed higher lignin content at most sampling time in maize cob, maize straw, sugarcane bagasse and rice straw might be due to low nitrogen source needed for production of lignases from these substrates by the *Pleurotus ostreatus* and *Lentinus squarrosulus* and similar observation of increased lignin content after pretreatment was reported by Adamafio *et al*. (2012).

Conversion of cellulose to simple sugars by cellulase-producing mushrooms selected for degradation in this work could be responsible for decrease in cellulose content observed at most sampling time in all selected lignocellulolytic substrates.The cellulose part of lignocellulosic substrates would have extensively utilize and converted to hexoses by selected mushrooms leading to decrease in cellulose after degradation as reported by some researchers (Akinfemi, 2010; Metri *et al*., 2018; Huang *et al*., 2019).Similar observation of decrease in cellulose content after degradation with *Pleurotus* specieswas reported by Akinfemi (2010) and Huang *et al*. (2019) from maize cob andcrop straw respectively.

The higher reducing sugar released from groundnut shell degraded by monoculture of *Pleurotus ostreatus* and *Lentinus squarrosulus* than co-culture of the two might be due to

high utilization of the released reducing sugar as carbon and energy sources by the co-culture than monoculture (Akpor, 2018; Hu *et al*., 2018) or the organisms might be having antagonistic effect on each other leading to decrease in released reducing sugar when grown together. Whilethe observed higher reducing sugar released in *Pleurotus ostreatus* and *Lentinus squarrosulus*-degraded maize cob than non-degraded one was probably because of the interaction between hydrolytic and oxidative enzymes released by these organisms when degrading maize cob, breaking down cellulose and hemicellulose to simple sugar (Adamafio *et al*., 2009; Ogunyewo and Olajuyigbe, 2016). Similar observation of increased reducing sugar content of maize cob when degraded by *Pleurotus ostreatus* was reported by Adamafio *et al*. (2009)

Increase in reducing sugar content observed in degraded maize straw could be due to the breaking down of different components of maize straw to reducing sugar by the enzymes produced by the organism which could be influenced by both genetic make up and environmental conditions (Huang *et al*., 2017; 2019).Furthermore, the observed increase in the reducing sugar of corn straw was after pretreatment(Huang *et al*., 2017).Higher reducing sugar content observed in degraded sugarcane bagasse could be due to ability of *Pleurotus ostreatus* and *Lentinus squarrosulus* to produce cellulase and xylanase which could have broken the holocellulose content of sugarcane bagasse to reducing sugar (Jonathan and Akinfemi, 2010; Dong *et al*., 2013; Shankarappa *et al*., 2015; Gani *et al*., 2018). Gani *et al*. (2018) reportedhigh reducing sugar when sugarcane bagasse was pretreated with alkaline and acid.

Ability of *Pleurotus ostreatus* and *Lentinus squarrosulus* to produce lignocellulolytic enzymes that can breakdown cellulose, hemicellulose and lignin to simple sugar could be responsible for high amount of reducing sugar recorded in degraded rice straw (Jonathan and Akinfemi, 2010; Belal, 2013; Nurika *et al*., 2019). Belal (2013) reported high reducing sugar in rice straw degraded with *Trichoderma reesei*for 14 days while Nurikaand others (2019) observed higher amount of reducing sugar after 21 days degradation of rice straw with *Serpula lacrymans*.

The observed higher reducing sugar in monoculture-degraded substrate than co-culture degraded culture is contrary to the report that co-culturing have synergetic effect on the

degradation of lignocellulose to fermentable reducing sugar (Wang *et al*., 2014). They also reported a better yield of reducing sugar in *Populus tomentosa* (Poplar wood) degraded with *Trametes orientalis* than when degraded with consortium of *Trametes orientalis* and other white rot fungi. However, the fluctuation in the amount of reducing sugar recorded with degradation time could be as a result of utilization of the released reducing sugar as source of energy by *Pleurotus ostreatus* and *Lentinus squarrosulus* during degradation as earlier observed by Bari *et al*.(2018).

The height of carbon dioxide produced per time in Durham's tube was used for selection of yeasts for ethanol production because carbon dioxide is produced along with ethanol by yeasts as reported by Dung *et al*.(2012). The selected yeasts were *Saccharomyces cerevisiae*which has been known for conversion of hexoses to ethanol. The hexoses obtained from degradation of lignocellulolytic substrates in this work were fermented to ethanolthrough glycolysis and conversion of pyruvate to ethanol via alcoholic fermentation pathway. Ability of selected*Saccharomyces cerevisiae*to produce ethanol from hexoses in this work could be due to its ability to produce pyruvate decarboxylase and alcohol dehydrogenase during alcoholic fermentation as reported by Zhang *et al*.(2021).Production of ethanol from pretreated lignocellulose carried out in this study has been observed by some researchers (Onoghwarite *et al*., 2016; Baz *et al*., 2017; Gani *et al*., 2018; Germec and Turhan, 2018;Kaur *et al*., 2018; Mokomele *et al*., 2018; Takano and Hoshino, 2018; Wu *et al*., 2018; Zhao *et al*., 2018; Cabanas *et al*., 2019; Huezo *et al*., 2019; Bonan *et al*., 2021; Jin *et al*., 2021; Kolajo, 2021).Bioethanol has been produced from maize stalk (Kolajo, 2021) sugarcane bagasse (Bonan *et al*., 2021) and rice straw(Jin *et al*., 2021).

The observed decrease in reducing sugar content recorded during fermentation of degraded substrates could be probably due to conversion of reducing sugar to ethanol during fermentation because ethanol is produced from reducing sugars (Bautista *et al*., 2018; Germec and Turhan, 2018; Kurambhatti *et al*., 2018; Mokomele *et al*., 2018; Zhao *et al*., 2018). Kurambhatti *et al*. (2018) observed decrease in reducing sugar concentration with fermentation period and at the same time increase in ethanol content with increase in fermentation day.

The observed decline in ethanol yield after initial increase in the yield in the first 72 hours of fermentation of degraded rice straw, maize straw and sugarcane bagasse fermented by*Saccharomyces cerevisiae* SA01 and *Saccharomyces cerevisiae* SA02 could probably be due to decline in reducing sugar in the fermentation medium and conversion of ethanol to other compounds (Tsunatu *et al*., 2017; Adelabu *et al*., 2018; Huezo *et al*., 2019; Mori *et al*., 2019). In line with this observation is the report of Mori *et al*. (2019) that exhaustion of fermentable sugarresults in oxidation of ethanol to acetate through acetaldehyde by many microorganisms which will later be converted into acetyl-CoA and then changed to carbon dioxide and water in Kreb's cycle. Highest yield of bioethanol observed at 72 hours of fermentation in this work is in line with the work of Tsunatu *et al*. (2017) and Adelabu *et al*. (2018) who also reported maximum bioethanol production at 72 hours of fermentation.

The observed higher reducing sugar in combined pretreated maize than single pretreated maize straw could be attributed to synergistic effect in both biological and sodium hydroxide pretreatment (Ma *et al*., 2010; Suhardi *et al*., 2013; Dai *et al*., 2015;Kumar and Sharma, 2017; Yang and Wang, 2019).The possible breaking down of the recalcitrant lignin in the lignocellulosic substrates might have exposed to enzymes produced by mushrooms used for biological pretreatment. Pretreatment of lignocellulose with acid/base prior to introduction of cellulase producing organism allows better access of the cellulase to cellulose in lignocellulolytic substrates (Saulnier *et al*., 2020; Kumar *et al*., 2021).Yang and Wang (2019) reported that combined pretreatment of grass resulted in higher yield of xylose and arabinose than single pretreated sample while Dai *et al*. (2015) observed that biological pretreated rice straw combined withsodium hydroxide pretreatment had higher yield of sugar than single pretreated one.

Presence of furfural and hydroxymethylfurfural in the hydrolysed combined pretreated maize straw could have prevented glucose from being available for ethanol production (Kupiainen *et al*., 2014). Furfural and hydroxymethylfurfural have been reported to have negative effect on metabolism of microorganism during fermentation (Lukajtis *et al*., 2018) andthis could be responsible for initial low yield of ethanol experienced in this work.

Higher yield of ethanol observed in combined pretreated maize straw than single pretreated one could be probably be because of higher reducing sugar recorded in combined pretreated maize straw and fermentable sugars are needed for ethanol production (Suhardi *et al*., 2013; Mori *et al*., 2019). Suhardiand others (2013) reported higher amount of ethanol in combined pretreated rice straw than single pretreated one.The utilization of reducing sugar by *Saccharomyces cerevisiae* SA01 and *Saccharomyces cerevisiae* SA02to produce ethanol might be responsible for the decrease in reducing sugar observed with fermentation day in this research (Liang *et al*., 2013; Taiwo *et al*., 2018; Mori *et al*., 2019).A similar report by Taiwoand others (2018) revealed a decrease in reducing sugar content and increase in ethanol content with increase in fermentation day.

The observed increase in ethanol with increase in the pH till optimum pH 5.5 of acetate buffer used to extract fermentable sugar from combined-pretreated maize straw has been reported by some researchers (Wakil *et al*., 2013;Abo *et al*., 2018;Adelabu *et al*., 2018; Tasnim and Farasat, 2018). Adelabu *et al*. (2018) reported increase in ethanol production from sorghum straw with increase in pH till pH 5.5 thereafter decrease in ethanol content was observed and Tsunatu *et al*. (2017) reported optimum initial pH of 4 when rice straw was fermented by *Saccharomyces cerevisiae*. Abo *et al*. (2018) observed increase in amount of ethanol produced from sugar molasses with increase in pH and reported highest amount of ethanol at pH 5.0. Each microorganism has pH range under which it can perform maximally below or above which the microbe would not be able to perform well and *Saccharomyces cerevisiae* has been known for production of ethanol under acidic condition (Nadeem *et al*., 2015).

The influence of temperature observed on the ethanol yield by the yeasts could be due to power of temperature on growth, metabolism, survival of fermenting organisms and fermentation (Tiwari *et al*., 2015). The recorded optimum temperature (30 °C) for production of ethanol in this study has been reported by some researchers (Nadeem *et al*., 2015; Taiwo *et al*., 2018; Tasnim and Farasat, 2018). Adelabu *et al*. (2018) obtained maximum production of ethanol by yeasts at 40 °C. Yeast are destroyed at too high

temperature and their activities are slowed down at very low temperature (Nadeem *et al*., 2015).

Easy assimilation of glucose than fructose as source of carbon and energy by *Saccharomyces cerevisiae* as reported by Mori *et al*. (2019) could be responsible for higher yield of ethanol from glucose than fructose by *Saccharomyces cerevisiae* SA01 and *Saccharomyces cerevisiae* SA02. Significant effect of different concentration of sugar on ethanol production recorded in this work could be due to different carbon to nitrogen ratio and this has been reported by some researchers (Liang *et al*., 2013; Nadeem *et al*., 2015; Mori *et al*., 2019).Initial increase in ethanol content observed in this study with increase glucose concentration was probably because the glucose was converted to ethanol. Similar observation was reported by Liang *et al*. (2013) that ethanol content increased with increase in sugar concentration until a maximum ethanol content was obtained and any further increase in sugar concentration resulted in decrease in ethanol content. Highest ethanol content was attained in this work when supplemented with 2 % glucose and similar result was obtained when Nadeem *et al*. (2015) supplemented fermentation medium with 2 % of glucose and had good yield of ethanol. Decrease in ethanol content when supplemented with more than 2 % glucose in this research could be due to inability of the yeasts to withstand the glucose concentration higher than 2 % which is in line with the work of Mori *et* al. (2019) who reported that each organism has limit of sugar concentration it can withstand in order to have optimum production of ethanol (Mori *et al*., 2019).

The use of corn steep liquor as source of nitrogen in this work improved the ethanol yield of *Saccharomyces cerevisiae* SA01 by 300 % (from 3.95 to 11.84 g/L) and that of*Saccharomyces cerevisiae* SA02 by 661 % (from 1.97 to 13.02 g/L). The reason being the richness in nutrients of corn steep liquor and a good source of nitrogen which is important for growth and metabolism of yeast (Taiwo *et al*., 2018; Hassabo *et al*., 2021). Utilization of corn steep liquor, a byproduct obtained during production of *Ogi*, with no positive economic value, as source of nitrogen for the production of ethanol is turning wastes to wealth. Similarly, Taiwo *et al*. (2018) replaced yeast extract with corn steep liquor and observed rapid utilization of reducing sugar and good yield of ethanol.

However,almost no ethanol was produced because of quite low biomass concentration when Liu *et al*. (2016) replaced yeast extract with different concentration of corn steep liquor as source of nitrogen.

Groundnut cake increased the yield of ethanol by 323 % and 641 % with *Saccharomyces cerevisiae* SA01 and *Saccharomyces cerevisiae* SA02 respectively. This shows that groundnut cake is a good source of nitrogen that can be used for fermentation of ethanol instead of high-cost nitrogen sources like yeast extracts and others (Singh *et al*., 2020). Similar record of good yield of β-Mannanase with groundnut meal as nitrogen sourcehas been reported(Umerie *et al*., 2000).

Soyabean meal is a byproduct of soya oil production which has tendency to be used as a source of carbon and nitrogen for production of ethanol (Lujan-Rhenals *et al*., 2017). Use of soya meal as nitrogen source led to increase in ethanol yield by 300 % with both *Saccharomyces cerevisiae* SA01 and *Saccharomyces cerevisiae* SA02 in this research which could be because soya meal is rich in nitrogen (Bhowmik *et al*., 2015; Singh *et al*., 2020). Bhowmik *et al*. (2015) replaced yeast extract with soya bean meal and reported better and quicker production of endotoxin than what was recorded with yeast extract.

Vegetal by-products (corn steep liquor, groundnut cake and soya meal) as nitrogen sources had better production of ethanol than animal by-products (fish meal and blood meal) as nitrogen sources by both *Saccharomyces cerevisiae* SA01 and *Saccharomyces cerevisiae* SA02 in this study. This could be probably due to the presence of more carbon in vegetal by-product than animal by-products which means vegetal by-product can be used as both carbon and nitrogen sources. Similar result was observed when Yatmaz *et al*. (2016) used fish meal and soya meal as nitrogen source for production of β-Mannanase where soya meal as nitrogen source had better yield of β-Mannanase than fish meal as source of nitrogen. However, Umerie *et al*. (2000) observed better production of lysine with blood meal than soyabean meal as nitrogen source with defatted meal but higher production was reported by soyabean meal with non-defatted meal.

The observed differences in the ethanol yield with various dilutions of the acetate buffer and corn steep liquor was probablydue to different ratio of carbon to nitrogenthat could have been formed with different dilution of acetate and corn steep liquor (Yatmaz *et al*.,

2016). These researchers observed different yields of β-Mannanase with different ratio of carbon to nitrogen.Hence, carbon to nitrogen ratio (C/N) in production medium is very important in order to have optimum yield by used organisms.

Microbial load and size play important role in the yield of ethanol by *Saccharomyces cerevisiae* SA01 and *Saccharomyces cerevisiae* SA02. Increase in ethanol yield observed with increase in inoculum load was possibly due to increase in the number of cell with no increase in the volume making the cell to metabolize reducing sugar quickly to bioethanol.The decrease in ethanol production observed with increase in inoculum size of *Saccharomyces cerevisiae* SA01 and *Saccharomyces cerevisiae* SA02 could be due to dilution of nutrients by higher sizes (volumes) of inoculum resulting in limited nutrients that were present in the fermentation medium which will directly lead to low ethanol yield. This was also observed by Adelabu *et al*. (2018) who reported decrease in ethanol production from sorghum straw with increase in inoculum size.

CHAPTER SIX

CONCLUSION

Pleurotus ostreatus and *Lentinus squarrosulus* are white rot fungi that are able to produce cellulase, xylanase and lignase/laccase which are important in breaking down cellulose, hemicellulose and lignin respectively.*Pleurotus ostreatus* and *Lentinus squarrosulus* can be used to pretreat lignocellulose to release fermentable reducing sugar. Degraded maize straw, sugarcane bagasse and rice straw had better yield of reducing sugar than groundnut shell and maize cob.The amount of reducing sugar released varied with the substrate, organism and degradation time. Better reducing sugar was obtained in combined pretreated maize straw than single pretreated maize straw which led to higher production of bioethanol from combined pretreated maize straw. High bioethanol content (14.99 g/L) by *Saccharomyces cerevisiae* SA01 and *Saccharomyces cerevisiae* SA02 was obtained by extraction with 60/40 ratio of acetate buffer (0.1M, pH 5.5) to corn steep liquor, supplemented with 2 % glucose, and fermented with 1% inoculum of 1.0 MacFarland standard at 30 °C for 3 days. Therefore, the use of cheap, readily available substrate and cost-effective pretreatment technique made bioethanol production from maize straw economically attractive.

Contributions to Knowledge

- The comparative quantification of reducing sugar released from different lignocellulosic materials which varied with organism and degradation time and the reducing sugar was higher from soledegradation with *Pleurotus ostreatus* compared with *Lentinus squarrosulus* and co-cultured strains.
- Maize straw, sugarcane bagasse and rice straw had better yield of reducing sugar than groundnut shell and maize cob.
- Combined alkaline pretreatment followed by biomass degradation using *Pleurotus ostreatus* yielded higher reducing sugar than single pretreatments.
- Corn steep liquor 'waste' was an effective nitrogen source for ethanol production using pretreated maize straw.
- Increased Inoculum load significantly increased ethanol production by both strains of *Saccharomyces cerevisiae*

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